Using Life Cycle Assessment as a Tool to Evaluate and Make Recommendations for Biopharmaceutical Manufacturing

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DECLARATION

I, Charnett Chau, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

Signature.....

Date.....

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ABSTRACT

Life cycle assessment (LCA) is a well-regarded methodology used to evaluate the environmental impacts of a system, essential to supporting the 2030 Agenda for Sustainable Development Goals. Due to the increasing need for companies to act more environmentally friendly, employing LCA to systematically and quantitatively evaluate their products and processes would be necessary. To date, little LCA work has been applied to biopharmaceutical production; this may be due to a lack of inputs and outputs data, methodology available or knowledge related to LCA. Hence, this project sought to develop guidance to apply LCA to biopharmaceutical processes, considering questions that companies would typically require to address. To this end, the LCA methodology was operationalised to the production of a major biopharmaceutical product, 6-APA, to demonstrate the advantages and limitations of LCA. As 6-APA represents the largest production mass output of the industry, industry-wide practical steps and policy considerations to reduce environmental impacts were drawn.

A series of LCA analyses, including sensitivity analyses, hot-spot analyses, scenario analyses and comparative study were conducted on the "average" 6-APA manufacturing process, modelled with input including that from industry contacts. This set of analyses ensured that recommendations drawn from the LCA study considered all factors, including the robustness and significance of results and the relationship between process parameters, specifically product titre, scale, location, and environmental impacts. Hot-spot analysis was conducted on nine scenarios where 6-APA production was considered to locate in different countries. Results concurred that the highest impacts in most environmental impact categories were derived from the supply of essential production materials and the electricity mix. This underscored the importance of considering the source (or the choice of suppliers) for the process inputs. The normalisation methodology was applied to estimate the relative impact of 6-APA manufacture globally and to assess the significance of the impacts generated. It showed that ecotoxicity impacts from coal energy generation in China were highly significant when production was scaled to global levels. This posed the question of whether the level of impacts generated in this single location was environmentally damaging. Hence, the thesis suggests that governments may wish to take steps to prevent potential environmental damages from possible over-concentrations of impacts. This thesis also highlights areas of further work, including improvements to inventory data, the assessment of later biopharmaceutical life cycle stages, and economic and social LCA, to complement and enhance the life cycle environmental impact assessment presented here.

IMPACT STATEMENT

Life cycle assessment (LCA) is a proven environmental impact assessment methodology. The employment of LCA has assisted many industries in developing their products, processes and systems to become more environmentally sustainable; it has further assisted governments to establish policies to lower our impacts on the environment at a national level. The 2030 Agenda for Sustainable Development (SDGs) that was adopted by all United Nations members particularly asks for responsible consumption and production (Sustainable Development Goal 12) (United Nations, 2015). This entails proper life cycle management of resources and materials and encourages companies to adopt sustainable practices to reduce wastes and emissions to the environment. Hence, LCA is essential to supporting the SDGs.

This project aims to play a role in the wider adoption of LCA by biopharmaceutical companies by encouraging its integration as part of process development procedures. This objective was achieved:

1. By developing specific guidance for the biopharmaceutical industry to allow companies to optimise their processes environmentally whilst meeting the needs of their various stakeholders;
2. Through the operationalisation of the application of the LCA methodology to a major biopharmaceutical product, 6-APA, to demonstrate the advantages and limitations of LCA amongst the recommendations that can be drawn to reduce environmental impacts on an industry-wide basis.

As 6-APA represents the largest production mass output of the industry, insights from the project can benefit the following parties:

- Biopharmaceutical companies – recommendations drawn from the analyses include practical steps to reduce environmental impacts—E.g. considerations for siting production facilities and the source for input materials.
- Governments – from understanding the significance of the global distribution of impacts associated with 6-APA production, policy considerations were drawn to prevent over-concentrations of impacts in one area.
- Academia - the limitations of the LCA conducted on 6-APA has indicated where future research is required to understand further the locational factors that will affect the environmental impacts associated with a product.

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ABBREVIATIONS

°C	Degree Celcius
%Change	Percentage change
1,4-DB	1,4 dichlorobenzene
6-APA	6-aminopenicillanic acid
7-ACA	7-aminocelphalosporanic acid
7-ADCA	7-aminodeacetoxy-cephalosporanic acid
AE	Accumulated exceedance
ALCA	Attributional life cycle assessment
API	Active pharmaceutical ingredient
BAT	Best available technology/technique
BR	Brazil
C footprint	Carbon footprint
CED	Cumulative energy demand
CER	Corporate environmental responsibility
CF	Characterisation Factor
CFC	Chloroflurocarbon
CIP	Cleaning-in-place
CLCA	Consequential life cycle assessment
CML	Centre of Environmental Science, Leiden University
CN	China
CO_2	Carbon dioxide
COSHH	Control of Substances Hazardous to Health
C_p	Specific heat capacity
CSR	Corporate social resposibility
CTU_e	Ecotoxicity comparative toxic unit
CTU_h	Human toxicity comprative toxic unit
DALY	Disability-adjusted life year
DE	Germany
DNA	Deoxyribonucleic acid
EA	Environmental assessment
EC	European Commision
ECDC	European Centre for Disease Prevention and Control
Eco 95	EcoIndicator 95
EDIP	Environmentl Design of Industrial Products
EEA	European Environment Agency
EI	Environmental index
EIA	Environmental impact assessment
EMA	European Medicine Agency
EoL	End-of-life
EPA	Environmental Protection Agency (US)
ES	Spain
EU ETS	European Emission Trading Scheme
EU-28	European Union, 28 member states
ExA	Enery/exergy analysis
FLASC	Fast life cycle assessment of synthetic chemistry

FTE	Full time employee
FU	Functional unit
g	Gram
GB	Great Britain
GHG	Greenhouse gas emission
GLO	Global
GSK	GlaxoSmithKline
GWh	Gigawatt hour
GWP	Global warming potential
GWP_{100}	Global warming potential, 100 year time-frame
HE	Heat exchanger
H_{fg}	Specific enthalpy of evaporation
HVAC	Heating ventilation and air conditioning
ILCD	International Reference Life Cycle Data System
IN	India
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organisation for Standardisation
J	Joule
JRC	Joint Research Centre (European)
K	Kelvin
L	Liter
LAPC	Local Air Pollution Control
LCA	Life cycle assessment
LCC	Life cycle costing
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
m	(symbol) mass flow rate / (unit) metre
m^3	Cubic metre
mAb	Monoclonal antibody
MDG	Millennium Development Goals
MFA	Material flow analysis
MJ	Megajoule
MRI	Midwest Research Institute
MY	Malayasia
N	Nitrogen
NaOH	Sodium hydroxide
NEPA	United States National Environmental Policy Act
NICE	National Institute for Health and Care Excellence (England)
NME	New molecular entity
NVOC	Non-methane volatile organic carbon
PAA	Phenylacetic aicd
PMI	Process mass intensity
POF	Photochemical ozone formation
PS	Production scale
PT	Product titre
PW	Purified water
RDR	Royal Decree
REPA	Resource and environmental profile analysis

RER	Europe
RNA	North America
RoHS	Restriction of the use of certain hazardous substances
RoW	Rest of the World
S	Sulphur
SDG	Sustainable Development Goal (The 2030 Agenda for Sustainable Development)
SETAC	Society of Environmental Toxicology and Chemistry
SG	Singapore
SIP	Steaming-in-place
S-LCA	Social life cycle assessment
STD	Standard deviation
t	Tonne
T	Temperature
TRACI	Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts
UBP	Environmental impact point
UN	United Nations
UNEP	United Nations Environment Programme
US	United States
W	Watt
WEEE	Waste electrical and electronic equipment
WFI	Water for injection
ZA	South Africa

Chapter 1: PROJECT MOTIVATION

1.1 INTRODUCTION

As the concepts of sustainable development become increasingly important within our society, every sector is becoming a subject of greater scrutiny in terms of their policies and strategies around sustainability. This includes the biopharmaceutical industry. Unlike the energy and chemical industries that were made to pioneer new approaches to reduce their environmental footprint following major polluting events (e.g. the Great London Smog (1952) and the Seveso disaster (1976)), the biopharmaceutical industry can now follow the examples set by the more mature industries (i.e. chemical industry) in terms of sustainability practices. These include exploiting green chemistry, employing effective onsite waste management, staying within emission limits set by governments and having corporate policies on health and safety. Historically, the biopharmaceutical industry was presumed to cause moderate environmental impacts. However, studies have emerged showing that their production processes are energy, material and waste intensive; and therefore are more comparable to industries that are known to be environmentally burdensome (Ramasamy, 2015). With this, it is now important to understand whether biopharmaceutical processes have major impacts on our environment.

Life cycle assessment (LCA) is a widely used and well-regarded methodology to evaluate environmental impacts, and it provides a powerful tool for understanding the impacts of the biopharmaceutical industry. There are other approaches employed by various industries, including the wider pharmaceutical industries, for measuring environmental sustainability. Examples include sustainability indicators, such as PMI and E-factors; and, other sustainability assessments, such as material flow analysis (MFA) and energy-exergy analysis (ExA). The indicators: PMI and E-factor, convey the intensity of input materials and waste generated from product manufacture, respectively. As for the environmental assessments: MFA and ExA, evaluate the input and output of materials and energy flows within a set, respectively. LCA is known as the most comprehensive tool to evaluate environmental sustainability. In addition to evaluating material and energy flows, it takes further steps to evaluate the impacts on our environment over a product's life cycle, from material extraction through to the product's disposal and recycle (Curran, 1994; Hunt and Franklin, 1996; Ramasamy et al., 2014).

LCA was developed in the 1960s to assist with environmental management and has since been a valuable tool in policy-making and process development. It has been particularly the case within the consumer and chemical industries to assist with working towards the goals of sustainable development (explored further in Chapter 2). Given the intrinsic link between

economic, environmental and social sustainability, optimising processes from an environmental perspective often leads to economic and social benefits. For instance, increasing resource-use efficiency may decrease supply costs and labour intensity. However, there are few impact assessments regarding biopharmaceuticals presented in the literature (see Table 1). Only ten studies assessed the impact of biopharmaceutical active pharmaceutical ingredients (APIs), and none addresses the impact of final formulated drug products (further literature review in Chapter 2). As a whole, this means that the impact of the industry is not yet well understood.

Furthermore, the use of LCA in the pharmaceutical sector is generally disjointed; although assessments on traditional pharmaceutical are more prevalent in literature, a review showed that they lack consistency (Emara et al., 2018b). Progress in predicting impacts associated with synthetic pharmaceuticals are being made, e.g. FLASC platform (Curzons et al., 2007; Jimenez-Gonzalez, 2002). However, Table 1.1 indicates slow progress in assessing biosynthesised therapeutics. The first LCA on a biotechnology-derived pharmaceutical was published in 2006 (Bruggink and Nossin, 2006); the low number of studies since the tool's introduction, 15 years ago, shows that there might be a barrier to its uptake.

This thesis acknowledges that the biopharmaceutical industry is a growing sector and contemplates further that if environmental sustainability is left unchecked, the accumulated impact has the potential to contribute significantly to existing global climate problems. Sustainable development has become a responsibility for every individual. As outlined in the 2030 Agenda for Sustainable Development (SDGs), the SDG 12 asks for responsible consumption and production and encourages companies to become as environmentally sound as possible (United Nations, 2015). The agenda suggests implementing sustainable practices and becoming environmentally transparent by reporting information around sustainability. For the biopharmaceutical industry to meet this agenda, it is important to find ways to overcome barriers to integrating LCA as part of process development procedures and improving the industry's transparency in this area.

Table 1.1: Summary of literature reporting life cycle assessment of pharmaceutical and relevant biotechnology-derived products. These studies are discussed in Chapter 2.

Product Type	Total	LCA Case Study*					Framework / Methods**	Review / Comments†
		Cradle-to-API (or Intermediate)	Cradle-to-Product	Gate-to- Product EoL	Focusing on Packaging	Focusing on Process Equipment / Material		
Pharmaceuticals in General	5				2	1	1	2
Synthetic Pharmaceutical (Chemically Derived)	21	12	6	2	4	3		1
Biopharmaceutical (Biotechnology Derived)	13	8				6	4	1
Semi-Synthetic Pharmaceutical (Biotechnology and Chemically Derived)	2	2						
Other Biotechnology Products	5		4			1		`

* Metrics used varied amongst the case studies. While most examine the life cycle environmental impacts, different LCIA were used and highlight mainly global warming potential (GWP) and cumulative energy demand. Out of the 20 LCA case studies for synthetic pharmaceuticals, six were exergy LCAs, three carried out inventory analysis, discussed process PMI and/or E factor, and one only discussed inventory results and GWP. For biopharmaceuticals, out of the eight case studies, two focused on inventory analysis and GWP. In addition, while a range of synthetic API has been examined, only monoclonal antibodies (mAbs) (six), vaccine (one) and antibiotics (two) have been studied. MAbs and vaccine case studies were not product-specific; studies typically compared single-use and multi-use technologies.

** All frameworks and methods provided in the literature suggest a cradle-to- "end of API production" gate, except one for biopharmaceuticals that advise cradle-to- "end of vial fill" gate. Recommended metrics varied.

† Review papers conclude the variability in metrics used and the challenges for pharmaceutical companies to adopt LCA as common practice fully. One review on pharmaceuticals, in general, showed insufficient studies on the end-of-life impact of therapeutics (i.e. ecotoxicity and human impact due to accumulation in the environment).

1.2 Barriers to the Integration of LCA with Biopharmaceutical Development

Conceptually, the barrier to the use of LCA by biopharmaceuticals can stem from multiple factors and may be attributed further to the age of the industry. The biopharmaceutical industry is relatively new as compared to other industries. While the first biotechnology-derived drug, penicillin, was formally produced at a large scale in the 1940s, it was not until the 1980s that genetic modification (recombinant DNA) technology emerged that biopharmaceuticals were classified differently from traditional pharmaceuticals. The primary focus of the industry is placed on the innovation of new therapeutics that can widen the range of diseases treatable. Consideration of the environment is only a secondary concern. This may be why companies have neglected to ensure the environmental footprint for their processes is captured and minimised.

In addition, before the introduction of biopharmaceuticals, governmental environmental policies were already in place to set limits and requirements on emissions and waste treatment. Hence, companies are required only to meet these guidelines without the added pressure to investigate the most environmentally preferable process. Furthermore, although biopharmaceuticals are a subcategory of pharmaceuticals, compared to traditional pharmaceuticals, which are chemically (or synthetically) derived, they are perceived to be less environmentally burdensome. This is mainly because the production of biotherapeutics does not necessarily involve the harsh chemicals that are often required for chemically-derived drug synthesis, which can incur undesirable emissions. Biopharmaceuticals are also often produced in smaller quantities as compared to traditional pharmaceuticals, suggesting that their environmental footprint as a whole is lower. Perceptions as such may have reduced further the need for biopharmaceuticals to conduct environmental assessments.

Another barrier to LCA use is the amount of information that would require disclosing in order to carry out the assessment and the subsequent reporting. As the commercialisation of biopharmaceuticals requires large investments, both time and money, companies are reluctant to share process specific details. Process information, such as unit operations and material inputs, can convey the requirements to manufacture products, which competitors can use as reference when developing biosimilars. To avoid competition, biopharmaceuticals are often patented. However, given that products would only be protected for a relatively short time frame, companies would choose not to disclose process specifics in order to maintain market competitiveness.

As a whole, overcoming these barriers to LCA use will require changing the mindset of biopharmaceutical decision-makers and/or governmental environmental requirements. In order to motivate the uptake of the LCA by companies, it must be demonstrated as beneficial for

companies to take leadership in improving their process sustainability and that its usage will not jeopardise return on investments. Otherwise, governments can potentially oblige companies to report product life cycle environmental impacts to inform environmental policy development specific to the industry. In reality, the two options may complement one another.

As highlighted in Figure 1.1, publicised academic findings prompt public expectations and policy formation over time. They occur when concerning topics have gone through iterations of research and awareness-raising, which have led to a consensus as to how specific issues should be rectified amongst our society. Equally, with increasing academic studies illustrating the environmental issues our global issues now face, our society has come to expect environmental initiatives beyond those set in law to be at least attempted.

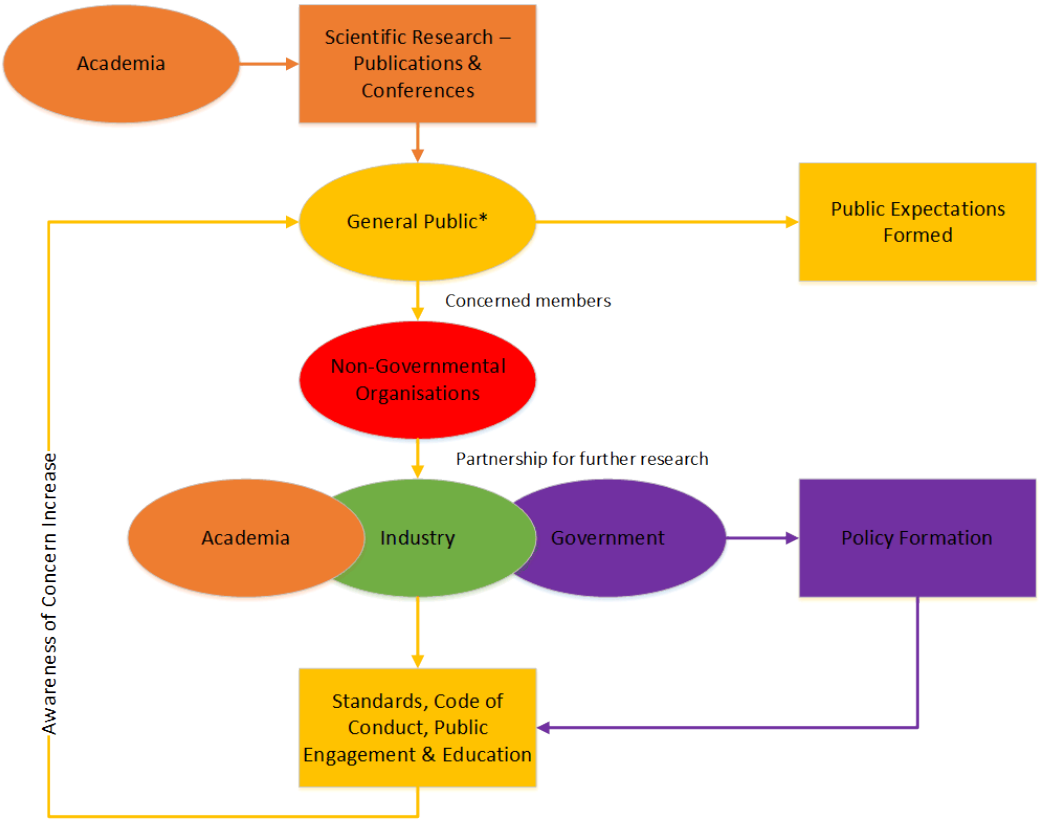

*Figure 1.1: Formation of public expectations and governmental policy. *General public composites all members of society including academic, government, shareholders and customers.*

Li and Yu (2011) have highlighted that having corporate environmental responsibility (CER) strategies, such as obtaining sustainable supply chains and adopting green practices, gives companies competitive advantages over those who do not. This is because CERs are usually considered alongside visions for business growth, show long-term thinking by business decision-makers, and are exploited as part of marketing campaigns to increase a company's reputation and provoke profit. The benefits of having CERs create motivations for biopharmaceutical companies to be more transparent in their sustainability reporting and to use LCA to assist with

business development plans. Governmental rulings can also provide additional motivation. If LCA is mandated, a company must carry out environmental reporting to avoid fines and scrutiny by the general public. Regardless of whether LCA is governmentally mandated or otherwise, once the biopharmaceutical industry fully adopts the tool, the abundance of studies detailing their impacts can inform the government in developing industry-specific environmental policies. This can further direct the industry towards becoming truly sustainable, aligning them further with the sustainable development goals. However, dedicated guidelines on biopharmaceutical process developmental planning and advice on how LCA results can be used and reported without disclosing process sensitive information would be essential for the tool's full adoption by the industry. This forms one of the main objections of this project.

1.3 Project Aims and Objectives

This thesis aims to (1) operationalise the application of LCA to a relevant case study showing the advantages and limitations of the methodology and (2) address our limited knowledge in the environmental impacts associated with the biopharmaceutical industry.

As a way to overcome barriers to LCA adoption, this project **demonstrates the benefit of applying the LCA to the biopharmaceutical industry** that can assist with process development and optimisation and enable the reporting of LCA results by biopharmaceutical companies without disclosing process sensitive information. **To begin understanding the many environmental impacts exhibited by the biopharmaceutical**, the LCA methodology was used to analyse global 6-APA production, the largest manufactured product by mass in the industry. Different LCA analyses, hot-spot analysis, sensitivity analysis, scenario analyses, and comparative analysis were carried out **to draw recommendations on the areas for improvement**. By analysing global 6-APA production, the project seeks to showcase the extent to which the supply and manufacturing processes and potential environmental policies can be optimised when sufficient LCA results on biopharmaceutical processes are collated.

A breakdown of the objectives and contents for each chapter are highlighted below:

Chapter 2 – Project Background (Literature Review)

A more in-depth explanation of the two subject areas of this project, sustainable development and the biopharmaceutical industry, is presented in Chapter 2. The chapter defined and discussed first the terminologies used and the history behind sustainable developments and the biopharmaceutical industry. This aimed to set out the scope of this thesis. The chapter then reviewed the biopharmaceutical companies' current economic, environmental and social sustainability and the current environmental policies that they must abide by. This aimed to give

context to the current activities being taken to drive the biopharmaceutical industry into becoming more sustainable. Next, a background on the introduction of environmental sustainability indicators and assessments, particularly LCA, was explored to give insights into their importance in supporting sustainable development. This gave reasons for the use of environmental measures by biopharmaceutical companies. The chapter then presents a review of the sustainability tools and metrics that biopharmaceutical companies currently employ and highlights the knowledge gaps in our understanding of the levels of environmental sustainability they achieve. These gaps were discussed alongside LCA's merits, and an argument on how they can be filled with the assessment's assistance is presented. Lastly, the chapter explored further the potential motivations for company decision-makers to adopt new initiatives and how LCA can be configured to best suit their needs. As part of this, exemplary use of LCA by other industries and how LCA can be used to support companies to align better with the SDGs were also presented.

Chapter 3 – Apply the Life Cycle Assessment Methodology to Biopharmaceutical Manufacture

Following the definitions in Chapter 2, the role of the assessment stages involved in LCA is explained in depth in Chapter 3. The chapter explores the roles and the different approaches available for each LCA stage to provide justification for the decisions made for applying the LCA methodology in subsequent chapters. Through this process, the chapter also provides alternative methodologies that can assist with answering questions beyond those posed in the chapter. The LCA methodology presented follows a "cradle-to-gate consequential product-oriented LCA" and shows how results can be reported. The approaches chosen acknowledge that consistent use of the tool by all manufacturers requires common goals to answer particular questions and generate results that drive toward a common purpose. Key stakeholders were considered during this process in order to define the goals and study the scope of the work. This influenced the inventory allocation and the choice of the life cycle impact assessment (LCIA) methodology used to analyse biopharmaceutical processes.

Chapter 4 – 6-APA Production in the US – A Case Study

In order to demonstrate the results and recommendations that may be derived from the LCA guidelines described in Chapter 3, a decision was made to assess the production of 6-APA using different analysis approaches. Chapter 4 presents the base-case study where 6-APA was assumed to be manufactured in the US. The choice of case study relates to the maturity of the manufacturing process and the high production mass. It was hypothesised that the study of 6-APA would form a base from which to gauge the overall environmental impacts associated with the entire biopharmaceutical industry.

The goal of the LCA case study emulated a manufacturer aiming to carry out process optimisation on their 6-APA production process. For this, a hot-spot analysis was carried out on the "average" manufacturing process of this product, which was advised by industry contacts. To check the significance and the robustness of the results, the results were normalised to global per person emissions, and sensitivity analyses were carried out, respectively. Scenario analyses were additionally carried out on the base-case model to understand how particular decisional parameters, product titre and production scale can affect the environmental impact associated with a product.

By testing the LCA model's sensitivity to parameter fluctuations and different impact assessment methods, the LCA methodology and case study results were shown to represent the 6-APA process adequately. Drawing from all analyses, the chapter presents the areas where environmental improvements were necessary and options to reduce impacts were diagnosed and highlighted. In addition, the chapter discusses the inadequacy of extrapolating environmental impact results on 6-APA production to predict other products, with reference to the scenario analyses conducted.

Chapter 5 – Comparative Study: The Effects of Production Location on Product Environmental Impacts

Chapter 5 presents a comparative study that further assessed the effects of another decision-lead parameter, production location, on the environmental impact profile of a biopharmaceutical product. Although the production process for 6-APA was assumed to be the same, location-specific variables such as the supply processes for raw materials, energy mix and water scarcity were modified to reflect production in nine different countries. The results demonstrated the correlations between these variables and the changes in environmental impacts and suggested that biopharmaceutical developers should consider manufacturing location as part of the process development program to minimise environmental footprints of manufacturing.

An analysis was carried out to estimate the global impact of 6-APA production. The approach taken for this estimation was first interrogated before recommendations were made based on the results of this chapter. However, when other decisional factors, such as local policies and economic incentives, were also considered, it revealed that governmental intervention might also be needed to ensure that local environmental thresholds are not exceeded due to the overproduction of one product type. Nonetheless, this chapter interprets the LCA results and recommendations for 6-APA production to their potential uses in governmental environmental policies. It highlights the importance of national and international policy as a key element in

ensuring that biopharmaceutical plants are operating in a manner that is consistent with sustainable development.

Chapter 6 – Conclusions and Future Work

A summary of the main contributions deriving from this work and future work suggestions are presented in this chapter. Contributions include the sources of high environmental impacts, which biopharmaceutical companies should consider during process development stages and further guidance on conducting and reporting LCAs on biopharmaceutical processes. The future works highlighted were derived from the limitations risen from conducting LCA on the production of 6-APA. Limitations included the difficulties in data gathering, particularly life cycle inventories (LCIs); the knowledge in fundamental factors that determine the environmental impacts associated with a process; the evaluation of 6-APA does not convey the impacts of a beta-lactam antibiotic API fully; and the problems deriving from comparing production processes based on their environmental impacts only. In addition, since this thesis operationalised the application of LCA only to the production of 6-APA, future works suggest the further operationalising of LCA to cover subsequent life cycle stages of this biopharmaceutical production intermediate.

1.4 PROJECT OUTCOMES

The following is a summary of the expected outcomes of this thesis:

- Application for the first time of the LCA methodology to a typical scale production process of 6-APA, and develop guidance for biopharmaceutical companies aiming to conduct LCA on their processes.
- A detailed step-by-step environmental impact assessment analysis, including assessing the interdependencies between the methodologies adopted and results obtained, and the advantages and limitations of the methodologies used.
- Recommendations on practical steps and policy considerations to reduce the environmental impact of 6-APA production at the global level, based on the LCA analysis undertaken.

Chapter 2: PROJECT BACKGROUND

2.1 INTRODUCTION

The main objective of this chapter was to expand on the motivations and the research groundings of this project (conveyed in Chapter 1). This chapter presents first the background and definitions of sustainable development and the biopharmaceutical industry. This includes the importance of both subjects and the key terminologies necessarily defined for this project. The chapter then discusses the current sustainability of the biopharmaceutical industry regarding the three pillars of sustainability: economic, environmental and social, and the current actions companies take to promote each sustainability aspect. Next, this chapter gives background to environmental assessments in sustainable development and their use within the broader pharmaceutical industry, with specific reference to life cycle assessment (LCA). The last section highlights the motivations for biopharmaceutical companies to adopt the use of LCA by referring first to the current sustainability status of the industry then further presenting the benefits of LCA. As part of the latter, exemplary uses of LCA in the past and how LCA can support companies to meet various Sustainable Development Goals are presented.

2.2 DEFINING SUSTAINABLE DEVELOPMENT AND THE BIOPHARMACEUTICAL INDUSTRY

From reviewing various sources, discrepancies were found on what "sustainable development" and "biopharmaceutical" mean and entail. As the project aimed to use LCA as a tool to assist the biopharmaceutical industry in aligning better with the goals of sustainable development, it was necessary to define the scope of both subject areas. The following sections give a brief history of "sustainable development" and "biopharmaceutical", which includes how they and associated key terminologies have emerged to have different interpretations. By defining the key terminologies used in this project, the scope in which the guidance on applying the LCA methodology to biopharmaceutical processes presented in this thesis would cover is presented.

2.2.1 Sustainable Development – Background and Key Terminologies

The concept of "sustainable development" dates back to the 1960s (Creech, 2012) despite only appearing as a phrase, for the first time, in the Brundtland Report (1987), where it was defined (see Table 2.1). The concept arose from the concern for our environment after the realisation that human activities can bring detrimental damages to the Earth's natural systems, on which we rely. The initial care for the environment can be attributed to resolving "nuisances" from industries (late 19th century) where noise, smell and visual pollution were an annoyance for members of the public (Palmer, 2015). However, it took major environmental events, such as

the “Great London Smog” in 1952 and the first nuclear accident in 1957, to occur before national and international plans were implemented to address environmental issues as a collective.

“Silent Spring” by Rachel Carson (1962) was said to be vital in introducing the concept of sustainable development (Creech, 2012). It demonstrated the “interconnections among the environment, economy and social well-being” (Carson et al., 1962) and the necessity to create harmony between human activities and the environment, including living conditions. The book highlighted how, due to the increasing food demands from our society, the agricultural operations flourished and progressed economically. However, this induced an increase in the use of pesticides linked heavily to animal health, human health and environmental damages (Creech, 2012; Mebratu, 1998).

The concept of sustainable development acknowledges that it is necessary to progress our society but not at the expense of our natural ecosystem. Hence, there is an inherent link between the environment and sustainability, particularly when discussing ways to create true sustainability. It is often implied that when a system is sustainable, it is environmentally friendly. However, over the years, it has become apparent that for a truly sustainable system, it must be economically, environmentally and socially sound (Figure 2.1 and defined in Table 2.1). This means that while a growing system requires a net positive cash flow (economic sustainability), the rate at which natural resources are consumed must not be higher than the rate it can be replenished (environmental sustainability) and that there are continual support for this system, i.e. sufficient workforce and need for the system (social sustainability).

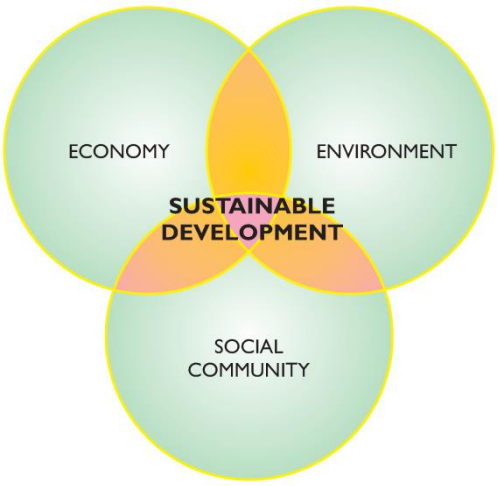

Figure 2.1: The three aspects of sustainable development.

With this understanding, the United Nations introduced the Millennium Development Goals (MDGs) in 2000 to steer the world towards being a sustainable development. The main focus of the MDGs was to make progress in combatting extreme poverty from all angles in a 15-year

timeframe. This required governments to implement movements and forge global partnerships for developments that allow an increase in accessibility to higher incomes, primary education, food, clean drinking water, healthcare and improved living environments. While the MDGs were successful in attaining goals for clean water, improving sanitation, and protecting areas, reports showed that climate change, also known as global warming, is an imminent issue that was not focussed on and would hold the most negative impact on those in extreme poverty where there is a lack of infrastructure (United Nations Secretariat, 2016). This outcome has highlighted how the combination of poor economic background and environmental issues in an area can affect the livelihood of its inhabitants, consolidating the interconnection between the three pillars of sustainability. Hence, one key learning from the MDGs was that more considerations for the environment were needed to achieve global sustainable development.

Another key learning from the era of MDGs was the importance of using the correct sustainability metrics to quantify progress. This was derived from the criticism that the most extreme poverty was overlooked due to using national average data as metrics (Fehling et al., 2013; United Nations Secretariat, 2016). It stemmed from concerns over the 15-year timeframe being too short for establishing proper infrastructure, particularly to tackle illiteracy, diseases incidences, and mortality rates in remote regions where extreme poverty is likely. Substantial improvements in easily accessible regions would have masked areas that required the most attention. Although there were flaws highlighted with the programme, it was said to have laid an adequate foundation to build sustainable targets upon (United Nations Secretariat, 2016). By considering the learnings gathered from the MDGs, the UN developed the 2030 Agenda for Sustainable Development – Sustainable Development Goals (SDGs) to include particular goals for major environmental issues, better-designed metrics and devised methods to monitor progress in all regions to assure that the agenda is inclusive - “leaving no one behind” (Development Initiatives, 2018). Here, distinctions must be made between the “Sustainable Development Goals” and the goal of sustainable development. The latter refers to achieving sustainable development as an overall goal and not the individual goals as set by the UN. However, both tend to follow the same ideology as the SDGs lay out many of the principles that industries are encouraged to follow (see 2.5.2).

As a whole, sustainable development does not concern only the environment but recognises that systems must also balance economic and social progression for a system to be truly sustainable. This means that for an industry to strive towards sustainable development, it must have an economically sound business that is backed by its stakeholders and ensure that the natural resources it requires can be continually supplied. Table 2.1 presents the terminologies that surround sustainable development and the definitions that are employed by this thesis.

According to the definitions provided, this thesis aims to promote the use of life cycle assessment (LCA) (Sections 2.4 and 2.5) to support the sustainable development of the biopharmaceutical industry. It focuses on how the application of LCA can improve the industry's environmental sustainability; and economic and social sustainability when all aspects are considered to operationalise the application of the LCA methodology.

Table 2.1: Key definitions surrounding sustainable development that are employed in this project.

Terminology	**Definition / Notes**
Sustainability	n. The ability in maintaining and continuing activities at a certain level over time. "Sustainability" is also frequently used to describe the level of avoidance in depleting natural resources or the level of competence to maintain an ecological balance. However, this explanation is too specific and is considered to define "environmental sustainability".
True Sustainability	n. A term used to describe a system where all aspects of sustainability: economic, environmental, and social (respective definitions below), harmonise and complement one another. The terms "sustainability" or "sustainable" alone can sometimes be used as "true sustainability", but the terms are too generic and therefore leaves it to readers to interpret. "True sustainability" avoids misinterpretations.
Sustainability Development	n. Describes a development (or system – e.g. business, product) that "is conducted without depletion of natural resources". It "…is, in essence, development that meets the needs and aspirations of the present generation without destroying the resources needed for future generations to meet their needs" (World Commission on Environment and Development., 1987).
Economic Sustainability	n. The ability for a system to function economically indefinitely. In development, economic sustainability is the ability to support economic growth over long periods (Barbier, 1987; Brown et al., 1987). Both systems and developments require a minimum net cash flow of zero. This is where the rate of income is at least equal to the rate of expenditure. For growth, positive cash flow is important such that further investment can be made. Poor management of money is why many new companies go bankrupt in their first two years (Doane and Macgillivray, 2001; Veleva and Ellenbecker, 2001).
Environmental Sustainability	n. The ability for a system to maintain ecological balance (Goodland, 1995). That is the maintenance of the Earth's natural cycles to provide continual services, such as purification of air and water; decomposition of wastes; and pollination of crops and natural vegetation, that we need for the future (Barbier, 1987; Goodland, 1995; Reddy and Thomson, 2015). Environmental assessments (EA), such as life cycle assessment (LCA), are used to quantify the burden exhibited by a system by balancing input and output parameters (Friends of the Earth, 2005). From such analyses, companies can focus

	on reducing their environmental footprints to move towards environmental sustainability.
Social Sustainability	n. The ability to maintain social wellbeing and social expectations. "Social sustainability" is often regarded as the hardest to define and maintain (Blackburn, 2007) because social wellbeing and social expectations vary between different communities as well as amongst members of the same community. McKenzie (2004) has defined social sustainability as "a life-enhancing condition within communities, and a process within communities that can achieve this condition." Life-enhancing conditions make this aspect of sustainability hard to quantify, as they are usually subjective. Indicators include, but are not limited to, the level of various forms of equality and the efficacy of the system that builds and maintain community responsibilities, which are subjected to the social expectations and living standards of a given community (Hutchins and Sutherland, 2008).
Millennium Development Goals (MDGs)	n. Refers to the eight international development goals set by the United Nations (UN) following the Millennium Summit in 2000, where the UN member states adopted the Millennium Declaration. The main aim of the MDGs was to reduce extreme poverty by 2015.
Sustainable Development Goals (SDGs)	n. Refers to the 17 international development goals set by the United Nations (UN) following the UN Sustainable Development Summit in 2015, where the UN member states adopted the 2030 Agenda for Sustainable Development. The SDGs were in the planning since 2012 as part of the Post-2015 Development Agenda, a process to develop a framework that would succeed the MDGs. Unlike the general goal of (attaining) sustainable development (defined above), the SDG aims to progress our society to achieving sustainable development globally.

2.2.2 The Biopharmaceutical Industry – Background and Key Terminologies

In this thesis, the biopharmaceutical industry is classed as a sub-section of the pharmaceutical industry. Although some "Big Pharma" supporters and a large portion of the financial sector considers all products deriving from life science companies to be biopharmaceuticals (Rader, 2008), others have agreed that pharmaceuticals are all medicinal products, which includes biopharmaceuticals but not all pharmaceuticals are biopharmaceuticals (PhRMA, 2013; Rader, 2008; Walsh, 2003). Other confusing or misleading definitions include one stated by Rader (2005) where BioSpace Glossary (website removed) stated that biopharmaceutical companies involve research "into new drugs as well as manufacturing, marketing, and distribution of pharmaceutical products". The range of interpretations prompted the necessity to summarise what processes and products pharmaceuticals and biopharmaceuticals encompass.

Unlike traditional herbal medicines, pharmaceuticals are compounds that cause biological activities in the human body, designed to treat various diseases (Table 2.2 for the types of pharmaceuticals and Table 2.3 for definitions). The terms "pharmaceutical" and "therapeutic" are often used interchangeably. Both can refer to either the specific active component(s) (active pharmaceutical ingredients – APIs) that induces biological activity or the overall formulated products, which can be in the forms of tablets, syrups, injections, sprays or patches. Until the 20th century, besides vaccines, pharmaceuticals were small molecules (<900 Daltons): natural compounds extracted from plants and fungi, chemically transformed derivatives of natural compounds, or chemically synthesised. With the increase in knowledge in biological systems, the extraction of macromolecules (large molecules) from animal and human to treat diseases was made possible. When techniques to produce biologically-derived products at a large scale, i.e. fermentation technology, the term "biotechnology" was introduced to represent the overarching method of harnessing and utilising biological systems to produce beneficial products. In the pharmaceutical industry, the first biotechnological process was the production of penicillin (the 1940s). In this thesis, enabling the production of penicillin was denoted as the birth of the biopharmaceutical industry (see Table 2.3 for the definition of biopharmaceutical); there may be discrepancies against this designation, but this considered definitions from various sources (Rader, 2008; Ronald A. Rader, 2005; Van Beuzekom and Arundel, 2006).

The term "biopharmaceutical" emerged in the 1980s (Walsh, 2003) when recombinant DNA technology was introduced, and it was initially used to describe therapeutics that were derived from the genetic modification of organisms. However, since the first recombinant products were macromolecules (large molecules), which are similar to the hormones and proteins in the human body, it was presumed that biopharmaceuticals are restricted to being such products. As recombinant DNA technology is the manipulation of biological systems to produce a certain product, it is classed as a biotechnology. As a way to distinguish the scientific breakthrough of utilising genetic engineering from the traditional manipulation of biological systems, the terms "modern biotechnology" and "traditional biotechnology" were introduced (Biotechnology Innovation Organization, 2017; Demain, 2007; Ronald A. Rader, 2005; Walsh, 2003).

"Traditional biotechnology" refers to the use of readily available living organisms to generate day to day products, including cheese, yoghurt, beer and drugs (OECD, 1998). Meyer & Schmidhalter (2014) highlighted that the first use of cell suspension cultures for commercialised production was for lactic acid in 1893, and then acetone, butanol and ethanol from *Clostridium sporogenes* in 1913. In addition to lactic acid, citric acid and gluconic acid came about for the preservation of food and flavouring of soft drinks, which has remained very popular to date. The breakthrough of biotechnology in the pharmaceutical sector was the ability to cultivate vitamins,

steroids and antibiotics between 1930 and 1955. Compared to recombinant proteins, traditional biotechnology-derived pharmaceuticals have greater production volumes. This is because (1) they are natural products that are easily obtainable and can be manufactured by many companies, and; (2) they can usually treat multiple indications. To reflect the market availability, the selling prices of these products are usually low to mid-ranged as comparable to other commodity chemicals (Bentley and Bennett, 2008).

"Modern (or novel) biotechnology" tends to be regarded as recombinant DNA technology. The technology involves modifying a host cells genome so that it will produce the desired molecule during fermentation. These molecules are usually modified proteins used to treat diseases either more effectively than old treatments or treat diseases that were not treatable previously (Kyriakopoulos and Kontoravdi, 2012). As the makeup of these proteins, i.e. the amino acids, are naturally found in every living being, it can be said that they are less likely to be rejected by the human immune system (Porter, 2001). Most of these therapeutics are injected into patients, unlike most small molecule drugs that are orally consumed.

Being only approximately 30 years old, the modern biotechnology sector of the biopharmaceutical industry has bloomed greatly. Products are usually high-valued but produced in low quantities. It was recorded that the industry generated a total of $228 billion in revenue in 2016 (Jakovljevic et al., 2017) with over 250 biologics (large recombinant molecules) on the market (Flyte, 2015; Otto et al., 2014). The global market is expected to reach $390 billion by 2024 (Mordor Intelligence, 2018) due to emerging products. The industry is continuing to expand with 4000 – 5000 drugs in research and development (R&D) and a yearly approval rate of four new molecular entities (NME); this is without considering biosimilars and biobetters that are also in development (Kinch et al., 2014). The success of modern biotechnology has led to the categorisation that biopharmaceuticals are therapeutics that are large molecules produced via modern biotechnology and that traditional biotechnology is used to manufacture readily existing small molecule therapeutics. However, once researchers began genetic engineering the productions of traditional products (e.g. increasing titres or purities), modern biotechnology became involved in producing small molecule therapeutics.

As the types of products produced by either mode of biotechnology became hard to differentiate, people started to expand the scope of biopharmaceutical to include traditional biotechnological products. In addition, the prefix "bio" in biopharmaceutical indicates the involvement of biological systems; this is true for both traditional and modern modes, and therefore makes sense to classify them similarly. Dictionaries have adopted this view (Cambridge English Dictionary, 2019; Merriam Webmaster, 2017).

Table 2.2: Types of pharmaceuticals and their production method.

First Discovery / Marketed	Pharmaceutical Type	Definition/Primary Production Method	Substance Type
1796 / 1798 (made available, non-profit.) (Cowpox/Smallpox)	Vaccines	Viruses were extracted initially from animals. Now - incubation of pathogens in egg or more recently produced through recombinant technology	Weaken or part of pathogens (viruses, bacteria and toxins)
1805 / 1826 (Morphine)	Plant / Fungi Derived	Substances that are naturally occurring and extracted from plant, root and fungi.	Alkaloids; steroids; salicylate
1832 / 1869 (Chloral Hydrate)	Chemically Synthesised	Substances obtained through organic chemistry, i.e. series of chemical reactions.	Various - chemical compounds (small molecules)
1897 / 1899 (Aspirin)	Semi-Synthetic	Substances that were chemically transformed from a product that is produced by living organisms/cells (plant, fungi, microorganism or mammalian).	Various – modified versions of natural compounds (small molecules)
1901 / 1922 (Animal Insulin)	Human / Animal Derived	Substances that are naturally occurring and extracted from human/animal.	Proteins - blood-derived clotting factors, polyclonal antibodies; peptides – hormones; toxins
1928 / 1942 (Penicillin)	Microorganism Derived	Substances that are naturally occurring and extracted from microorganisms. Production via cell culture fermentation.	Metabolites – antibiotics; toxins; alkaloids; vitamins
1975 / 1982 (Human Insulin)	Recombinant Therapeutics	Substances derived from the genetic modification of animal and microbial cells, which are subsequently grown in cell cultures to produce products.	Various – both small and large molecules Current focus: monoclonal antibodies, hormones and vaccines

Like the term “pharmaceutical”, there were some confusions with the scope of “drug”. While some sources have said that drugs refer specifically to tabletted small molecule pharmaceutical products, others have stated that drugs include all formulated pharmaceutical products (i.e. containing either small or large molecule APIs) (Ronald A. Rader, 2005). Furthermore, the terms “biologic” and “biosimilar” (generic versions of biologics) in US Food and Drug Administration (FDA) and European Medicines Agency (EMA) articles are referred to as products that are derived from biotechnology and are “biological drugs”; this will class biologics, which are not necessary small molecule products, as “drugs” (Dranitsaris et al., 2011; EMA, 1999; FDA, 2012; HHS, 2002). Some sources further categorise that “biologics” and “biosimilars” are specialised biotechnology-derived therapeutics, such as vaccines, monoclonal antibodies and hormones, which are designed to target specific diseases (Conner et al., 2014; Weise et al., 2011). Hence, biologics and biosimilars are biological drugs, which are interchangeable with biotechnology-derived drugs/therapeutics. The term “biopharmaceuticals”, like “pharmaceutical”, can refer to the component within biological drugs that causes biological activity or the final formulated therapeutic.

Table 2.3: Key definitions surrounding the pharmaceutical industry that are employed in this project.

Terminology	Definition
Pharmaceutical / Therapeutic	n. A medicinal product where the biologically active component is either chemically synthesised or extracted from living organisms or produced via biotechnology. Can refer to the final tabletted or formulated product, or the active pharmaceutical ingredient only.
Biopharmaceutical	n. A medicinal product where the biologically active component is produced via biotechnology. Can refer to the final tabletted or formulated product, or the active pharmaceutical ingredient only.
Active Pharmaceutical Ingredient	n. The biologically active component of a pharmaceutical product.
Drug	n. A medicinal product where the biologically active component is either chemically synthesised or extracted from living organisms or produced via biotechnology. Typically refer to the final tabletted or formulated product.
Biological (drug)	n. A medicinal product where the biologically active component is produced via biotechnology. Typically refer to the final tabletted or formulated product.
Biologics	n. Recombinant large molecule products, derived from modern, recombinant DNA, biotechnology.
Biosimilar	n. The generic form of a biologic that may be produced via a different method but function as efficiently or better.
Biotechnology / Bioprocess	n. (1) "The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services." (OECD, 2005) n. (2) An umbrella of unit operations or processes that are used to process biological materials. There are two major types: traditional and modern biotechnology. (Further differences are given below).
Traditional Biotechnology	n. (1) A process or technology used to cultivate, extract and purify naturally occurring products from living cells. Sources include microorganisms, plants, animals and human.
Modern Biotechnology	n. (1) A process or technology involved in the generation, production, extraction and purification of recombinant products. This requires the genetic engineering of whole organisms or living cells to allow the production of materials that are not native to them and approaches that assist in developing potential products and optimise the cultivation process.

The different interpretations highlighted have served to show how the industry is forming its identity. By gathering information from different sources, terminologies within the biopharmaceuticals were defined (Table 2.3). As a whole, products from the biopharmaceutical industry are manufactured via biotechnology. Since biotechnology involves manipulating living cells and/or their components to generate the product, this excludes natural substances existing in plants, such as morphine, unless the plant was genetically modified to produce a novel substance. Although secondary metabolites, such as penicillin, can be produced naturally in bacterial or fungi cells, specific cultivations are necessary to manipulate and promote production. For simplification purposes, this project considered products that form part of a final drug product and; requires fermentation/cell culturing technology for their production as products of the biopharmaceutical industry.

2.3 The Biopharmaceutical Industry and Sustainable Development

Having defined "sustainable development" and "the biopharmaceutical industry", this chapter will now progress to providing a background to the sustainability of biopharmaceuticals. As described in Table 2.1, the promotion of all three aspects of sustainability concurrently is needed to generate sustainable development. The extent to which biopharmaceutical companies embrace this is discussed. Since there is limited knowledge on the environmental sustainability aspects of the industry, the environmental laws that companies must abide by are presented as a guide to their current performance.

2.3.1 The Biopharmaceutical Industry and the Three Pillars of Sustainability

Although the biopharmaceutical industry has grown into a high-value business, valued at $240 billion in 2018 (Mordor Intelligence, 2018), individual companies within this sector are not necessarily economically sustainable. Due to the inherently high risk in drug development: high capital investment, lengthy development timelines, clinical hurdles and a short repay period due to expiry of patent protections, biopharmaceuticals, especially new start-ups, are economically unstable. It was quoted to cost approximately $953 million over a 10-year development period for a drug to be commercialised (Herper, 2017). This leaves approximately ten years to achieve a return on investment before patent expiry. Since the large capital is funded primarily by investors and shareholders, they place pressure on the drug development company to guarantee a return on investment. Otherwise, future partnership opportunities may not be possible (Paul et al., 2010). Figure 2.2 highlights the actions taken by biopharmaceutical companies to secure investment by shareholders.

Due to the level of economic unsustainability, company activities tend to surround securing revenue and preparing for lower-income when patents of existing drugs expire. This is important for biopharmaceuticals to satisfy shareholders and for the long-term maintenance of the company. Companies typically aim to expand profit margins, secure sales and generate a promising pipeline (Figure 2.2). To achieve these objectives and satisfy shareholders, other stakeholders may also need to be satisfied to increase social sustainability. For instance, holding relevant corporate social responsibilities (CSRs), a declaration of a company's position on societal and ethical matters, will gain support from customers and employees if company activities align with their belief. By gaining stakeholders' support, it may increase sales and/or increase workplace efficiency. These are also examples of how developing social sustainability can also improve economic sustainability.

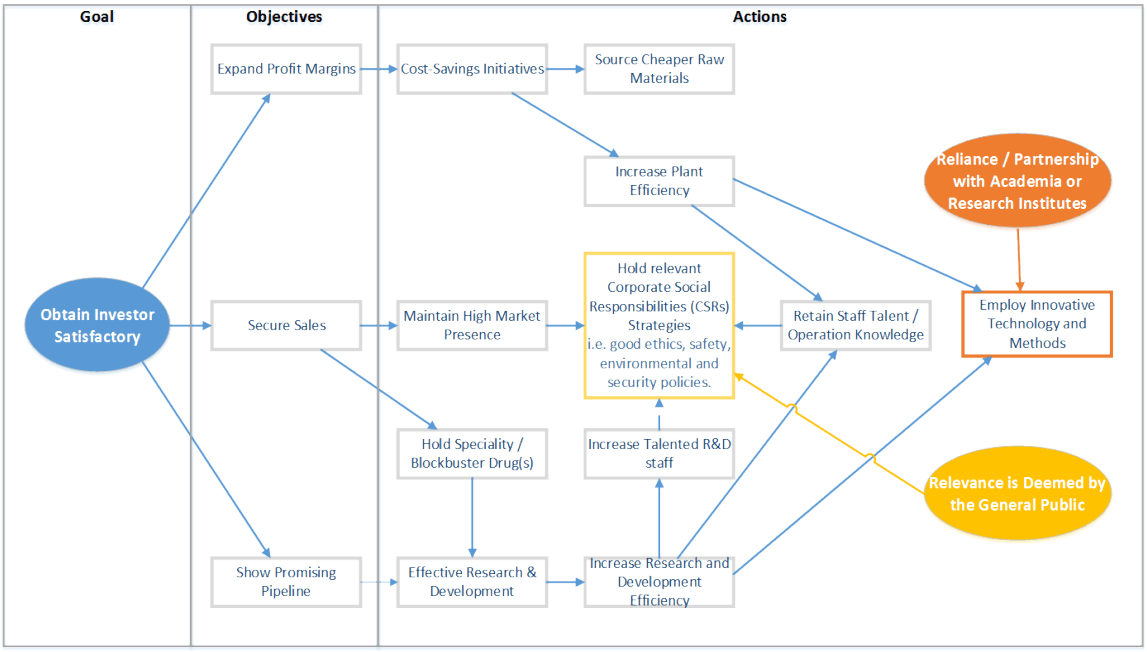

Figure 2.2: Actions that biopharmaceutical companies take to secure investments and increase the potential return on investment to satisfy investors. These actions are usually derived from three objectives shown in the figure. Holding relevant corporate social responsibilities improves social sustainability, and employing innovative technology that is more efficient can benefit environmental sustainability. Oval = stakeholder's influence; Rectangle = company objectives and actions.

In the context of social sustainability, the biopharmaceutical industry is invaluable to our society and therefore has support from the general public as a whole. As an industry, it promotes the well-being of members within communities with the products it supplies; however, companies must meet social expectations to maintain their social sustainability. Basic social expectations are set by law and are followed by most biopharmaceutical companies. Our global society, in general, has grown to agree on various community responsibilities that are deemed "enhancing" and necessary for everyone to abide by (O'Dwyer and Owen, 2005). Many are translated into national and international governmental policies to set the standard of how we should act. Indicators have been established to monitor these. For example, most governmental agendas require the levelling of various types of equality, including gender and ethnicity (Blackburn, 2007; McKenzie, 2004). An indicator for this is statistics on gender ratio within workplaces, which reveals the efficacy of procedures in place. If inadequate, society has learnt to scrutinise and require the company to take actions to rectify their misconduct.

Further examples of baseline expectations for businesses set out by law include adequate health and safety, treatment of workers, and environmental care. Individuals who do not meet governmental requirements can be fined and scrutinised by the general public, reducing their social support (Tolson 2008). As an example, Pfizer received a settlement fine of $486 million to investors in August 2016 (Garden City Group LLC, 2016). Allegedly, the company misled investors on the safety of their drugs, which prompted the securities lawsuit that resulted in the fine.

Scrutiny was reflected by the drop in company share prices at the time (Garden City Group LLC, 2016).

In addition to meeting governmental requirements, most companies also have CSR to state their position on how they are strategising to tackle public concerns. Where they exceed society's expectation, research has shown that companies benefit by gaining competitive advantages (Hutchins and Sutherland, 2008; Li and Yu, 2011). Since environmental issues are topical, holding corporate environmental responsibilities (CER) strategies will gain social support (Li and Yu, 2011). Johnson and Johnson, as an example, is a biopharmaceutical manufacturer that aimed to partner only with suppliers that have two or more public sustainability reports (Johnson & Johnson, 2015). This meant that vendors who do not comply with environmental management standards (e.g. ISO 140001, an NGO standard) are unlikely to be partnered. It also showed leadership in driving fellow companies to become more environmentally conscious whilst proving that companies can be disadvantaged for not keeping up with social expectations.

CER strategies that biopharmaceutical companies implement can range from commitments directly associated with the environmental sustainability of their processes to supporting the wider community. Examples include reducing plant emissions and waste, installing sustainable production infrastructure, increasing cleaner energy resources, and supporting community-wide environmental initiatives like local environmental conservation work. Activities such as reducing waste and input materials can include implementing more efficient technology and operational methodologies, which helps the company function more economically (Figure 2.2). Hence, activities to increase a company's environmental sustainability has the potential to increase social sustainability (through reporting activities as CER) and increase economic sustainability.

Although there is pressure from the public to act "environmentally" sustainably, it may not override biopharmaceutical companies' need to prioritise profit-making to supplement their capital costs. While companies do conduct cost-saving activities and must abide by environmental laws (Section 2.3.2), there are not many reports or studies in the literature regarding the environmental consequences of biopharmaceutical activities (see Section 2.4.1). Inferring from the steps taken by the industry to evaluate the environmental impacts of their processes, optimising their environmental sustainability is not yet a priority. To gauge better the foundation of activities that biopharmaceutical companies carry out to reduce their environmental footprint, the environmental laws they must abide by are presented next. It provides a discussion on whether the laws in place are sufficient in applying pressure onto companies to act as sustainably as they can be.

2.3.2 Environmental Law that Biopharmaceutical Abide by

Like all industries, there are both national and international laws, such as Local Air Pollution Control (LAPC) within the European Union, that biopharmaceutical manufacturers abide by. Table 2.4 summarises the environmental legislation that can affect and influence manufacturing decisions. Since different countries and/or regions may hold different environmental concerns, the legislations reflect this by applying differing rules (Tolson, 2008). It was evident that in the EU, there is much concern for air and wastes, whereas, in the US, the focus is on water. Both areas of legislation affect manufacturing industries, as they must monitor their waste outputs and emissions to air and water within limits set in law. While laws regarding air, water and waste ensure proper management of emissions from a process, laws associated with land and wildlife affect decisions on manufacturing locations.

Although there is an extensive list of environmental policies, most focus on reducing emissions, particularly for tackling climate change, and were put in place through a reactive approach. For instance, industries with high emissions are addressed first (Fiksel, 2006). Since the scales of production of biopharmaceuticals are relatively small, emissions per manufacturing plant are comparably smaller. Therefore, some legislations do not necessarily apply for or may not be limiting biopharmaceuticals. One example is the Climate Change Levy, European Emission Trading Scheme (EU ETS). This scheme was created to reduce emissions from approximately 12,000 companies (Centre for Climate Energy Solution, 2016; European Commission (EC), 2016), and only a few pharmaceutical companies who manufacture bio-therapeutics with "heavy-energy using installations", such as GlaxoSmithKline, were mandatorily added (SEPA et al., 2013). Otherwise, there are no specific emission limits for biopharmaceutical companies. This presents little pressure for companies to strive for a greener process besides the pressure of staying cost-competitive.

Table 2.4 Summary of laws and policies to do with environmental issues that affect manufacturing. Source: Coxall and Hardacre (2020), European Parliament (2020), Tolson (2008), and US EPA (2021).

	US Law	UK / EU Law
Air	• Clean Air Act Amendment 1970 • Clean Air Act 2015 • Toxic Substances Control Act (1976) • The Kyoto Protocol • Montreal Protocol • United Nations Framework Convention on Climate Change	• Local Air Pollution Control (LAPC) • Local Air Pollution Prevention and Control (LAPPC) • Clean Air Act (1993) • Climate Change Agreements 2006 • Climate Change Levy 2001 • Climate Change Levy, European Emission Trading Scheme (EU ETS) • EU Regulations on Substances that Deplete the Ozone Layer, Environmental Protection (Control on Ozone – Substances) Regulations 2002 • The Kyoto Protocol • Montreal Protocol • United Nations Framework Convention on Climate Change
Water	• Clean Water Act 1972 • Interim Clean Water Act 1995	• The Water Industry Act 1991 • The Water Resources Act 1991

	• Coastal Zone Management Act 1972 • Oil Pollution Act 1990 • Proposition 65 1986 • Safe Drinking Water Act 1974	• Control of Pollution (Oil Storage) (England) Regulations 2001
Land	• Comprehensive Environmental Response, Compensation and Liability Act 1980 • Emergency Planning and Community Right-to-Know Act 1986 • Federal Land Policy and Management Act 1976 • National Environmental Policy Act 1970	• Town and Country Act 1990 • The Town and Country Planning (England and Wales) Regulations 1999 • The Environmental Act 1995 • Contaminated Land (England) Regulations 2000
Waste	• Resource Conservation and Recovery Act 1976 • Environmental Protection Agency Act, 1992	• Environmental Protection Act 1990 • Environmental Protection (Duty of Care) Regulations 1991 • 7th Environment Action Programme • Control of Pollution (Amendment) Act 1989 • Controlled Waste (Registration of Carriers and Seizures of Vehicles) Regulations 1991 • Hazardous Waste Regulations 2005 • Waste Electrical and Electronic Equipment (Producer Responsibility) (WEEE) Regulations 2004 7 Restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS) • List of Wastes Regs 2005 • Producer Responsibility Obligations (Packaging Waste) Regulation 2005
Wildlife	• Endangered Species Act 1973	• Wildlife and Countryside Act 1981

In addition to the environmental policies in Table 2.4, there are guidelines and directives developed to assist industries in lowering their environmental footprint. Two that are particular for manufacturing industries are Control of Substances Hazardous to Health (COSHH) (HSE, 2005) and the Best Available Techniques (BATs), which is part of the European Directive on reducing industrial emissions (Directive 2010/75/EU, 2010). COSHH is concerned with the handling and disposal of substances. This is applicable to some of the materials used and disposed of by the biopharmaceutical industry. However, many materials are not perceived as hazardous to health. As suggested in LCA reviews of the industry, the environmental impact profiles of many of the materials necessary for biopharmaceutical manufacture are not yet quantified (see Section 2.4.2.1). Hence, it is unclear whether process materials are truly environmentally harmless or should there be threshold considerations for all materials. While materials may not be hazardous to human health, safe extraction and disposal for all materials to prevent unwanted consequences to the environment should be thought out. COSHH may hold the potential to evolve to guide the handling of a wider range of materials by considering more than the health and environmental threats of the materials alone and include threats associated with its generation and supply.

For BATs, there are reference documents for various industries, which gives guidance on the technologies that should be used for certain processing (European IPPC Bureau, 2019). Guides most relevant to the biopharmaceutical industry include BATs for the “Manufacture of organic fine chemicals” (European Commision, 2006), “Common waste water and waste gas treatment/management systems in the chemical sector” (Brinkmann et al., 2016) and “Waste

treatment" (Pinasseau et al., 2018). The document on organic fine chemicals, also refers to pharmaceuticals (chemically and biologically synthesised) and highlights the typical operations and processes used for their manufacturing and key environmental issues within the sector in general. It states that the key issues are "emissions of volatile organic compounds, waste waters with potential for high loads of non-degradable organic compounds, relatively large quantities of spent solvents and non-recyclable waste in high ratio" (European Commision, 2006). However, these emissions are not particular to biopharmaceutical processes, the resources employed and the emissions of subsequent processes are largely dependent on cell growth and purification requirements, which do not usually require harsh chemicals. Environmental issues stemming from biological waste inactivation is more likely. In addition, there were no data available on raw materials consumption, which means the currently available BATs is not a comprehensive overview. Acknowledging that input materials are part of the technology to manufacture a certain product, governments may want to give guidance on the best available techniques (and materials) that have the lowest environmental footprint over their life cycle.

With current emission thresholds not being a limiting factor for biopharmaceuticals and little guidance present to reduce emissions from biopharmaceutical processes, governments pose little incentives for biopharmaceutical companies to lower their emissions. The issue with this is that although a single company may not have high emissions than those from other industries, the biopharmaceutical industry comprises many companies with smaller processes. Figure 2.3 shows that the industry spans across the world with over 1200 manufacturing facilities. The industry is also continuing to expand, as stated in Section 2.2.2. Hence, the risk is that all emissions combined may result in high emissions as the industry's impacts go unchecked.

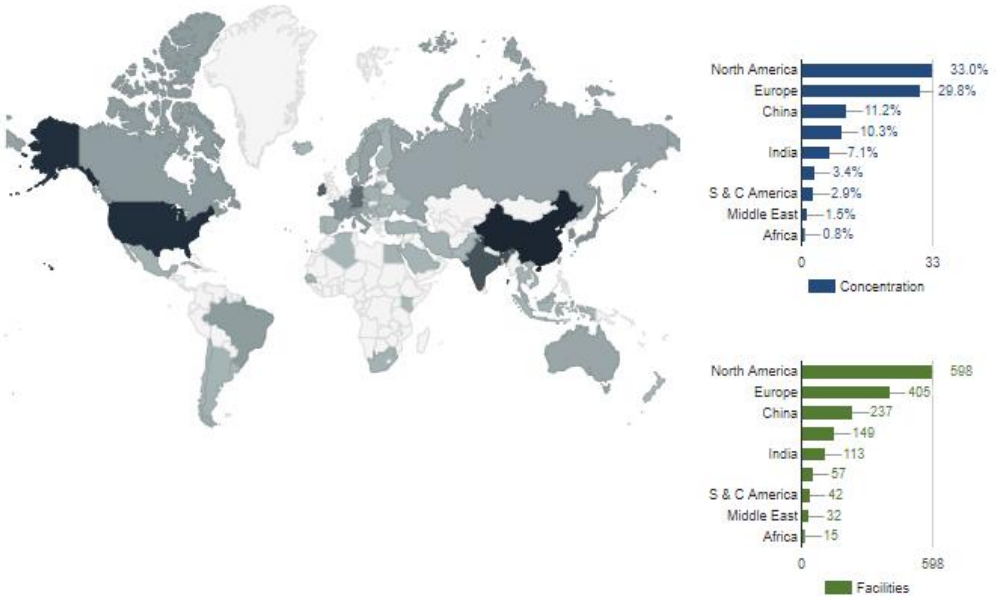

Figure 2.3: Map of biopharmaceutical facilities, last update 26 October 2020 by BioPlan Associates Inc. (2020)

As many governments have pledged to the 2030 Sustainable Development Agenda, environmental policies may evolve to a more preventative approach to coincide further with sustainable development and green chemistry principles. Governmental policies are continuously evolving to reflect our society's needs based on knowledge built through publicised research (as shown in Figure 1.1) (Aniekwe et al., 2012; Hagendijk and Irwin, 2006; Lavis et al., 2002). Due to the maturity of other industries, there is industry-specific (BATs) guidance. In the future, there are potentials for new regulations to govern biopharmaceuticals on their environmental footprint. However, the use of environmental assessments (EA), particularly life cycle assessment (LCA), would likely be necessary.

2.4 The Role of Environmental Assessments in Sustainable Development

As a way to build a case for the use of LCA for measuring the environmental sustainability of biopharmaceutical processes, this section reviews the role of environmental measures in assisting companies to become more sustainable. First, the different sustainability indicators and environmental assessments (EA) are introduced to highlight their role in sustainable development. Then, a more specific review is then provided on the current usage of these tools, particularly LCA, by the biopharmaceutical industry, with reference to the chemical and pharmaceutical industry. Here, the gaps in LCA studies and the issues that should be addressed in this project are discussed.

2.4.1 Environmental Sustainability Indicators and Assessments

Governments worldwide are said to have begun introducing environmental policies and advocating sustainable development in the 1960s and 1970s, following damaging environmental events (Section 2.2.1). The events highlighted the need for waste management and process safety regulations to set limits on the waste being generated by industries and how to avoid best polluting our land and water resources. Naturally, by setting limits on waste and emissions, they became metrics used to report environmental performances of processes. Sustainability indicators or metrics are referenced values of a system used to track sustainability levels. The first sustainability indicators included the amount of waste (emissions to air, land and water) produced (for indicators specific to the chemical and pharmaceutical industries, see Section 2.4.2.1.). Due to the realisation that fossil resources are finite, metrics that were routinely used also included the extent of resources required for a process and the efficiency of their use. Since indicators may not necessarily detail how a process affects the environment, environmental assessments (EAs) are necessary to highlight undesirable impacts. EAs are typically used to assist developmental planning and understand the environmental sustainability of systems to assist process optimisation.

The formal use of different EAs emerged simultaneously as concerns and policies were introduced to reduce the environmental footprint of industries (between the 1960s - 1970s). The major assessments that are in use today are environmental impact assessment (EIA), material flow analysis (MFA), energy/exergy analysis (ExA) and life cycle assessment (LCA). Each was designed to understand and rectify different environmental problems but are applicable to all industries.

EIA was introduced in the 1960s (Isah, 2012) and became widely used due to the 1969 United States National Environmental Policy Act (NEPA). It is a required exercise for developers to show local planning authority how the environment is affected (positive and negative) by the proposed development; for new processes and facilities (Friends of the Earth, 2005). Several stages are involved in EIA; scoping, analysis, mitigation, determining significance and follow up (Manuilova et al., 2009). The aim is to quantify the environmental effects that exist and are likely to occur in future activities; results are designed to be checked against tolerance levels, and thresholds set out in standards, guidelines, and governmental policies. Hence, results are typically quantified resource utilisation and emissions. Typically, environmental indices or factors (scores) are applied to individual material indexes (mass inventory of input and outputs) to generate environmental indexes (EIs), which are then summed to form a cumulative impact score. The EU also adopted this practice in 1985, which is now Directive 2011/92/EU, "on the assessment of the effects of certain public and private projects on the environment".

A drawback of this methodology is the uncertainty that comes from estimating future impacts of a given project; and formulating a single, cumulative impact on the local, regional and global environment (Manuilova et al., 2009; Ramasamy, 2015). Heinz et al. (2006) has shown the use of EIA applied to the production of insulin where EIs were assigned to inputs and outputs of the process. Although this assisted in generating a quantitative figure on the overall impact level of the production process, this may not be entirely accurate. Since individual environmental issues do not necessarily relate to one another, the overall score does not convey how the environment is affected. Weightings are typically applied on each process parameter, i.e. water consumption, energy consumption, greenhouse gas (GHG) emission and waste outputs, in terms of their level of importance and urgency before an overall score is summed. However, weightings are subjective and must vary to reflect the needs of individual local, regional and national environmental policies to be beneficial. Ideally, the impacts should be separated into different categories to give a more meaningful interpretation of the data presented.

MFA, previously known as a study of social and/or industrial metabolism, predates most environmental assessment with documentation of its concepts since the 19th century (Fischer-Kowalski and Hüttler, 1998). Metabolism used in the context of biology is where raw materials

are converted via a series of biochemical reactions down various metabolic pathways; in the social and industrial context, it regards the flow of material and substances due to human/labour processes. It was not until the late 1950s that discussions on potential shortages of future energy supply provoked the necessity to actively develop the approach to understand how we utilise our resources (Graedel, 2019). By the late 1960s, there were two approaches pioneered: (1) focusing on socio-economic systems, which assists in understanding how materials are used and converted, and (2) focusing on the ecosystem system, which also considers the feedback of materials into the environment.

Depending on the study goals, the two approaches may be combined to assess a system's sustainability. Ultimately, MFA is a tool that can support decision making regarding resource and environmental management. In MFA, the key materials or "goods", indicator substances, the system boundaries and the processes within are identified before quantifying the mass flows of matters. It then requires the use of the mass conservation principle to account for mass changes in the system. "Goods" are the materials of interest, whilst indicator substances are usually pollutants that allow the tool to identify sources and sinks of pollution and to quantify the impact of potential resource recovery (Brunner and Rechberger; 2004). Because MFA strives for transparency and manageability, the simplification can cause potential environmental issues to be overlooked. Understanding the quantities of substances flowing into the environment may not be sufficient to recognise the level of impact it can have in the local surrounding, which means further analysis may be required.

To specifically tackle potential shortages and pollution deriving from the use of fossil fuels, particularly after the 1973 oil crisis, **ExA** was developed (Ediger and Çamdalı, 2007). It was primarily used to assist with improving the efficacy of energy generation within industries by finding ways to reduce the utilisation of fossil resources. ExA is a tool used to look at the quantity and quality of energy sources, which can help quantify the energy efficiency of a given system. Energy analysis alone looks solely at quantity, whilst exergy analysis is "a method that uses the conservation of mass and conservation of energy principles together with the second law of thermodynamics for the analysis design and improvement of energy and other systems" (Ranjan et al., 2016). Combined, ExA evaluates the amount of energy available before the system reaches equilibrium, which indicates the efficiency and the quality of the energy system (Park et al., 2014; Woudstra, 2016). This is important to aid decision making on the choice of equipment and operations that would be embedded into a process, as it ensures effective use of energy resources (Ramasamy, 2015). It is also said to be a key instrument for energy and environment policy generation (Dincer, 2002). Because the focus of the analysis is energy, the method does not deal with other non-fossil related environmental issues. Furthermore, the inefficient use of

fossil fuel will indicate unnecessary GHG emissions, but the quantification of this and the subsequent impacts on global warming are not analysed. Although ExA is still relevant for calculating the efficacy of renewable energy sources, to be in line with sustainable development, it would be important to analyse the system further to understand and prevent other environmental impacts.

The practice of **LCA** is said to have begun in 1969 (Hunt and Franklin, 1996) in the consumer sector. It was the year that Coca-Cola Company ran an internal study to evaluate drinks packaging with the help of the Midwest Research Institute (MRI). Although it is not the full LCA that is employed today, the study quantified and compared the inventories (raw materials, fuels and energy used, as well as the outputs to the environment) for the manufacturing of glass bottles, plastic bottles and aluminium cans. Activities included raw material extractions, container fabrication, their use (and reuse if applicable) and disposal. With this as the first life cycle inventory analysis, the foundation for LCA was laid and led the MRI to be commissioned by other companies and the US government to develop and carry out such analysis. Through these projects, the inventory analysis method, which included data collection and mass and energy balancing activities, was refined; and impact assessment methodologies were made possible. They formed the underlying life cycle impact assessment (LCIA) methods used in modern LCAs (see Section 2.5.2.1 for further details).

However, it wasn't until the early 1990s that LCA was fully developed as a recognised methodology by the Society of Environmental Toxicology and Chemistry (SETAC) (Klöpffer, 1997; Zbicinski, 2006). The "Code of Practice" workshop series ran by SETAC defined LCA as a process that evaluates and "quantifies the energy and material used and wastes released to the environment" (inventory analysis), the impact associated with this (impact assessment) and identifies the areas where environmental improvements are possible (improvement assessment) (Consoli, 1993; Zbicinski, 2006). With time, the LCA methodology was modified, and in 1997, it was developed into a standardised framework by the International Organisation for Standardisation (ISO). The ISO framework agrees with the "Code of Practice" on the need for goal definition, inventory analysis and impact assessment but does not regard improvement assessment as an actual LCA analysis; it was deemed an activity that can be performed only after LCA and therefore shown as an optional analysis (Zbicinski, 2006). Hence, the improvement assessment phase was changed to "interpretation", which is when insights are extracted from impact assessment results through other forms of analyses. Interpretation aims to generate information to meet the study goals by interpreting results such that the intended audience can relate and understand.

Figure 2.4 outlines the general framework of LCA and shows examples of its applications. The methodology has four distinct phases. Although assessments start at goal and scope definition, the phases can be carried out iteratively to ensure that all phases align with the primary phase. The goal and scope definition includes the goal, what will be studied, the questions that need answering, and any assumptions needed to enable the study (Finkbeiner et al., 2006; Klöpffer, 1997). The other phases are life cycle inventory analysis (LCI), where data is collated; life cycle impact assessment (LCIA), where inventory data is converted to environmental impacts; and interpretation, where LCI and LCIA results are interpreted to meet the goals (Finkbeiner et al., 2006; International Organization for Standardisation, 2006a).

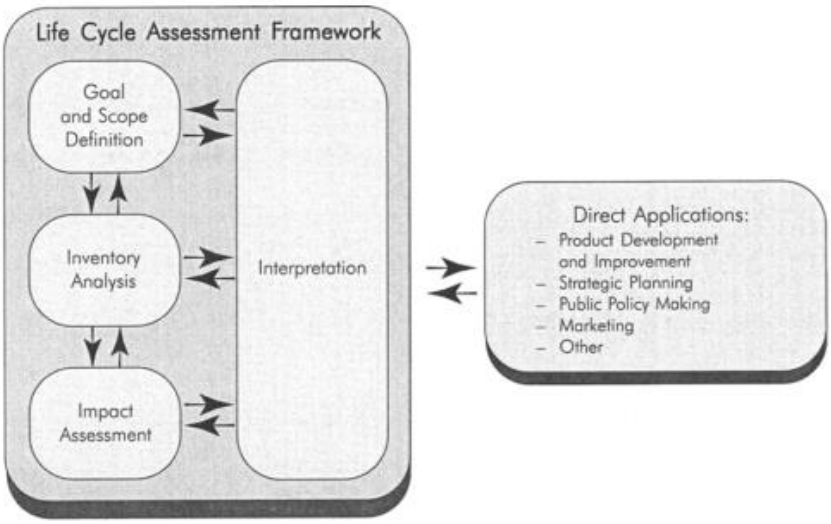

Figure 2.4: ISO life cycle assessment framework (Klöpffer, 1997)

LCA allows the generation of an **inventory (LCI)** of inputs and outputs throughout a system's life cycle, including greenhouse gas (GHG) emissions and energy demand amongst all other substances that are used to quantify most forms of environmental impacts by means of **life cycle impact assessment (LCIA)**. For instance, **climate change** caused by GHG emissions, **acidification** caused by sulphur, NOx and ammonia emissions, etc. (Figure 2.4). The environmental impacts, which the LCA tool has been developed to quantify, include and not limited to: acidification, climate change, ecotoxicity, eutrophication, human toxicity, ionising radiation, land use, photochemical ozone formation, ozone depletion and water consumption. In summary, they are calculated by aggregating and characterising the materials (or elementary) flows into environmental impact categories, which can be further classified into damage categories (as Figure 2.5), before the optional exercises such as normalisation and weighting are conducted (see Chapter 3).

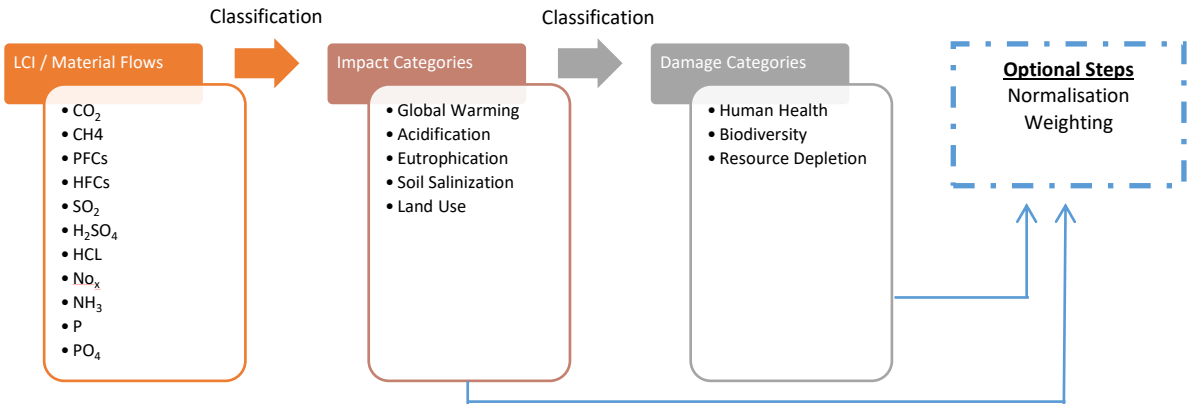

Figure 2.5: Summary of life cycle impact assessment procedure with example materials flows and impact categories.

Although the discussed environmental assessments emerged concurrently, LCA holds elements of all other assessment methods (above). For inventory building and analysis, the concepts from MFA and ExA are important for materials and energy balancing. There are similarities between EIA and LCA whereby an inventory of inputs and outputs are necessary; the difference lies with system boundaries. Traditional EIAs deal with single industrial plants and concentrates on emissions affecting the direct surrounding environment, and LCA assumes a system boundary and calculates a cumulative impact over a product or system's whole life cycle. Impact assessment for LCA differs from EIA such that results are not related to emission thresholds but instead details a process' contribution to various environmental impact categories. Furthermore, LCA is a tool sometimes used as part of EIA to understand the indirect influences of a production system and give further insight. When a comprehensive analysis of environmental impacts is required, i.e. a necessity to acquire information on all elements, life cycle assessment (LCA) would be most beneficial. LCA is a tool that enables a well-rounded representation of a given system, which is especially useful if the system in question (in this case: the biopharmaceutical industry) is not well understood in terms of its environmental sustainability.

To demonstrate the knowledge on the environmental sustainability of the biopharmaceutical within the literature, the following section provides a background on the principle it follows, green chemistry. Then, a review on the environmental indicators and assessments employed by biopharmaceuticals and, as the base for comparison, the wider pharmaceutical industry is presented.

2.4.2 Green Chemistry and the Sustainability Indicators Employed by the Pharmaceutical (and Biopharmaceutical) Industry.

Following the implementation of policies that aimed to rectify the over polluting effects of manufacturing industries, countries began to broaden their focus on pollution prevention in the

late 1970s and 1980s (Murphy, 2020). This environmental approach requires reducing materials and using more environmentally friendly materials to prevent waste and minimise unnecessary waste treatment processes (Anastas and Williamson, 1996). As the US Pollution Prevention Act 1990 and the UK Environmental Protection Act 1990 encouraged companies to prevent and reduce pollution at its source, programs also emerged to reward those that can prevent pollution in innovative ways (Anastas and Kirchhoff, 2002; Linthorst, 2009; Wardencki et al., 2005). Since the chemical industry extracts, manufactures and uses a wide range of substances that are known to be polluting, notions to make chemical processes more sustainable came to light, which has derived the concepts of green chemistry.

Green chemistry is an approach applied to chemical processes to prevent pollution and promote environmental sustainability. Although the term "green chemistry" first appeared in 1990, its full concept was established over the following years through symposiums and workshops such as "Benign by Design: Alternative Synthetic Design for Pollution Prevention" in 1993 (Linthorst, 2009). According to Linthorst (2009), it was in Anastas and Williamson (1996) that ultimately defined the underlying principles of green chemistry that is still in place today. As knowledge in pollution prevention grew, the principles of green chemistry have expanded to be more precise (Table 2.5). Although green chemistry was designed for the chemical industry, the principles are adopted by both the traditional pharmaceutical and biopharmaceutical industries (de Marco et al., 2019; Malerba and Orsenigo, 2015).

Table 2.5: Aims and principles of green chemistry described in Anastas and Williamson (1996) and Wardencki et al. (2005).

First account by Anastas and Williamson (1996)	**12 Principles by Wardencki et al. *(2005)***
Alternative feedstock and starting materials - Use of benign feedstock. - Reduce the amount of feedstock. - Reduce the intrinsic toxicity of feedstock through structural modification or replacement.	Prevention - Minimise waste and waste treatment processes – by reducing the amounts of materials necessary for production. - (All principles below assist with the prevention of pollution).
Alternative Synthetic transformation and alternative Reagents - Elimination or reduce toxic substances through substitution to more benign chemicals. E.g. replace metal catalyst with visible light.	Atom economy - Maximise the incorporation of materials in the final product.
Alternative reaction conditions - Reduce energy consumption. - Reduce solvent use (reaction condition often link to substances being utilised). - Use alternatives to solvents such as supercritical fluids (SCFs).	Less hazardous chemical syntheses - Processes should use and generate substances with no or little toxicity to human health and the environment where possible.
Alternative products and target molecules - Design safer chemicals; reduce the toxicity of a molecule without lowering its functional efficacy.	Designing safer chemicals - Design effective chemicals whilst minimising their toxicity.
	Safer solvent and auxiliaries - Avoid auxiliary substances (e.g. solvents, separation agents) when possible. - Use alternatives to solvents or use safer solvents.
	Design for energy efficiency - Minimise energy requirements. - Desired process conditions include ambient temperature and pressure.
	Use of renewable feedstocks

	- Raw materials and feedstocks chosen for a process should not be depleting where possible.
	Reduce derivatives - Avoid unnecessary derivatisation (e.g. temporary modification of physical/chemical processes) to avoid additional use of reagents and generation of waste.
	Catalysis - Selective catalytic reagents are preferred over stoichiometric reagents.
	Design for degradation - Chemical products should not persist in the environment and break down into inert substances at end-of-life.
	Real-time analysis for pollution prevention - Develop analytical methods to monitor processes (real-time or in-process) to prevent, and pre-empt, hazardous substance formation.
	Inherently safer chemistry for accident prevention - Choose substances with intentions to minimise potential chemical accidents, such as harmful releases, explosions and fires.

As shown in Table 2.5, a key concept theme to prevention is reduction, and it is applied to input materials, the energy required, and waste outputs of chemical processes. Various metrics have been developed to understand and track a process' performance in upholding these principles. The first to be introduced was **E-factor** by Sheldon (1992), which highlighted the amount of waste generated from the production of 1 kg of a product; this mass ratio of waste to product is said to be one of the simplest yet effective methods in conveying the resource and waste prevention efficacy of a process. Through the 25 years of implementing E factors, Sheldon (2017) has summarised the typical values for individual industry segments (Table 2.6) and confirmed that as the number of processing steps increases (required to achieve more refined substances), the E-factor becomes higher. This supports the green chemistry principle to reduce derivatives.

Table 2.6: E factors of chemical productions, from Sheldon (2017)*.*

Industry segment	**Tonnes per annum**	**E factor ($kg_{waste}/kg_{product}$)**
Oil refining	10^6-10^8	<0.1
Bulk chemicals	10^4-10^6	<1-5
Fine chemicals	10^2-10^4	5-50
Pharmaceuticals (small molecules)	10-10^3	25->100

Other mass-based metrics, **process mass intensity (PMI)** and **atom economy,** were also developed to convey process efficiency. PMI conveys the mass ratio of all input materials (excluding water) to produce 1 kg of a product; the atom economy expresses the molecular weight of the desired product as a percentage of the total molecular weight of its reactants (Jiménez-González et al., 2012). More specific indicators are used to address concerning aspects of a process. Prominent examples include **C footprint**, the percentage carbon deriving from fossil fuel source; **CO_2 production**, the mass ratio of carbon dioxide (produced) to a product; **water intensity**, the mass ratio of all water used to a product; and **solvent intensity**, the mass ratio of

all solvent (excluding water) to product (Watson, 2011). Non-mass-based process sustainability indicators that companies may employ are **steps per product** and the **number of catalytic steps,** particularly for traditionally synthesised pharmaceuticals (Watson, 2011).

A survey revealed that the most popular indicator used by pharmaceuticals is PMI (Watson, 2011). Since pharmaceutical companies typically require high capital investments, attaining cost savings is a priority. Hence, the preference for using PMI can be attributed to the fact that referencing process materials allows a better representation of the economic performance of a process than waste produced (E factors) (Jiménez-González et al., 2012). Even so, reviews on sustainability metrics employed by the industry concur that most companies do not use an extensive set of indicators, i.e. typically limited to those stated above (Jiménez-González et al., 2012; Sheldon, 2017; Veleva et al., 2003; Watson, 2011). Reports stated further that to assess the sustainability of a product or a process; it is necessary to go beyond process metrics (Jiménez-González et al., 2012; Sheldon, 2017). They stated that companies should interpret how the product or process affects the environment on a wider scale, including understanding the eventual safety and environmental effects of their outputs. This coincided with the principle of developing real-time analysis for pollution prevention. An example approach that is employed at the product development stage is the "pentagon of ecologically correct thinking", which assists the choice of production methods, reagents, personnel, time and product quality (de Marco et al., 2019). Analyses can also be employed to help assess the toxicity outputs of a process (product, co-product and waste) and predetermine the waste management procedures required for scaled-up manufacturing. This can ensure process outputs are compliant with green chemistry principles and governmental waste policies. Above all, analytical results can inform the environmental consequences of materials supplies and other product life-cycle stages and quantify the environmental impacts of product manufacture.

Veleva et al. (2003) suggested that there are five levels of sustainability reporting: Level 1: Company Compliance/Conformance Indicators; Level 2: Company Material Use and Performance Indicators; Level 3: Company Effect Indicators; Level 4 Supply Chain and Product Life-cycle Indicators; and Level 5: Sustainable Systems Indicators. While companies aim to comply with governmental regulation (Level 1), Veleva et al. (2003) showed that companies may not necessarily report indicators for this form of performance and only simply state the regulation or voluntary initiatives they are compliant with. They stated further that Level 2, followed by Level 3 are the predominant type of indicators. Level 2 indicators are eco-efficiency measures for a process (e.g. PMI). They are easily measured and has a direct link to financial savings. On the other hand, Level 3 indicators may require additional forms of calculation to

obtain total process emissions and environmental impacts, typically **global warming potential** (GWP) (also known as climate change) or **total CFC-equivalent emissions**.

Veleva et al. (2003) further stated that the lesser reported indicators are Levels 4 and 5. For their calculation, companies are required to take account of the environmental, health and safety profiles of input materials (including production and transport) and understand how production processes affect the wider society. Indicators for Level 4 arise from applying life-cycle thinking to a product or process in order to understand its sustainability performance in a wider context, which can be compared to national and global thresholds and generate Level 5 indicators (Veleva et al., 2003). Hence, if Level 4 reporting is not sufficient, Level 5 indicators cannot be established.

Examples of Level 5 indicators are "per cent of water from local sources used within average local recharge rate" and "per cent of the total energy used from renewable sources harvested sustainably". Veleva et al. (2003) found no companies reported Level 5 indicators at the time, but there has been a development in relating process performance to global thresholds and limits in recent years. For instance, the development of earth carrying capacities and their incorporation as normalisation factors in LCA. Normalising LCA results with carrying capacities interprets environmental impact results (Level 4 indicators) to how damaging the system is (Level 5) (Sala et al., 2015).

Since the use of fossil fuel has become a concern, **carbon footprint** (total greenhouse gas (GHG) emission) and **cumulative energy demand** (CED - from fossil fuel) are popular Level 4 metrics for pharmaceutical companies to report (Arango-Miranda et al., 2018; Veleva et al., 2003; Watson, 2011). Other forms of environmental impacts were found not to be routinely reported, meaning that this level of reporting is not yet mature (Schneider et al., 2010; Sheldon, 2017; Veleva et al., 2003; Watson, 2011). There are traditional **EIA,** and **LCA** carried out on pharmaceuticals; however, as stated previously, EIAs are insufficient to inform specific effects on the environment, and it appears that LCAs adoption by pharmaceutical, particularly biopharmaceutical, companies are low.

2.4.2.1 Life Cycle Assessments on the Biopharmaceutical and the Wider Pharmaceutical Industries

LCA is noted as a desirable tool for companies to implement as it can generate comprehensively both an "input and output" inventory and the environmental impacts associated with a product or a process (Levels 3 and 4 indicators as Veleva et al. (2003))(Sheldon, 2017; Watson, 2011). It can also provide detailed insights on the risk of a system by allowing analysis to be broken down into different stages of its life cycle and then brought together to form a complete interpretation

of all impacts. Hence, results can be generated and managed by different operative leads to find solutions for reducing impacts. This is an example of how LCA is a flexible methodology. However, although there are LCA studies on pharmaceutical products, most are of synthetic active pharmaceutical ingredients (API), i.e. not biopharmaceuticals (Table 2.7). This may be due to limited data availability (Jiménez-González and Overcash, 2014).

Table 2.7 highlights LCA papers relevant to pharmaceutical products and/or processes and the sustainability metrics used. Of the 48 pieces of literature, three are reviews of LCA studies available in the literature. They concurred that there was a lack of understanding of biopharmaceutical processes (Emara et al., 2018a; Jiménez-González and Overcash, 2014; Sheldon, 2017). Emara et al. (2018a) further emphasised the need to model pharmaceutical toxicity in the environment at their end-of-life (EoL). Only eight papers evaluated the fate of pharmaceuticals at their EoL, but none of which regarded biopharmaceuticals. Nine papers discussed the implementation of LCA (in general) by pharmaceutical and/or biotechnology companies by posing considerations and challenges to overcome barriers, including proposing an LCA framework for others to follow. One of the nine consideration/frameworks discussed pharmaceuticals in general, while others are particular to biopharmaceutical production. Furthermore, only Brunet et al. (2011), Harding (2008) and Ramasamy (2015) complemented their proposed LCA framework with case studies.

Out of the total 38 LCA case studies in Table 2.7, only ten papers were carried out on biopharmaceutical production processes. Four studies focused on comparing the production of monoclonal antibodies (mAbs) via conventional stainless-steel equipment and via single-use technology; one study compared mAbs production in fed-batch and perfusion fermentation configurations. The three other studies were on antibiotics production. Nevertheless, seven additional studies were found relevant to biopharmaceuticals: enzyme production and their use in industries (2), the use of solvents (2), pharmaceutical packaging (2) and lysine production via biotechnology (fermentation-derived) (1). Although the range of biopharmaceutical products assessed using LCA is limited, some lessons were learnt from understanding traditional pharmaceutical products and other sectors and have been incorporated into biopharmaceutical process design. For instance, past LCAs have made clear that both the use of enzyme catalysts and moderating the use of solvents are environmentally preferable to alternatives. These process approaches have become innate in designing new pharmaceuticals (including biopharmaceuticals) production schematics. This could be a reason why the biopharmaceutical industry is not seen as a risk to the environment. As these process choices were evaluated previously, there was no need to evaluate new processes that already consist of these particular

implementations. Hence, there are no specific benchmarks for their employment in biopharmaceutical processes.

Noted from review papers and the limitations highlight in most case studies, the input and output inventories (LCIs) for many materials specific to the manufacture of biopharmaceuticals are often not available in LCA databases. This has often lead to oversimplified LCAs (Jiménez-González and Overcash, 2014). Of the ten case studies conducted on biopharmaceutical processes, four did not quantify the life cycle inventory (LCI) into impact categories and focused only on energy usage and climate change potential. Arguably, the two measures are currently regarded as urgent environmental issues, which may be why they were prioritised over other impacts. The shortened analyses indicate that LCA practice in the biopharmaceutical industry is currently establishing its position for stakeholders to form gradual acceptance and understanding of its results. Regardless, the fair amount of LCA consideration and framework suggestion papers, the range in LCA methodologies applied and the variety in impact categories reported (as depicted in Table 2.7) showed that the practice of LCA is not yet mature.

Biopharmaceutical companies may benefit from following similar life cycle inventory developments as synthetic pharmaceuticals, for instance, those carried out by big pharmaceuticals such as GSK and Pfizer (Curzons et al., 2007; Jimenez-Gonzalez, 2002). However, this would require a mass gathering of process data. It could be that big pharmaceuticals, with the extensive pipeline and data, would need to lead and initiate the use of LCA on biopharmaceuticals and begin the benchmarking of a range of products. Nonetheless, as new or small biotech companies are often those who develop biopharmaceuticals, it would also be necessary to assess and pool their environmental performances to attain a benchmark for the industry. Despite the limited data available for conducting a comprehensive LCA on a biopharmaceutical process, small biopharmaceuticals can still benefit from analysing their processes using average data in the literature, and databases, to diagnose potential environmental concerns for rectification at the developmental stages. In this light, proper guidance for companies to apply the LCA methodology to their process with considerations for companies' aims in conducting the analysis may be needed to encourage its uptake.

Under the green chemistry principle of designing safer chemicals, pharmaceutical companies, in general, should consider the fate of their products. However, the dose and potency of drugs are important to tackle the disease they are designed for; hence, activities to lower their toxic effects are not typically carried out at their design but at waste treatment levels. While there are characterisation factors (CFs), which converts quantities of substances in the environment to their potential effects (see Chapter 3 – Section 3.2.3), for some pharmaceuticals developed (Alfonsín et al., 2014; Fantke et al., 2017; Muñoz et al., 2008; Ortiz de García et al., 2017), they

are not well integrated with assessing the impact of product leakages from production facilities. Instead, CFs for pharmaceuticals are often used to compare urban waste treatment technologies (Morais et al., 2013; Remy et al., 2015). The reason for this could be that waste generated from pharmaceutical production is typically treated until they meet a certain standard before their release. This led to the assumption that the effects of any leakages are negligible; only when unused drugs are disposed of and an accumulation of different substances occur in societal waste streams that they may become an issue. Nonetheless, CFs of pharmaceuticals could be implemented better to measure the sustainability of pharmaceuticals over their life cycle. This would assist companies in providing better advice on the most environmentally preferred disposal method for unused drugs.

As there is insufficient data on the effect of pharmaceuticals in the environment (Emara et al., 2018a), there is potential for pharmaceutical companies to assist with the generation of CFs by providing data on their degradation and potency. However, toxicity effects must consider the fate and risk of organisms ingesting the substance, which can only be quantified fully through field research. This may also be the reason why there are no toxicity modelling for more recently developed pharmaceuticals/biopharmaceuticals. Ultimately, monitoring biopharmaceuticals in waste streams will be required to quantify their effect in the environment before CFs can be developed.

To begin addressing the gaps in the literature concerning LCA and biopharmaceutical processes, companies must first consider that the use of LCA is valuable for meeting their sustainability agendas, whether economically, environmentally or socially driven. It is then that LCA studies on their processes will become more prevalent in the literature, and hence, enable better critical reviews on the overall environmentally sustainability of the industry. With companies conducting LCAs, current issues on data availability and CFs can begin to resolve as well. In the next section, this chapter presents how LCA is a valuable tool for the companies to utilise by summarising their needs, which LCA can assist with fulfilling, and presenting exemplary examples of their use.

Table 2.7: Literature on the application of life cycle assessment (LCA) on pharmaceutical and selected biotechnology products. "Analysis/Outputs" show the types of analysis and the metrics the paper presents. "Study Focus" shows the portion of pharmaceutical products that the study or review paper is interested in. "Associated Pharmaceutical Type" shows the type of pharmaceutical that the product or process under study attribute to. "API" = active pharmaceutical ingredient. "CED" = cumulative energy demand, a sustainability indicator. "Cost" indicates cost metrics. "E factor" = waste to product ratio, a sustainability indicator. "Eco 95" = EcoIndicator 95, an LCIA methodology that quantifies the inputs and outputs of materials into environment scores, as traditional environmental impact assessments. "Exergy" indicates exergy analysis results, i.e. primary energy usage and or exergy resource consumption. "GWP" = global warming potential, an environmental impact category. "LCI" = life cycle inventory; indicates inventory analysis results. "LCIA" = life cycle impact assessment, indicates more than three environmental impact categories presented. "PMI" = process mass intensity, a sustainability indicator. "Social" indicates social metrics. "Water usage", a sustainability indicator.

#	Literature Focus and Title	Reference	LCA Approach	Analysis / Outputs	Study Focus - Category	Associated Pharmaceutical Type (Production Method)
	LCA Considerations and Methodologies					
1	A generic approach to environmental assessment of microbial bioprocesses through life cycle assessment (LCA)	(Harding, 2008)	**Framework and Case Study: Cradle-to-gate**	LCI & LCIA	Biotechnology products	Biopharmaceuticals (Biotechnology-Derived)
2	Ranking potential impacts of priority and emerging pollutants in urban wastewater through life cycle impact assessment	(Muñoz et al., 2008)	**Fate Modelling Methodology and Case Study**	Characterisation factors & LCIA	Final drug products	Synthetic Pharmaceuticals (Chemically-Derived)
3	LCA tool adaptation to pharmaceutical processes	(Martins et al., 2010)	**Framework: Cradle-to-gate**	LCI & LCIA	APIs and final drug products	Biopharmaceutical (Biotechnology-Derived)
4	Cleaner design of single-product biotechnological facilities through the integration of process simulation, multi-objective optimization, life cycle assessment, and principal component analysis	(Brunet et al., 2011)	**Framework and Case Study: Cradle-to-gate**	LCI & LCIA	Biotechnology products	Biopharmaceuticals (Biotechnology-Derived)
5	LCA tool for sustainability evaluations in the pharmaceutical industry	(Mata et al., 2012)	**Framework: Cradle-to-grave**	LCI & LCIA	APIs and final drug products	Biopharmaceuticals (Biotechnology-Derived)
6	Comment on "Life cycle comparison of environmental emissions from three disposal options for unused pharmaceuticals."	(Daughton, 2012)	**N/A**	N/A	Drug disposal	Synthetic Pharmaceutical (Chemically-Derived)
7	Challenges of developing decision-support tools based on life cycle assessment (LCA) for the biopharmaceutical industry	(Ramasamy et al., 2013)	**N/A**	N/A	APIs	Biopharmaceuticals (Biotechnology-Derived)
8	Life cycle assessment as a tool to support decision making in the biopharmaceutical industry: Considerations and challenges	(Ramasamy et al., 2014)	**N/A**	N/A	APIs	Biopharmaceuticals (Biotechnology-Derived)
9	PPCPs in wastewater – Update and calculation of characterization factors for their inclusion in LCA studies	(Alfonsín et al., 2014)	**Fate**	Characterisation factors	Final drug products	Synthetic Pharmaceuticals (Chemically-Derived)
10	A framework to support environmentally-based decision-making in the biopharmaceutical industry	(Ramasamy, 2015)	**Framework and Case Study: Cradle-to-gate**	LCI & LCIA	APIs	Biopharmaceuticals (Biotechnology-Derived)
11	A framework for evaluation of environmental sustainability in pharmaceutical industry	(Raju et al., 2016b)	**Framework and Case Study: Cradle-to-gate**	LCI & LCIA	Final drug products	Synthetic Pharmaceuticals (Chemically-Derived)
12	Life Cycle Management in the Pharmaceutical Industry Using an Applicable and Robust LCA-Based Environmental Sustainability Assessment Approach	(Emara et al., 2018b)	**Framework: Cradle-to-grave**	LCI & LCIA	All product stages	All Pharmaceuticals
	Environmental Impact Reviews					

13	The evolution of life cycle assessment in pharmaceutical and chemical applications – a perspective	(Jiménez-González and Overcash, 2014)	N/A	LCI & LCIA	All product stages	Synthetic Pharmaceuticals (Chemically-Derived)
14	The: E factor 25 years on: The rise of green chemistry and sustainability	(Sheldon, 2017)	N/A	E factor, PMI and LCI	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
15	Modelling pharmaceutical emissions and their toxicity-related effects in life cycle assessment (LCA): A review	(Emara et al., 2018a)	Fate	LCI and LCIA	Final drug products	All Pharmaceuticals
	Case Studies					
16	Developing environmentally-sound processes in the chemical industry: a case study on pharmaceutical intermediates	(Jödicke et al., 1999)	Cradle-to-gate	LCI & Eco 95	Pharmaceutical intermediates	Synthetic Pharmaceuticals (Chemically-Derived)
17	Life cycle assessment in pharmaceutical applications	(Jimenez-Gonzalez, 2002)	Cradle-to-gate	LCI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
18	Limited LCAs of pharmaceutical products: merits and limitation of environmental management tool	(de Jonge, 2003)	Cradle-to-gate (product); Cradle-to-grave (packaging)	Exergy	Final drug products and packaging	Synthetic Pharmaceuticals (Chemically-Derived)
19	Cradle-to-gate life cycle inventory and assessment of pharmaceutical compounds	(Jiménez-González et al., 2004)	Cradle-to-gate	LCI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
20	Assessment of bio-based pharmaceuticals: the Cephalexin case	(Bruggink and Nossin, 2006)	Cradle-to-gate	LCI, toxicity potential & risk potential	APIs	Semi-Synthetic Pharmaceuticals (Biotechnology and Chemically-Derived)
21	Fast life cycle assessment of synthetic chemistry (FLASC™) tool	(Curzons et al., 2007)	Cradle-to-gate	LCI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
22	EHS & LCA assessment for 7-ACA synthesis	(Henderson et al., 2008)	Cradle-to-gate	LCI & LCIA	Product intermediates and production technologies	Semi-Synthetic Pharmaceuticals (Biotechnology and Chemically-Derived)
23	Enzymes for pharmaceutical applications - a cradle-to-gate life cycle assessment	(Kim et al., 2009)	Cradle-to-gate	LCI & LCIA	Enzymes (Biotechnology products)	All Pharmaceuticals
24	Decision support guideline based on LCA and cost/efficiency assessment	(Fred et al., 2010)	Gate-to-Grave	LCIA & Cost	Final drug products and drug disposal methodologies	Synthetic Pharmaceuticals (Chemically-Derived)
25	Cradle-to-gate inventory of vancomycin hydrochloride	(Ponder and Overcash, 2010)	Cradle-to-gate	LCI	APIs	Biopharmaceuticals (Biotechnology-Derived)
26	Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors	(Stephenson et al., 2010)	Cradle-to-gate	LCI & GWP	Biodiesel	N/A
27	Sustainable route design for pharmaceuticals - why, how and when	(Poechlauer et al., 2010)	Cradle-to-gate	E-factor & GWP	APIs	All Pharmaceuticals

28	LCA approach to the analysis of solvent waste issues in the pharmaceutical industry	(Raymond et al., 2010)	Cradle-to-grave	LCI & LCIA	Solvents (Process materials)	Synthetic Pharmaceutical (Chemically-Derived)
29	Life cycle assessment of fine chemical production: a case study of pharmaceutical synthesis	(Wernet et al., 2010)	Cradle-to-gate	LCI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
30	An environmental life-cycle assessment comparing single-use and conventional process technology	(Flanagan et al., 2011)	Cradle-to-gate	LCI & GWP	APIs and production technologies	Biopharmaceuticals (Biotechnology-Derived)
31	A life cycle assessment of injectable drug primary packaging: comparing the traditional process in glass vials with the closed vial technology (polymer vials)	(Belboom et al., 2011)	Cradle-to-grave	LCI & LCIA	Packaging	All Pharmaceuticals
32	A systematic evaluation of the resource consumption of active pharmaceutical ingredient production at three different levels	(Van Der Vorst et al., 2011)	Cradle-to-gate	LCI & Exergy	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
33	Reducing the environmental impact of single-use systems	(Jobin and Krishnan, 2012)	Cradle-to-grave	Water Usage, GWP & CED	Production technologies	Biopharmaceuticals (Biotechnology-Derived)
34	Life cycle comparison of environmental emissions from three disposal options for unused pharmaceuticals	(Cook et al., 2012)	Gate-to-grave	LCI & LCIA	Drug disposal methodologies	Synthetic Pharmaceuticals (Chemically-Derived)
35	Is it better to remove pharmaceuticals in decentralized or conventional wastewater treatment plants? A life cycle assessment comparison	(Igos et al., 2012)	Gate-to-grave	LCIA	Final drug products and drug disposal methodologies	Synthetic Pharmaceuticals (Chemically-Derived)
36	An environmental life-cycle assessment comparing single-use and conventional process technology: comprehensive environmental impacts	(Pietrzykowski et al., 2013)	Cradle-to-gate	LCI & LCIA	APIs and production technologies	Biopharmaceutical (Biotechnology-Derived)
37	Environmental assessment of enzyme use in industrial production - a literature review	(Jegannathan and Nielsen, 2013)	Cradle-to-gate	LCI & LCIA	Enzymes (Biotechnology products)	All Pharmaceuticals
38	Exergetic sustainability assessment of batch versus continuous wet granulation based pharmaceutical tablet manufacturing: a cohesive analysis at three different levels	(de Soete et al., 2013)	Cradle-to-gate (pharmacy)	Exergy	APIs and final drug products	Synthetic Pharmaceuticals (Chemically-Derived)
39	Reduced resource consumption through three generations of Galantamine HBr synthesis	(Van Der Vorst et al., 2013)	Cradle-to-gate	LCI & Exergy	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
40	Increasing the sustainability of membrane processes through cascade approach and solvent recovery—pharmaceutical purification case study	(Kim et al., 2014)	Gate-to-gate	CED and Cost	Solvents (Process materials)	All Pharmaceuticals
41	Combined simulation-optimization methodology to reduce the environmental impact of pharmaceutical processes: application to the production of penicillin V	(Brunet et al., 2014)	Cradle-to-gate	LCI, LCIA & Cost	APIs	Biopharmaceuticals (Biotechnology-Derived)
42	Environmental resource footprinting of drug manufacturing: Effects of scale-up and tablet dosage	(de Soete et al., 2014a)	Cradle-to-gate	LCI & Exergy	Final drug products and packaging	Synthetic Pharmaceuticals (Chemically-Derived)
43	Life cycle analysis within pharmaceutical process optimization and intensification: case study of active pharmaceutical ingredient production	(Ott et al., 2014)	Cradle-to-gate	LCI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
44	Environmental sustainability assessments of pharmaceuticals: An emerging need for simplification in life cycle assessments	(de Soete et al., 2014b)	Cradle-to-gate	LCI, Exergy	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
45	Life cycle inventory improvement in the pharmaceutical sector: assessment of the sustainability combining PMI and LCA tools	(Cespi et al., 2015)	Cradle-to-gate	PMI & LCI	APIs	Synthetic Pharmaceuticals (Chemically-Derived)

46	Guidelines for sustainability assessment of water technologies	(Remy et al., 2015)	Gate-to-Grave	LCIA	Final drug products and water treatment technology	Synthetic Pharmaceuticals (Chemically-Derived)
47	Life-cycle and cost of goods assessment of fed-batch and perfusion based manufacturing processes	(Bunnak et al., 2016)	Cradle-to-gate	LCI, LCIA &Cost	APIs and production technologies	Biopharmaceuticals (Biotechnology-Derived)
48	Life cycle assessment and costing of urine source separation: Focus on nonsteroidal anti-inflammatory drug removal	(Landry and Boyer, 2016)	Gate-to-grave	LCIA & Cost	Final drug products and drug disposal methodologies	Synthetic Pharmaceuticals (Chemically-Derived)
49	Comparison of environmental sustainability of pharmaceutical packaging	(Raju et al., 2016a)	Cradle-to-grave	LCI & LCIA	Packaging	All Pharmaceuticals
50	The environmental footprint of morphine: a life cycle assessment from opium poppy farming to the packaged drug	(McAlister et al., 2016)	Cradle-to-gate	LCI & GWP	Final drug products and packaging	Synthetic Pharmaceuticals (Chemically-Derived)
51	Life cycle assessment of multi-step rufinamide synthesis - from isolated reactions in batch to continuous microreactor networks	(Ott et al., 2016)	Cradle-to-gate	LCI, PMI & LCIA	APIs	Synthetic Pharmaceuticals (Chemically-Derived)
52	The potential ecotoxicological impact of pharmaceutical and personal care products on humans and freshwater, based on USEtox™ characterization factors. A Spanish case study of toxicity impact scores	(Ortiz de García et al., 2017)	Gate-to-Grave	Characterisation factors, Human Toxicity and Ecotoxicity	Final drug products	Synthetic Pharmaceuticals (Chemically-Derived)
53	Single-use and sustainability: Continued studies using LCA tools	(Whitford, 2018)	Cradle-to-gate	LCI & LCIA	APIs and production technologies	Biopharmaceuticals (Biotechnology-Derived)
54	Sustainability Assessment of Blue Biotechnology Processes: Addressing Environmental, Social and Economic Dimensions	(Pérez-López et al., 2018)	Cradle-to-gate	LCI, LCIA, Cost & Social	Production technologies	N/A
55	Environmental sustainability of the solar photo-fenton process for wastewater treatment and pharmaceuticals mineralization at semi-industrial scale	(Foteinis et al., 2018)	Gate-to-grave	LCI	Drug disposal methodologies	Synthetic Pharmaceuticals (Chemically-Derived)

2.5 Motivations for the Biopharmaceutical Industry to Adopt LCA

In the previous sections, the use of LCA was reviewed to have many benefits and was recommended as the next steps for companies to act sustainably in the literature (Section 2.4.2). However, the review of the current sustainability of the biopharmaceutical industry (Section 2.3) and the limited assessments present in the literature (Section 2.4.2.1) suggested there is likely a barrier to the adoption of LCA. To overcome any barriers to the use of this environmental assessment, companies must be motivated by the potential values LCA can bring. In this section, the motivations for the biopharmaceutical industry to adopt LCA are presented. First, how LCA can assist companies' sustainability agendas (as highlighted in Section 2.3.1) is summarised. Next, the section highlights how the biopharmaceutical industry and best utilise LCA to meet both company and global objectives, exemplary uses of LCA by other industry and how LCA can support industries in achieving certain aspects of sustainable development goals (SDGs) are presented.

2.5.1 The Needs of Biopharmaceutical Companies and LCA

Due to the drug development timeline, companies can go through phases of limited income. This makes them reliant on their various stakeholders such as investors, customers, employees, and research institutes to ensure the smooth running of their business and obtain a positive cash flow. The reliance on stakeholders can pose an incentive for biopharmaceutical companies to use LCA to compare technologies for process optimisation, which may improve eco-efficiency. An example present in literature is the comparison amongst different fermentation configuration, which has confirmed that switching to single-use technology can reduce cleaning reagents (Pietrzykowski et al., 2013; Ramasamy, 2015) (see Section 2.5.2 for other exemplary uses by other industries). The goals and scope of LCA studies can focus on reducing cumulative inputs and minimising waste treatment processes or set per stakeholder's other agendas. As Li and Yu (2011) suggested, having CERs can give rise to competitive advantages; actively reducing environmental footprints will gain approvals from the general public and increase the company's social standings as added benefits.

As society becomes more aware of global environmental issues, there are increasing expectations for companies to report their environmental performances and take leadership into promoting sustainability. Since governments are committed to upholding the 2030 Sustainable Development Agenda (SDGs), policies are continually evolving to reflect current social and environmental needs. LCA has been historically associated with governmental policy generation. Since current environmental policies are largely generalised and may not be applicable to biopharmaceuticals, it poses an opportunity to use LCA to allow policymakers to

begin guiding the industry to become more environmentally sustainable. However, it would be of interest for companies to begin aligning themselves better and taking an active role in embracing the SDGs before governmental enforcement; the use of LCA would assist this (see Section 2.5.3). While most companies have learned to publicise their greenhouse gas emissions, it is becoming necessary to comprehensively report other environmental impacts of governments and other stakeholders' concerns. As the primary use of LCA is to evaluate the wide range of environmental impacts associated with a system, reporting requirements can be met. Mass environmental sustainability reporting on processes will also allow benchmarking of impacts, which can assist companies in tracking their environmental performances and contribute to fulfilling SDG targets.

LCA as a comprehensive assessment can assist the biopharmaceutical industry in generating environmental performance data on their processes and allow companies to become more transparent in their environmental reporting. However, a barrier to carrying out studies could be the data intensiveness of LCA and the potential for disclosing process sensitive information. As biopharmaceutical companies may not have proficient experience in carrying out LCA, guidance specific for companies outlining the activities and potential reporting guide would be beneficial; see Chapter 3. By developing guidance on conducting LCA with considerations for the agendas of biopharmaceutical companies, this thesis aims to incentivise the adoption of LCA by the industry.

2.5.2 Exemplary Use of Life cycle assessment (LCA)

This section presents two examples of how the use of LCA has transformed particular industries. The examples provided illustrate how LCA recommendations for future development can be assessed to clarify the potential consequences to the environment before its implementation.

2.5.2.1 Consumer Industry – Product Optimisation

The Coca-Cola Company proved the usefulness of the first form of LCA, resource and environmental profile analysis (REPA), in 1969 (Hunt et al., 1992; Jensen et al., 1997). The REPA study aimed to aid decision-making on whether the company should outsource the manufacture of drink containers and inform the choice between refillable glass bottles, disposable plastic bottles, and aluminium cans. Although the study's assessment criteria and impact assessment methodologies may differ from the LCAs employed today, the study considered various impact associated with each stage of the product life cycle. At the time of the study, fossil fuels for energy generation was a cause of concern as it led to major pollutions. Hence, there was much focus on energy reduction and the analysis reflected this. Plastic is much lighter in weight and easier to fabricate compared to glass and aluminium. This meant the employment of plastic

required less electricity and fuel for container manufacture and transport, respectively, which caused fewer emissions from fossil fuel usage. Since solid waste generation was not perceived as an imminent issue, largely due to limited knowledge of the impacts associated with disposing of waste to landfill, the generation of plastic waste was seen as less environmentally burdensome than recovering and washing glass bottle for refills and redistribution. Hence, Coca-Cola decided to switch their drinks packaging from glass to plastic bottles (Hunt and Franklin, 1996). Without the need to recapture glass bottles for reuse, the company began outsourcing container production. As a whole, the move enabled The Coca-Cola Company to reduce operational cost whilst reducing GHG emissions from producing and handling their products.

This study sparked interest for other companies to look into their packaging life cycles. For instance, Mobil Chemical Company found polystyrene foam trays as a better choice over pulp trays due to similar reasoning as Coca-Cola's study. It was said that the "most prominent" use of LCA by the government was for assessing packaging (Jensen et al., 1997). In 1972, the US Environmental Protection Agency was the first government body to commission studies on packaging to begin regulating this field. The move affected the glass, steel, aluminium, paper and chemical (plastic) industries (Hunt and Franklin, 1996; Hunt et al., 1974). Nine beverage container materials were assessed, and seven environmental aspects were focused upon: raw materials, energy, water, industrial solid wastes, atmospheric emissions, waterborne wastes, and post-consumer solid wastes. Hunt et al. (1974) reported that glass was the most environmentally preferred in five categories if they were reused more than 19 times, but least preferred in terms of raw materials usage and post-consumer solid waste if they are only used once. The study further showed that aluminium and steel containers were most preferred in minimising post-consumer solid waste due to their recyclability. This emphasised the importance of reuse and recycling to minimise the environmental burden. However, the production of metals caused high industrial solid waste and atmospheric emissions. This meant that the choice of packaging is dependent on the most concerning environmental aspect of where they are produced (Hunt et al., 1974; Jensen et al., 1997). For instance, in locations where atmospheric pollution is of great concern, aluminium container manufacture will not be preferred. Hence, this study must be reviewed against environmental thresholds in order for decision-makers to put in place suitable policies.

2.5.2.2 Chemical Industry - Eco-efficiency and Process Optimisation

The first use of LCA by the chemical industry coincides with the introduction of green chemistry and the finalisation of LCA by the International Organisation for Standardisation (ISO) (Jenck et al., 2004). Early LCA studies compared process materials that were found to be polluting. Examples included the comparisons between different surfactants by the Ecosol group; and

between oleochemicals and petrochemical surfactants by Henkel (Jenck et al., 2004). The results were that oleochemicals had environmental superiority, and hence, by switching away from petrochemical surfactants, production processes were deemed more environmentally efficient.

Since the concept of sustainable development requires the balance of economic, environmental and social sustainability, it is necessary to assure that environmental initiatives will not jeopardise a company's return on investment; otherwise, the business will not be sustainable. Eco-efficiency is the efficiency of a business or system that creates economic value whilst reducing ecological side effects and resources use (DeSimone and Popoff, 2000). BASF, a multinational chemicals manufacturing company, initiated eco-efficiency analysis in 1996 (Jenck et al., 2004) and have developed a method that incorporated cost analysis and LCA (Saling et al., 2002). They used this to compare dying processes and built new plants that incorporated the more economically and environmentally favourable processes. Existing processes were also modified as a result. By analysing the whole life cycle of the jean dying processes, BASF was able to decide strategically between their "options for action" and ultimately raised their share of the jeans dyeing market "from 2% to 40%" (Jenck et al., 2004; Saling et al., 2002). The company used the combined methodology (cost analysis and LCA) further to compare other products. This led to higher-value products reaching customers at a lower operation cost (DeSimone and Popoff, 2000) - an objective that biopharmaceutical companies may wish to strive for. Examples include Luviset polyurethane copolymers developed to use in hair sprays to reduce volatile organic compound (VOC) emissions; and Neopor building insulations to retain heat and reduce energy consumption (Jenck et al., 2004).

Other chemical companies have used LCA principles to create better products. Procter & Gamble developed a fabric softener to allow a 99% reduction in sewage treatment, which subsequently increased their company's ecotoxicology profile (Jenck et al., 2004). The Dow Chemical Company also developed Synalox, a biodegradable lubricant with improved efficacy as compared to fossil-fuel based ones (DOW, 2014; Jenck et al., 2004). As the pharmaceutical industry, in general, was derived from the chemical industry, many environmental learnings were considered when developing pharmaceutical production processes, particularly for traditionally chemically synthesised drugs. Biopharmaceutical manufacture relies on biochemical reactions; lubricants and solvents, typical of chemical processes, do not necessarily apply in production processes but are still necessary for plant processes such as machinery maintenance and cleaning.

2.5.3 Using LCA to meet Sustainable Development Goals

As LCA, amongst other environmental assessments, was developed to promote the environmental sustainability aspect of the three pillars to true sustainability, it is considered vital

in supporting the sustainable development agenda (Kan, 2019; Weidema et al., 2018). In addition to quantifying environmental impacts, if required, LCA can also be used to assemble insights on economic and social impacts in the form of life cycle costing (LCC) and social life cycle assessment (S-LCA). This proves further that LCA is a well-rounded and flexible tool for assessing sustainability. However, in this project, the focus is on the environmental aspect. Potential future works could see an approach to combine LCA, LCC and S-LCA to attain a multi-criteria decisional tool to assist with the sustainable development of the biopharmaceutical industry (see Chapter 6).

Among the 17 goals depicted in Figure 2.5, SDG 6 to 9 and 11 to 15 considers the environment. Table 2.8 further highlights how LCA can assist in tackling particular targets within each goal. For many of the SDGs, LCA can be used to validate process choices in terms of their environmental sustainability. By conducting life cycle inventory (LCI), input and output flows of current and proposed processes and systems, such as water infrastructures of energy technology, can be compared to assist decision making on which should be employed. When results are checked against local or national policy limits, decisions can also be made as to whether optimisation and alternate processes are necessary. Life cycle impact assessments (LCIA) can examine the effect on the environment, indicating areas that may require attention in the future and assuring that overall anthropogenic impact does not exceed global limits. Results from the analyses can be used as a performance tracker and indicate how different industries are assisting their respective countries to be in line with their SDG commitments.

Figure 2.6: The 17 Sustainable Development Goals – Source: (UN Department of Global Communications, 2020).

SDGs 8, 9 and 12 are most concerned with the sustainability of businesses and industries. As presented in Table 2.8, LCA would be beneficial in understanding the eco-efficiency of manufacturing processes to assist business growth whilst helping companies become more

environmentally responsible. SDG 9 particularly asks for the continued upgrade of industry infrastructures and scientific research into this. The ability to carry out hot-spot analysis to diagnose high impact areas using LCA will give rise to a priority list for companies to direct research for minimising their environmental footprint. Furthermore, as companies and businesses are part of our communities, steps should be implemented to align themselves to SDG 11 by complementing and strengthening regional developmental plans. For instance, companies could consider how might their processes contribute towards local thresholds when evaluating potential process for optimisations purposes. They can also opt to partake in tackling global issues, such as climate change, by actively seeking and comparing processes that may lower their overall greenhouse gas emissions (SDG 13).

As a whole, the use of LCA can support our society in meeting multiple SDGs. From understanding the individual targets within each SDG, it was found that industry could contribute to **SDG 8, 9, 11, 12 and 13** with the assistance of LCA**.** There are potentials for biopharmaceutical companies to actively strive to meet these goals and present themselves as leaders in sustainable development. In Section 2.5.1, it was reviewed that to maintain approval from company stakeholders, and companies would benefit from aligning themselves with governmental agendas. By meeting the stated SDGs, companies have the potential to gain public support and competitive advantages, particularly when LCA is used in conjunction with cost-saving tools. For these reasons, this project has aimed to develop LCA recommendations and guidance to align the biopharmaceutical industry with the concepts set within SDG 8, 9, 11, 12 and 13.

Table 2.8: The Sustainable Development Goals that the use of life cycle assessment can contribute towards. (Source: (United Nations, 2015)). ***Bold Italics*** *indicate how LCA can be used to assist industries.*

Goal	Target	How can LCA Contribute?
SDG 6 Clean Water and Sanitation	"6.3. By 2030 improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater substantially increasing recycling and safe reuse globally." "6.4. By 2030, substantially increase water-use efficiency across all sectors and ensure sustainable withdrawals and supply of freshwater to address water scarcity and substantially reduce the number of people suffering from water scarcity." "6.6. By 2020, protect and restore water-related ecosystems, including mountains, forests wetlands, rivers, aquifers and lakes." "6.a. By 2030, expand international cooperation and capacity-building support to developing countries in water- and sanitation-related activities and programmes including water harvesting, desalination, water efficiency, wastewater treatment, recycling and reuse technologies".	• LCA can be used to assess new technologies and water management regimes before their implementation to assure any new developments are eco-efficient.

SDG 7 Affordable and Clean Energy	"7.2. By 2030, increase substantially the share of renewable energy in the global energy mix." "7.3. By 2030, double the global rate of improvement in energy efficiency." "7.b. By 2030, expand infrastructure and upgrade technology for supplying modern and sustainable energy services for all in developing countries, in particular least developed countries, small island developing States, and land-locked developing countries, in accordance with their respective programmes of support."	• LCA can be used to assess new technologies and energy production infrastructures before their implementation to assure any new developments are eco-efficient.
SDG 8 Decent Work and Economic Growth	"**8.3**. Promote development-oriented policies that support productive activities, decent job creation, entrepreneurship, creativity and innovation, and encourage the formalization and growth of micro-, small- and medium-sized enterprises, including through access to financial services." "**8.4**. Improve progressively, through 2030, global resource efficiency in consumption and production and endeavour to decouple economic growth from environmental degradation, in accordance with the 10-year framework of programmes on sustainable consumption and production, with developed countries taking the lead."	• ***LCA results can direct research to improve aspects of industry operations. This can improve the efficiency of processes both economically and environmentally.*** • ***When multiple studies are presented, benchmarking of industrial processes can occur, policies, holding specifications and targets, can be developed to guide future developments.***
SDG 9 Industry, Innovation and Infrastructure	" **9.4** By 2030, upgrade infrastructure and retrofit industries to make them sustainable, with increased resource-use efficiency and greater adoption of clean and environmentally sound technologies and industrial processes, with all countries taking action in accordance with their respective capabilities." **"9.5** Enhance scientific research, upgrade the technological capabilities of industrial sectors in all countries, in particular developing countries, including, by 2030, encouraging innovation and substantially increasing the number of research and development workers per 1 million people and public and private research and development spending."	• ***LCAs on a single system can diagnose areas for which improvements are necessary to decrease its environmental burden. Research can be conducted to develop technologies that can enable this.***
SDG 11: Sustainable Cities and Communities	**"**11.6. By 2030, reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management." "11.a. Support positive economic, social and environmental links between urban, per-urban and rural areas by strengthening national and regional development planning." "11. By 2020, substantially increase the number of cities and human settlements adopting and implementing integrated policies and plans towards inclusion resource efficiency, mitigation and adaption to climate change, resilience to disasters, and develop and implement, in line with the Sendai Framework for Disaster Risk Reduction 2015-2030, holistic disaster risk management at all levels."	• Cradle-to-gate, gate-to-gate or gate-grave LCA on processes can quantify the emissions specific to a city comprehensively. The life cycle inventory (LCI) of the assessment can indicate the potential municipal waste that arises from the level of waste management. • LCA can be used along economic and social assessments to assist developmental planning and therefore support sustainable development on a local level. • LCA results can be used to assure that developments are resource-efficient when appropriate interpretation analyses are used.
SDG 12: Responsible Consumption and Production	**"12.1** Implement the 10-year framework of programmes on sustainable consumption and production, all countries taking action, with developed countries taking the lead, taking into account the development and capabilities of developing countries." **"12.2** By 2030, achieve the sustainable management and efficient use of natural resources."	• ***With developed countries take the lead to first diagnose and improve their processes, it can set examples for developing companies to follow green methodologies made available and possible via research efforts.***

	"12.4 By 2020, achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment." **"12.5** By 2030, substantially reduce waste generation through prevention, reduction, recycling and reuse." **"12.6** Encourage companies, especially large and transnational companies, to adopt sustainable practices and to integrate sustainability information into their reporting cycle." **"12.7** Promote public procurement practices that are sustainable, in accordance with national policies and priorities." **"12.a** Support developing countries to strengthen their scientific and technological capacity to move towards more sustainable patterns of consumption and production."	
SDG 13: Climate Action	**"13.2** Integrate climate change measures into national policies, strategies and planning."	• Utilising LCA results for benchmarking will allow the anticipation of future environmental impacts. Strategies and plans can be developed accordingly.
SDG 14: Life Below Water	"14.1 By 2025, prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution." "14.3 Minimise and address the impacts of ocean acidification, including through enhanced scientific cooperation at all levels." "14.7 By 2030, increase the economic benefits to Small Island developing States and least developed countries from the sustainable use of marine resources, including through sustainable management of fisheries, aquaculture and tourism." "14.c Enhance the conservation and sustainable use of oceans and their resources by implementing international law as reflected in UNCLOS, which provides the legal framework for the conservation and sustainable use of oceans and their resources, as recalled in paragraph 158 of The Future We Want.	• LCA can be used to focus particularly on marine pollution due to land-based activities. Hot-spot analysis can be carried to diagnosed processes that contribute significantly to marine emissions; this will direct efforts towards problematic areas. • LCA results can be used to assure that developments and management regimes are resource-efficient and environmentally sustainable when appropriate interpretation analyses are used. • LCA as an environmental impact benchmarking tool can assist policy development in enhancing conservation and sustainable use of oceans and their resources.
SDG 15: Life on Land	"15.2 By 2020, promote the implementation of sustainable management of all types of forest, half deforestation, restore degraded forests and substantially increase afforestation and reforestation globally." "15.5 Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species."	• LCA can use used to assess different forestry techniques before their implementation to assure the most environmentally preferred method is employed. • Using end-point metrics, LCA can assess a systems damage, particularly on biodiversity and via hot-spot analyses, diagnose areas that can benefit from the introduction of rectification measures.

2.6 CONCLUSION

As a whole, this chapter has defined sustainable development and the biopharmaceutical industry, and that this project is focused on the environmental sustainability aspects of the three pillars. While knowledge of the overall environmental burdens of biopharmaceutical processes are currently limited, the industry is continually developing its operations worldwide. The

increase in demands for more effective drugs will only add to the existing environmental impact the industry already generates. To best prevent detrimental damage globally, it is necessary to consolidate the level of impact now. This will help us determine the actions required to ensure the environmental sustainability of companies and assist them in attaining a sustainable development status.

The chapter showed that LCA could be the answer to establishing the environmental impact of the biopharmaceutical industry and can be used as a tool to make recommendations for future development. By incorporating the merits of MFA, exergy analysis and traditional EIA, LCA analyses the life cycle, from raw material extraction to the end-of-life or recycling, of a given product, process or system, primarily by compiling information on the different environmental impacts it is associated with (Klöpffer, 1997). LCA is also a flexible tool. It has proven to benefit companies in the past and can equally benefit biopharmaceuticals in the future. Studies on biopharmaceutical processes can deepen understanding of their environmental performances and aid decision-making. Furthermore, there are further potential drivers for biopharmaceuticals to adopt the use of LCA, which includes aligning better with governmental agendas and increasing companies' reputation to the public.

However, the methodology is time consuming due to the intensity of data required for the assessment. Data gathering may be a hurdle for biopharmaceutical processes as this may disclose confidential manufacturing parameters that companies may not want. Ways to encourage the gathering and the disclosing of data may be required. Guidance on how to operationalise the LCA methodology to biopharmaceutical process can motivate the adoption of LCA by the industry. The guidance should consider the goals of business decision-makers to ensure the smooth running of the company and secure economic sustainability. To assist the industry in conforming to social expectations, the guidance must also consider ways of reporting LCA results without disclosing sensitive process information. In Chapter 3, the standard LCA methodology is first explained before specific considerations (or guidance) for analysing biopharmaceutical processes are presented.

Chapter 3: APPLYING THE LCA METHODOLOGY TO BIOPHARMACEUTICAL PRODUCTION

3.1 INTRODUCTION

The previous chapter has highlighted the gaps in knowledge on the environmental sustainability of the biopharmaceutical industry, which the adoption of life cycle assessment (LCA) can fill. This chapter explains further the standardise principles and methodology of LCA according to ISO standards 14040:2006 and 14044:2006 (Finkbeiner et al., 2006; ISO, 2006a, 2006b) and provides guidance for the application of LCA on biopharmaceutical production. The guidance was developed with considerations for the key drivers that would encourage biopharmaceutical companies to assess the environmental impacts associated with their manufacturing processes. It focuses on applying the LCA methodology to biopharmaceutical active pharmaceutical ingredient (API) and product intermediate production. Moreover, the considerations it provides have informed the LCA methodologies used in subsequent chapters.

The chapter is structured into two parts. First, the standardised LCA methodology is described in the order of the four different phases: goal and scope definition, life cycle inventory (LCI), life cycle impact assessment (LCIA) and interpretation. Then, the guidance for biopharmaceuticals companies is outlined by first summarising the key drivers for conducting LCA and then describing how considerations for each driver would affect the approaches in which LCA is conducted at each LCA phase.

3.2 THE STANDARDISED PRINCIPLES OF LIFE CYCLE ASSESSMENT

3.2.1 Goal and Scope Definitions

As per the ISO standards on LCA, the goal and scope definitions phase sets the study purpose, system boundaries and assumptions (ISO, 2006b, 2006a). As part of defining the goal, the LCA practitioner examines and sets the questions that the study ought to answer and the intended application of the study by considering the audience of the results. These activities determine the way that the LCA is conducted, i.e. the choice of LCA modes and approaches that are composed to form the LCA methodology, which forms part of the scope. The scope considers the system boundaries, the functional unit and the analyses that would best fit the intended goals and applications of the LCA results. The following paragraphs summarise this. Although data collection and allocation procedures, impact assessment methodologies, and critical review processes are also defined at the goal and scope definition phase, they are carried out under

subsequent phases: life cycle inventory (LCI), life cycle impact assessment (LCIA) and interpretation respectively, and are explained in subsequent sections.

There are many questions that LCA can answer by employing different combinations of LCA approaches, i.e. techniques used to conduct LCA. Table 3.1 summarises the LCA types (the LCA mode and focus of the study), which determines the research questions the LCA can answer, the functional unit of the system and the allocation approach required when the studied system is multifunctional. The table was adapted from Schrijvers et al. (2020), where they have summarised the "building block of archetypes of LCA goal and scope definitions" and showed that research questions are composed of the three parameters of interest that determine the LCA modelling approaches that should be used. Schrijvers et al. (2020) stated that by examining the question "the reason for carrying out the study", "the functional unit" (FU) and "the perspective of LCA" should be revealed.

"The reason for carrying out the study," asks for the study focus. It asks whether the focus lies with the production/treatment or the consumption of a product or a service. The former suggests a process-oriented LCA approach, and the latter, a product-oriented LCA approach. The "functional unit" (FU) provides a quantitative description of the function (a unit measurement) of the system. It is the reference flow of the system, whereby the inventory and impact results are generated per FU (ISO, 2006b; Klöpffer, 1997). Typical FUs are mass- and economic-based (e.g. "the production of 1 kg/£1 of product A"), depending on the goal, a time factor can be added to the FU (e.g. "the consumption of 100 kg of product A over one year"). Lastly, "the perspective of LCA" seeks whether the interest lies with "accountability of impacts" or "consequences on global impact" and determines which LCA mode should be conducted and therefore the data requirements for the study. There are two LCA modes attributional LCA (ALCA) and consequential LCA (CLCA). Ekvall et al. (2015) provided the definitions for both modes, which were summarised from the ILCD handbook (JRC-IEA, 2010):

- "The attributional LCI model describes its actual or forecasted specific or average supply chin plus its use and end-of-life value chain, all embedded into a static technosphere.
- The consequential LCI model describes the supply chain as it is theoretically expected in consequence of the analysed decision, embedded in a dynamic technosphere that reacts to a change in the demand for different products." (Ekvall et al., 2015)

Hence, attributional LCA (ALCA) is required for environmental impacts accounting; otherwise, consequential LCA (CLCA) is used. They largely determine the allocation approach required and the type of data required (Section 3.2.2). Arguably there are several other modes of LCA (Guinée et al., 2018) (see Appendix A – Table A.1 for full descriptions and data requirements). However, while the data requirements for such LCAs may be different, the underlying LCA mode can be

categorised to either ALCA or CLCA by understanding whether the study interest lies with environmental impact accounting or understanding the consequences/net-impacts of a system, respectively.

Table 3.1: The required allocation approach for each LCA approach when they are applied to the same system where material X is converted to product A, co-product B and co-product C via process Y. Table adapted from Schrijvers et al. (2020).

LCA Type (Mode and Focus)	Functional Unit	Research Question	Allocation Approach
Attributional process-oriented LCA	The treatment of X and the production of product A, co-product B and co-product C.	What is the accountability for impacts of the treatment of X and the production of A, B and C via process Y? How can we decrease the accountability for impacts of the treatment of X and the production of A, B and C via process Y? Do the treatment of X and the production of A, B and C via process Y have lower consequences on global impacts than treatment and productions of these flows via alternative processes?	No allocation; this is a system expansion already.
Attributional product-oriented LCA	The consumption of product A from process Y.	What is the accountability for impacts of the consumption of product A via process Y? How can we decrease the accountability for impacts of the consumption of product A from process Y? Does the consumption of product A from process Y have a lower accountability for impacts than product A from process Z?	Partitioning must be applied to identify the inventory/impact that is attributed to product A from process Y. To attribute the life cycle impact of the consumption comprehensively, more information on the material is required and partitioned accordingly. This is dependent on the scope of the study.
Consequential process-oriented LCA (1)	The treatment of X and the production of product A, co-product B and co-product C.	What are the consequences on global impacts of the treatment of X and the production of product A, co-product B and co-product C via process Y? How can we decrease the consequences on global impacts of the treatment of X and the production of product A, co-product B and co-product C via process Y? Does the treatment of X and the production of product A, co-product B and co-product C via process Y have lower consequences on global impacts than treatment and productions of these flows via alternative processes?	No allocation; this is a system expansion already.
Consequential process-oriented LCA (2)	The production of product A from process Y.	What are the consequences on global impacts of the production of product A from process Y? How can we decrease the consequences on global impacts of the production of product A from process Y? Does the production of product A from process Y have lower consequences on global impacts than the production of product A from process Z	The substitution approach is applied on the outgoing of co-products b and c as emissions avoided. Equally, if materials X is a waste product, by utilising it to created product A, substitution can also be applied to the ongoing flow of material X.
Consequential product-oriented LCA	The consumption of product A from process Y.	What are the consequences on the global impacts of the consumption of product A process Y? How can we decrease the consequences on global impacts of the consumption of product A from process Y?	If the supply of product A is constrained and consumption by the marginal user is imminent, substitution applies to product A. Suppose the supply of product A is not constrained. The substitution

		Does the consumption of product A from process Y have lower consequences on global impacts than product A from process Z?	approach is applied on the outgoing of co-product b and c as emissions avoided. Equally, if materials X is a waste product by utilising it to created product A, substitution can also be applied to the ongoing flow of material X.

Further to the LCA approaches highlighted in Table 3.1, the overall questions posed (the objectives of the LCA study) would often determine the system boundary approach required for the study. The system boundaries of the LCA study defines what should be evaluated; this follows the reason, or the focus, of the system. The system of interest can often be separated into foreground and background, where the foreground system is the focal system that is of most interest and can be modified and optimised by the intended audience. This typically includes processes and operations that are identifiable at the "known site" (Clift and Wright, 2000). The background system supports the foreground system. They include "processes and operations which cannot be identified and localised" and activities that "the environmental impacts cannot be assessed on a site-dependent basis" (Clift and Wright, 2000). For instance, the supply and disposal of materials. Figure 3.1, developed by Ramasamy et al. (2014), highlights the different system boundary approaches, cradle-to-grave, cradle-to-gate and gate-gate, for a process-oriented LCA. In the context of the figure, the "Use Phase" is the foreground system, whilst the "Supply" and "End-of-life" phases are situated in the background.

The **cradle-to-grave** approach assesses the system's entire life cycle, which begins at raw material extraction (the "cradle" aspect) and evaluates all processes through to the end-of-life and recycling of materials (the "grave" aspect). Hence, "cradle" sits within (and begins at) the supply phase amongst other activities (potential "gates", endpoints for a study), such as raw materials conversion to secondary materials, before the use phase, where the materials are used; which itself is a potential "gate". The end-of-life phase contains the "grave" aspect of the study and includes the series of events (potential "gates") after the use of the process, for example, the decommissioning of equipment. Cradle-to-grave is the most comprehensive approach and can assist with understanding all environmental impacts associated with the system of interest. The **cradle-to-gate** approach means choosing a point in the product/process life cycle to end the evaluation, while the **gate-to-gate** approach means choosing the points in the life cycle to start and end the evaluation. Cradle-to-gate is the most popular approach found in the literature; it is often used to evaluate the impacts associated with the production of a product and when it is assumed that the intended audience is not held responsible for the use and/or the final disposal process of the product. Gate-to-gate studies often focus on the foreground system only. They are often used for comparing processes with similar supply chains or when the goal focuses on understanding only the direct inputs and outputs of a process. Note

that cradle-to-gate and gate-gate LCAs can include waste treatment of waste from the processes within the studied system (should the scope permits) and shall not be deemed a cradle-to-grave LCA if the subject of the study is not disposed of or recycled.

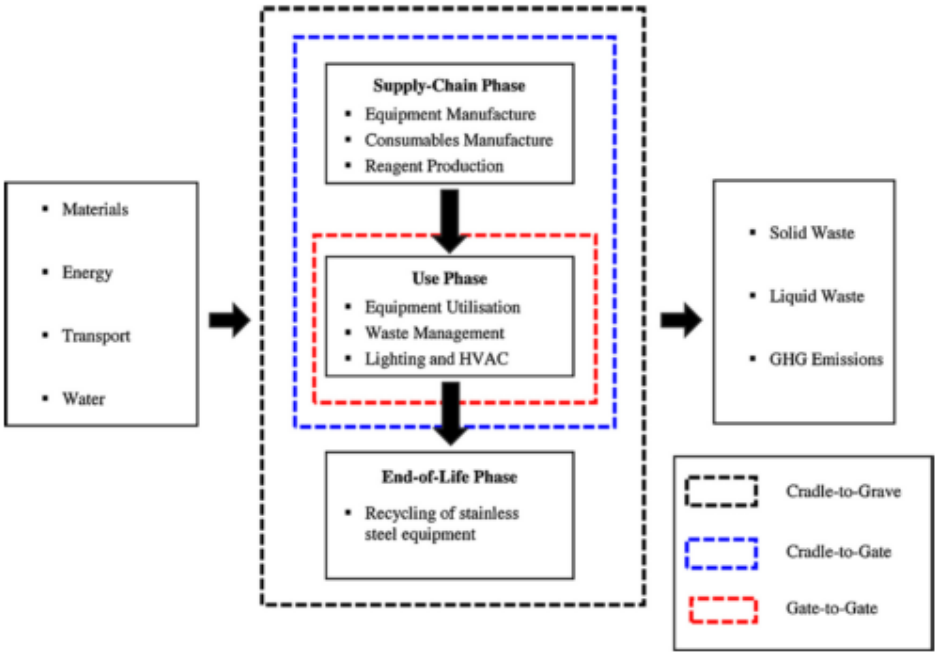

Figure 3.1: LCA system boundary approaches (Ramasamy et al., 2014). "Cradle" refers to the initial extraction of natural substances, "gate" refers to an event or action in a product's/process' life cycles (e.g. material fabrication, factory product, distribution, use by the public, etc.) before and after which point evaluation stops and; "grave" refers to the disposal or recycling of the product/process.

Lastly, while the goal (or proposed questions) can determine the LCA approaches and analyses that must be conducted on LCI and LCIA results (Sections 3.2.3 and 3.2.4 respectively) as part of results interpretation (Section 3.2.5), the iterative nature of LCA means that the choice of analysis can determine the LCA approaches required for the study too. There are known analyses typical to LCA; each has a certain requirement for the LCA mode and approaches needed to be used. They are:

- **Hot Spot Analysis:** the goal of which is to find the areas with the highest environmental burden. This usually is where either ALCA or CLCA on a single system is analysed, and the resulting impacts are allocated to analysis groups, which can be a sub-process or an entity of the system.
- **Improvement Assessment:** the goal of which is to locate areas where environmental improvement can be made. Typically, ALCA on a single system is firstly carried out, and then CLCAs are conducted to compare different improvement scenarios and seek out changes in environmental impact. This is where the allocation approach substitution is used to give environment credits to co-products (avoided impacts by not producing elsewhere).
- **Comparative Study:** the goal of which is to compare two or more systems to support decision-making. A direct comparison can be made as long as the functional unit (FU) and the system boundary approach employed by each system are the same.

3.2.2 Life Cycle Inventory Analysis (LCI)

Inventory analysis aims to generate an inventory table of all elementary flows (inputs and outputs) of the system (all activities within the scope) per FU (Martins et al. 2010). Once LCI is completed, the data can provide information on a specific resource and emission intensity, e.g. carbon emission and non-renewable resource; this indicates the environmental burden that the system carries. As a whole, the LCI phase of the LCA study involves data gathering on the system of interest; tallying and allocating the data to the functional unit(s) by using the allocation approach set as part of the goal; and, checking and validating the data for completeness (Figure 2.4). The underlying principle of LCI is that energy and mass are conserved in a given system, i.e. they can be neither created nor destroyed, and so, energy and mass balancing is often used to check that all elementary flows are accounted for. This is particularly important when primary data and calculations are used to generate the inventory.

As shown in Figure 3.2, taken from the ISO 14044, many considerations and steps ensure that the inventory is representative of the system that is being assessed. The major considerations are the source and type of the data. This includes how the data is collected and calculated, whether data cut-off should be applied, and whether the correct allocation approach is used. Different types of data could be utilised: average data or marginal data (ISO, 2006b; Ramasamy et al., 2014). They are chosen based on whether the goal of the LCA is to generate a snapshot of the environmental impacts or understand the change in impacts over time, respectively. It is often stated that ALCA uses average data and CLCA uses marginal data; however, depending on the goal, average data can be used for conducting CLCAs (Ekvall et al., 2015).

The data source can be either primary, secondary, or a mixture. Primary data, which are preferred over secondary data, are specific to (and can be gathered directly from) the system being studied, i.e. recorded process data from industry or own experiment/surveys. Secondary data are collated from the literature and other data sources, which may not be specific to the system under study. Both forms of data may feed into the LCI directly or used as a base to calculate the inventories for the system. For example, experimental data can be obtained from either own experiments (primary data) or literature studies (secondary data) but may require scaling to industry working conditions if the LCA aims to understand the future environmental impact of a process. Note that, whether primary or secondary data, the units used should be consistent; so that each material and substance can be tallied (Martins et al. 2010).

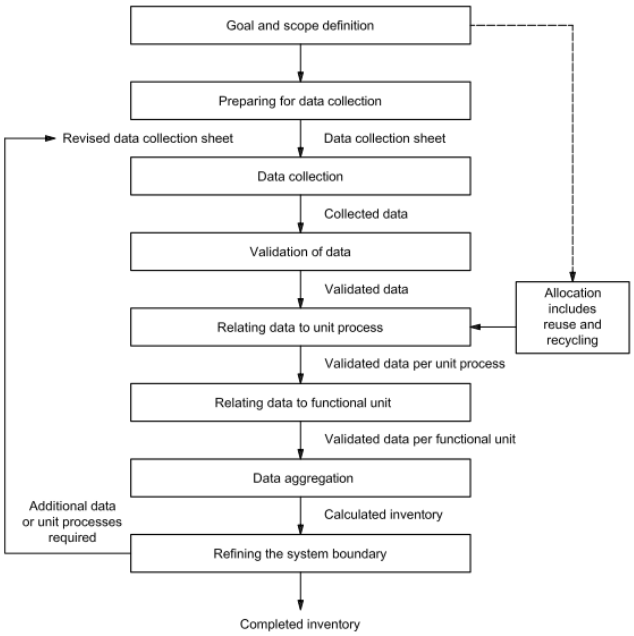

Figure 3.2: Inventory analysis procedure (ISO, 2006a)

While the LCI phase is a data-intensive phase, there are established databases containing average cradle-to-gate and gate-to-gate LCIs, i.e. input and outputs for many products and processes, which reduces time spent gathering data. Example databases include Ecoinvent (Ecoinvent, 2019), Gabi (Sphera, 2020a), US LCI (National Renewable Energy Laboratory, 2012) and Swiss Input/Output (Jungbluth et al., 2011). These databases are routinely updated to reflect current regional, national or global average environmental burdens attributable to particular processes. They are particularly useful for gathering data on the background systems, supply and end-of-life phases of an LCA, where primary data would be hard to collate.

An option to reduce the amount of data processing required is to employ a data cut-off approach. LCA handbooks have often suggested a completeness criterion of >95% (EC JRC, 2010). This means that no more than 5% of the total mass (and energy) inputs can be omitted from data collection (Klöpffer and Grahl, 2014; Zampori et al., 2016). The recommendation ensures LCA results will stay representative of the system being analysed. A data cut-off criterion that is typically suggested in the literature is 0.05% of the estimated cumulative mass of inputs (Klöpffer and Grahl, 2014; Seliger et al., 2011). This means that if a material input is <0.05kg and the total input for a process is 100kg, this input can be omitted. However, a problem would arise if most inputs fit this criterion and lead to a cumulative omission of over 5% mass. Hence, if a cut-off criterion is employed, the study must be checked for data completeness.

Upon collecting all the necessary data on the system, they are related to the functional unit (FU) and may be sorted into analysis groups. Analysis groups are used to split the LCI of the overall product/system into categories. Groups can be simple: for instance, supply, use and end-of-life

phase; or more detailed, such as raw material extraction, material fabrication, transport, product manufacture. Analysis groups are used particularly in hot-spot analyses to diagnose which group of activities is most material-, energy-, and waste-intensive and generates the greatest impacts once LCIA is carried out (Section 3.2.4). Note that assumptions may be needed to allocate the inventory. For example, allocating inventories associated with equipment fabrication to 1 kg of product often requires an assumption on how much product can be produced over the equipment's lifespan.

When there are multiple products, the allocation approach specified in the goal and scope definition must be followed and checked. There are three approaches, as mentioned in Section 3.2.2. "Partition" is where the inventory, and therefore impacts, are allocated (i.e. partitioned) amongst products and co-products. The amount allocated can be based on either the mass and economic value ratio between the products of the system. "Substitution" is where co-products are substituted with the amounts of impacts avoided in the global system by its production, i.e. impact is subtracted by the amounts of impacts that would be generated in another system. Lastly, "systems expansion" is where the FU unit includes both product and co-product (Heijungs, 2014; Schrijvers et al., 2020); this means neither partition nor substitution is applied. Note that both Heijungs (2014) and Schrijvers et al. (2020) discussed that system expansion is often confused with substitution and has distinguished the two approaches in their papers; this thesis follows their distinction between the two.

3.2.3 Life Cycle Impact Assessment (LCIA)

Life cycle impact assessment (LCIA) converts the inventory of elementary flows to their environmental effects. The steps within this phase include classification, characterisation, normalisation (optional) and weighting of the environmental impacts (optional) (Figure 3.3). How the steps are carried out depends on the goal of the study, where LCIA methodologies, set models and methods that calculate the material and substance flows into environmental impacts, are chosen.

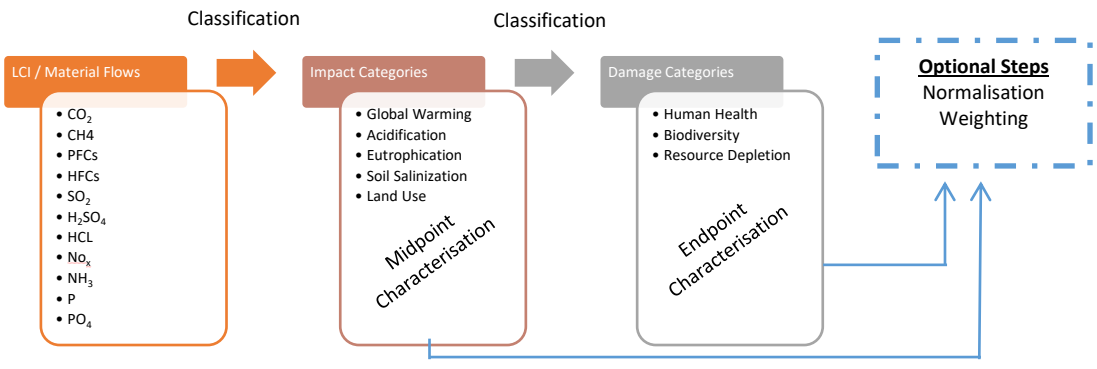

Figure 3.3: A summary of the life cycle impact assessment procedure with example materials flows and categories.

Classification and characterisation work in synergy with one another. In short, classification is the grouping of elementary flows into specific categories, and characterisation then converts the flows into common units of environmental effects (Thinkstep, 2015). Two types of LCIA metrics are quantifiable: **environmental impacts (problem-oriented metrics)** and **environmental damages (damage-oriented metrics)**. The difference between the two types is that impacts are direct translations of the life cycle inventory obtained (through **midpoint characterisation**), while damages are translations of environmental impacts to concerns (through **endpoint characterisation)**. It is said that environmental damages are better understood and more relatable to the wider audience (Thinkstep, 2015). However, calculations on the overall damages are seen as less accurate because they involve further assumptions on how different impact categories synergise (Bare et al., 2000; Thinkstep, 2015).

Regardless of which LCIA metric is calculated, the conversion of elementary flows into environmental impacts and subsequently environmental damages rely mainly on characterisation factors (CFs) developed. Table 3.2 is presented to demonstrate the characterisation procedure by using climate change as an example. Quantities of greenhouse gases are converted to their impact on climate change (or global warming potential) based on their potentials for causing an impact as compared to carbon dioxide; hence, expressed in terms of kg_{CO2eq} (kilogram equivalence to carbon dioxide). Characterisation ends with the tallying of characterised values to form the total. While the calculation for climate change follows the methodology developed by the Intergovernmental Panel on Climate Change (IPCC) (ISO, 2006b; Klöpffer, 1997), there are various methods developed for calculating other impact and damage categories. Hence, different CFs are available. Popular LCIA methodology packages include ReCipe (Goedkoop et al., 2008), ILCD recommendations (JRC, 2011), CML-IA (CML, 2016) and TRACI (Bare, 2011). The choice of which methodology package is used for environmental impact calculations rest again on the study goals (see Section 3.3.4 for a demonstration on how the goal would affect the choices of impact assessments).

Table 3.2: Example characterisation factors (CF) for global warming potential (GWP) (100-year time-frame)

Substance	**CF**	**GWP_{100} of 1kg of Substance**
Carbon Dioxide (CO_2)	1	1 kg_{CO2eq}
Methane (CH_4)	28	28 kg_{CO2eq}
Nitrous Oxide (N_2O)	276	276 kg_{CO2eq}

After characterisation, normalisation can be used to express impacts in terms of benchmarked values. Many LCIA methods contain normalisation methods, and the reference values are commonly average impacts per person per year, which is relative to a reference system or a

geographical area such as Europe or the entire world (Thinkstep, 2015). Normalising results using average emissions per year expresses impacts as a faction of the total anthropogenic emissions that normally occur. This can indicate the significance of the impacts generated by the system. Impacts can also be normalised to global carry capacities for each impact category (Sala et al., 2020) to express impacts as fractions of global thresholds. This can help identify critical impact categories that need rectifying.

After normalisation, weightings can be further applied to normalised results. Commonly, weight factors are subjective as they are developed according to the level of concerns relative to the various impact categories. Since each country has different environmental concerns, weighting factors can be developed to reflect an area's order of concern for each environmental impact category. Once impacts are weighted, impacts can be ranked in the order of their environmental urgency or be summed into a single environmental score. If an impact category of great concern ranks low against others impact after weighting, there is low urgency to reduce said impact. If weighted scores are summed, the overall score can be compared against other systems weighted using the same methods. Although this allows a summarised comparison, a concern is that impact categories that generated high impact scores may be masked by other impact categories that scored low and therefore overlooked.

3.2.4 Interpretation

The interpretation phase aims to interpret the activities carried out throughout the LCA according to the goal and scope definitions set. It is where conclusions and recommendations are made on the study (Finkbeiner et al., 2006; ISO, 2006b). The phase involves two distinct sets of activities. (1) Interpret, using further analyses, on both LCI and LCIA results to answer the questions set at the goal and scope definition phase (Thinkstep 2015; Klöpffer 1997; Finkbeiner et al. 2006). This is where critical assessments such as hot-spot analysis, improvement assessment and comparisons are carried out. (2) Check whether the LCA process model and methodologies used and the results devised from the previous phases are consistent with the ideals and scope of the study. This requires data completeness checks and sensitivity analysis, which quantifies the quality and/or robustness of the LCA (ISO, 2006b). Commonly, the quality checks on the LCA conducted are used to support the legitimacy of the conclusions and recommendations drawn.

To quantify data completeness of the LCA, the practitioner must know whether there are data omitted to estimate the percentage of omitted entries. If unknown, uncertainty/sensitivity analysis can be conducted to check the LCA model's sensitivity towards either the parameters or the LCIA methodology used for the study to confirm its robustness. By varying parameters by

a percentage, which should be indicative of the data's uncertainty level, the percentage changes in the environmental impacts would indicate the uncertainty range in the LCA results; hence, suggesting its robustness. If the model is sensitive to certain parameters, it suggests that either steps to reduce the uncertainty of these parameters are needed, or further diagnoses are needed to confirm whether gaps in the data or errors in the assumptions and calculations are made. This can prompt LCA practitioners to revise the LCA study through revising data cut-off criterion, system boundaries and calculation approaches. Methodology sensitivity analysis is an optional test to understand whether the LCIA methodologies used are comparable to other existing methods. This is particularly useful if a new assessment approach is being employed. Diagnosing the differences in the results derived between the methodologies would reveal the potential advantages and errors in the ones employed. Further guidelines on ways to carry out sensitivity analysis can be found in International Organization for Standardisation (2006), Zampori et al. (2016) and Finkbeiner et al. (2006).

3.3 GUIDANCE FOR THE BIOPHARMACEUTICAL INDUSTRY

In this section, guidance on conducting LCA on biopharmaceutical manufacturing processes, particularly the manufacture of active pharmaceutical ingredients (APIs) and product intermediates deriving from fermentation processes, is provided. This guidance was developed to inform the choices for operationalising the LCA methodology to biopharmaceutical production from the perspective of a biopharmaceutical decision-maker. More specifically, it acknowledges a company striving to reduce their environmental footprint and adhering to the goals of sustainable development but understands that there are barriers to overcome. The guidance considers the drivers, aims and concerns of the decision-makers for conducting LCA and provides the LCA approaches and other considerations for each LCA study phase that can address the different drivers.

The decision made to focus on API and product intermediate manufacture was as follows. Mata et al. (2012) stated there are two main production phases for biopharmaceuticals, API production and "medicine" production, which denotes the formulation of API with excipients ready for distribution and use. The two production stages typically occur at different facilities, and either stage can be contracted to other companies, which means under different managements. When product intermediates must first be produced before its transformation to an API at another facility, up to three manufacturing sites could be involved in producing the final biopharmaceutical drug product. From this piece of knowledge, a presumption was made that due to logistical reasons, particularly for data gathering, LCAs would likely be conducted on a per-site basis. Since there are limited LCA on biopharmaceuticals in general, a systematic

approach would be to understand the environmental impacts of the first stages of a product's life cycle (the API or product intermediate production stage) before evaluating subsequent stages.

Furthermore, literature studies have shown that impacts associated with formulating small molecule pharmaceuticals into tablets are much lower than those generated by API production (de Jonge, 2003; de Soete et al., 2014a; McAlister et al., 2016). As biopharmaceuticals can also be formulated into tablets, the existing studies suggest that the production of biopharmaceutical APIs and product intermediates are likely more impactful than formulation. These studies gave the confidence to focus on first the biopharmaceutical production stages. Nonetheless, this section provides the key drivers that encourage biopharmaceutical companies to carry out LCA in general. While the considerations being described subsequently are focused on API and production intermediate productions, it may not be limited to this production phase. For instance, the LCA approaches that support companies' aims in conducting LCA can be replicated, where applicable, for later production phases.

3.3.1 Drivers for Conducting Life Cycle Assessment

As a way to encourage the adoption of the LCA tool, the guidance considers how can companies' key drivers (or aims) be addressed or answered and provides the LCA approaches necessary for conducting an LCA strategically. Table 3.3 highlights the drivers for carrying out LCA from a company's perspective and the LCA phases that they each affect. This list was compiled with considerations for the sustainability of the biopharmaceutical industry and the barriers preventing companies from fully adopting the use of LCA (addressed in Chapters 1 and 2). The thesis contemplated that Drivers 1 to 6 were essential to consider for companies adopting the LCA methodology. In subsequent sections, guidance is provided on how the drivers would influence the LCA approaches taken to evaluate the environmental impacts of a process as well as the choices adopted for this project. Driver 7 was noted as optional because there are separate cost and social sustainability assessments readily available, which could be used independently to LCA. Companies may not require cost and social metrics integrated with the environmental evaluations of their processes. For these reasons, economic and social metrics were not considered. This project opted to focus on providing comprehensive guidance on evaluating the environmental aspects of a process.

Table 3.3: Key drivers considered for assessing biopharmaceutical production. LCI – life cycle inventory, LCIA – life cycle impact assessment.

Key Drivers		Background and rationale behind the drivers	Associated LCA phase
1	To obtain comprehensive environmental impact results associated with biopharmaceutical manufacturing.	Literature review showed few literature studies on biopharmaceutical processes that quantified an extensive set of environmental impacts. A full understanding of the environmental impact generated by the industry is necessary to ensure processes are environmentally sustainable from all aspects. Allows companies to be transparent on their environmental footprint and leverage results as part of their corporate social responsibility (CSR strategy).	Goal and Scope, LCI and LCIA
2	To reduce the pressure on companies to gather data on processes they may not have ownership over. Have the option to use secondary data when data is scarce and have procedures to understand the uncertainty of the LCA.	It was understood from LCA reviews and case studies that environmental information on some materials used in biopharmaceutical processes are not available.	LCI and Interpretation
3	To align LCA results with current governmental reporting of environmental emissions/impacts where applicable.	This would help companies compare their impact against governmental emissions and/or impact thresholds (current and future).	LCI, LCIA and Interpretation
4	To diagnose areas of high environmental impact to direct process optimisation activities.	Lowering the environmental burdens of a process often lead to eco-efficiency. Areas of high impact pose the most potential for reduction.	Goal and Scope and Interpretation
5	To enable the comparison of processes to assist with process development and environmental optimisation in general.	As proven by other industries in the past, this ability of LCA is essential to assist companies in making environmentally preferable choices.	Goal and Scope, LCI, LCIA
6	To increase a company's transparency on their environmental footprint without disclosing process sensitive information when reporting LCA results.	Reporting on sustainability is becoming a social responsibility, but the amount of process data required for LCA could be a barrier to its use. Procedures in place to prevent the disclosure of sensitive information would assure companies that competitors	Reporting/representation of all phases
7	The inclusion of economic and social metrics for better comparison. (Optional)	To attain true sustainability, economic and social sustainability should be considered.	LCI

3.3.2 Goals and Scope Considerations for Biopharmaceutical Manufacturing

While the focus of the guidance is on the production of APIs and product intermediates that are produced via the use of biotechnology, the focus of the LCA study, whether process-oriented or product-oriented, would change depending on the goals set. Both the process- and product-oriented approaches would allow the generation of comprehensive environmental impact results (Driver 1 in Table 3.3) and diagnose high impact areas (Driver 4). However, only the product-oriented approach would allow a fair comparison between two processes for process optimisation purposes (Driver 5). Although processes may produce the same product, their throughput and co-products may differ; this in turn means that the overall function of the systems is different and therefore incomparable if the process-oriented approach was used for the assessment.

Similarly, either the attributional or the consequential LCA approach would suffice Drivers 1, 4 and 5. However, when processes that generate different co-products are compared, the attributional product-oriented approach requires the use of the partitioning method as the allocation approach. This means that the inventory and impacts are allocated amongst all products, and the benefits of producing certain materials or substance overs others would be

masked. Due to the use of the substitution method, the consequential approach allows the comparison between processes by taking into account the consequences on the global environmental impacts due to the production of the co-products. Therefore, this approach can inform the net environmental impacts associated with the product or process and is particularly beneficial when choosing between waste treatment processes. For instance, process A may produce less waste than process B, but the waste must be incinerated for energy recovery; on the other hand, waste from process B is recycled. The attributional product-oriented approach would allocate impacts to both the primary product and the product produced via the waste treatment process; the consequential approach allocates all impact to the primary product but considers the emissions avoided arising from producing energy and the recycled material, and enable a more direct comparison between the overall processes.

Hence, the consequential product-oriented LCA approach was chosen for the assessments conducted as part of this project (Table 3.4). Since the focus of the study is on the API and/or production intermediate production process, a cradle-to-gate system boundary approach was chosen. Note that in Table 3.4, the wording "consumption of" is used instead of "production of". This was to ensure the focus of the study relates to the product and not the process. However, assuming that all APIs and product intermediates produced are used up by the next life cycle stage(s), i.e. the production rate = the consumption rate, the impacts that are allocable to the consumption of an amount of product would be the same as those allocable it producing the same amount of product. Under this assumption and from this point forward, the thesis will use "production" in place of "consumption" when describing the research questions and functional units.

The Choice of Functional Units: Two FUs were adopted for the LCA studies conducted as part of this project. These were "X" per year (where X denotes the total amount of product produced over the one year) and 1 kg of the product. The choice made for the first FU considered Drivers 3 and 6 in Table 3.3, where companies may want to adhere to governmental environmental reporting and requirements and increase their transparency in sustainability reporting. Firstly, annual reports on a company's environmental footprint are increasingly becoming expected due to increasing social adherence to sustainable development (Friedman and Miles, 2001; Zamil and Hassan, 2019). In addition, it was found that per year representations of impact are in line with current governmental reporting on air emissions (Azapagic and Perdan, 2011; OECD, 2019), land emissions (European Environment Agency, 2018) and national annual water consumption (OECD, 2019). Because targets to reduce GHG emissions (Le Quéré et al., 2018) and acidification and eutrophication exceedances of critical loads (European Environment Agency, 2018) are on

a per year basis, expressing LCA results per year would indicate whether the impacts associated with product manufacture are within national thresholds.

Table 3.4: LCA approach (taken from Table 3.1) that this thesis has adopted.

LCA Element	LCA Methodology Description	Notes
LCA Approach	Consequential product-oriented LCA	
Research Questions – answerable via the chosen approaches.	What are the consequences on the global impact of the consumption of product "A" (process "Y")? How can we decrease the consequences on the global impact of the consumption of product "A" from (process "Y")? • What accounts for generating the highest impact? Does the consumption of product "A" from process "Y" have lower consequences on global impact than product A from other processes?	"Consumption" refers to consumption by the next stages of the product's life cycle; for an attributional product-oriented LCA, the term can be replaced with "production" if the product is assumed to be consumed imminently at the gate. "A" refers to an API or product intermediate. "Y" refers to the company's process being studied. "X" refers to an amount, nominally "1."
Functional Unit	The consumption of "A" from process "Y" over a one-year period. (Annual reporting) Or The consumption of "X" kg of "A". (Process development)	
System boundary	Cradle-to-gate	
Allocation Approach	Substitution	Multi-product systems in biopharmaceutical product are unlikely. Applicable to waste treatment outputs.

The second FU (1 kg of product) coincides with the LCA studies in literature, and when compared to other potential metrics, it was considered best to enable fair and consistent comparisons between processes. Metrics considered included volumetric measurements, activity-based units, dose and vial, but each was deemed unfeasible for different reasons. Firstly, volumetric measurements cannot express the functionality of the product consistently as product concentrations in a solution may differ from process to process. For activity-based metrics, as APIs and product intermediates are not the final drug product, their activity levels would change when subjected to different environments by the end of the formulation stage (Mire-Sluis, 1997). This makes allocating environmental impacts difficult.

Moreover, potencies are expressed as a function of mass, i.e. IU/mg, mg/IU and nmol/IU (Knopp et al., 2019); since the measure of mass has less variability and arguably more precise (Mire-Sluis, 1997), a mass-based metric, i.e. mg, g, and kg, is a more straightforward way of expressing the function of a system. Lastly, the use of dose, or vial, as a functional unit would require assumptions on what constitutes one dose to allow the allocation of impacts consistently, making impact allocation inaccurate. The inaccuracy stems from the multiple factors that affect the amount of an API in "one dose" of a final product; factors include the indication the drug would treat, the form of the final product and the age and weight of the patient. Since one drug can have multiple indications and formulations, assuming one specific dose would not be fully informative unless the mass of the API or the specific indication is informed

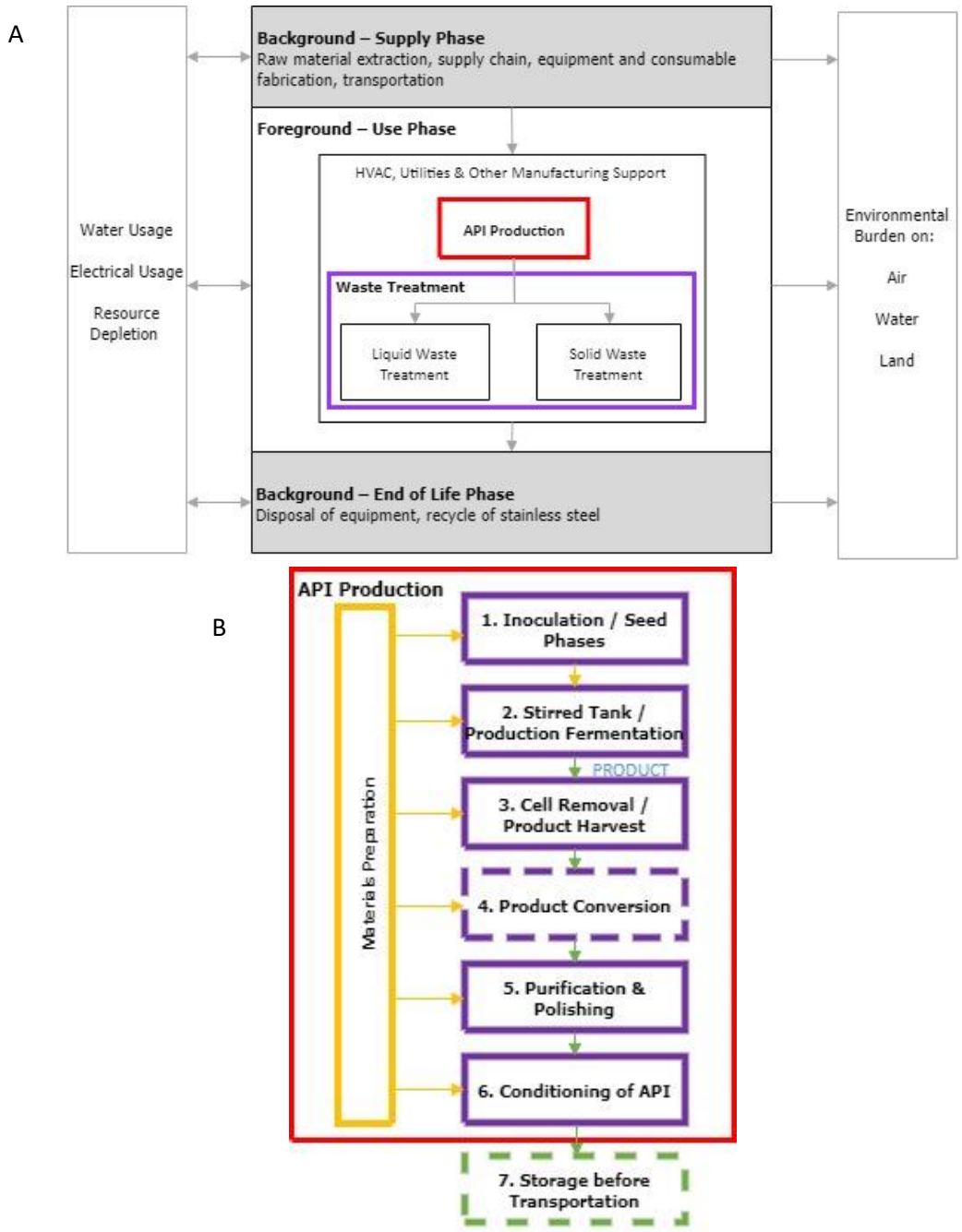

Figure 3.4: (A) Cradle-to-gate system boundary of biopharmaceutical production (until the end of active pharmaceutical ingredient (API) production, but can equally be used for biopharmaceutical product intermediates which are derived from biotechnology. (B) Typical biopharmaceutical production schematic where 4. Product Conversion is optional; see Table 3.5 for a description of each processing block.

Explaining the System Boundaries: In Figure 3.2, (A) shows the generic cradle-to-gate system boundaries for the production of biopharmaceutical API (and product intermediate) that will support the goals used for this project. It supports Driver 1 (Table 3.3) such that it comprehensively includes all the activities that are associated with the production of the product. The boundaries include all operations at the manufacturing facility, i.e. both direct production process and the support processes, such as steam generation, water purification processes, cleaning processes, and on-site waste treatment, to be within the use phase. The supply and end-of-life phases consider the supply of operating materials, the fabrication of equipment, manufacturing waste disposal and plant renewal. Note that the end-of-life phase does not include the disposal of the product. In addition, the figure highlights high-level indicators that contribute toward different environmental impacts and are relevant to all three life-cycle phases. Section 3.3.2 discusses the data gathering required to achieve a comprehensive life cycle inventory required for the boundaries set.

Figure 3.2 (B) shows the seven processing steps for producing a biopharmaceutical API (product intermediate); Table 3.5 accompanies the figure with a description of each block's functions. In this thesis, operations involved with manufacturing have been grouped into processing blocks. This will serve three purposes: to diagnose the group of operations that needs modification (Driver 4), to allow efficient comparison amongst manufacturing processes (Driver 5), and enable the masking of the underlying process when reporting LCA results to the public (Driver 6).

It was recognised that a manufacturing process consists of a series of unit operations, each made up of multiple pieces of equipment and that each is insufficient alone. As described in Table 3.5, multiple unit operations are required to carry out a specific function within a process; changing one operation will require another to be modified. This means that optimisations carried out on a process would likely occur over a group of operations based on their function. Hence, allocating unit operations into the processing blocks when conducting LCA would allow quick diagnosis (hot-spot analysis) on which areas of the process require attention. Furthermore, a unit operation can appear to support multiple functions (sub-processes) within the overall process. Hence, when comparing processes for process optimisation, the use of processing blocks would prevent the comparison of similar operations with different responsibilities within the manufacturing process.

Table 3.5: Operations involved within each biopharmaceutical API manufacturing stage

Processing Block	Function / Typical Unit Operations Involved
1. Inoculation and Seeding Cultures	This block initiates the overall production; vials of cells are thawed to be grown in flasks and subsequently bench reactors as the cells increase in volume. Cells are typically grown in flasks up to 1L in incubators. As cells multiply, they are transferred to larger containers. They can be grown in larger flasks, disposable bags or bench-scale reactors.
2. Production Fermentations	Cells are continued to grow in bioreactors when cell volume has reached the stage where they require mechanical agitation to prevent them from clumping and settling. Once the required mass of cells is present, the condition of the cell culture is altered to switch the cell's core action from one of reproduction to product formation. Bioreactors size can range up to 200m^3 capacity. The size will depend on the target production mass. The configuration of bioreactors is determined during process development, whereby batches can operate either in parallel or staggered mode.
3. Cell Removal / Product Harvest	This stage is to remove host cells and large host cell proteins. If the product is expressed intracellularly, homogenisation is used. Otherwise, filtration and/or centrifugation are typically used as the first downstream operations. There are different designs of unit operations capable of homogenisation, filtration or centrifugation. The specific equipment employed depends on the nature of the host cell, product and impurities resulting from fermentation. The combination and order of these unit operations can also differ between manufacturing processes. Other unit operations that can be used include solvent extraction, precipitation or chromatography.
4. Product Conversion (Optional)	This is optional. Some products are modified post-fermentation either through a chemical or enzymatic reaction. Examples include converting penicillin or cephalosporin to their respective beta-lactam rings; insulin can be produced by combining two protein chains. For both chemical and enzymatic reactions, specific conditions must be met in terms of pH, temperature, buffer and solvent ratios. This can involve batch or flow-through reactors and mixing units to prime the feed media before the conversion occurs.
5. Purification and Polishing	It is necessary to remove host-cell proteins, viruses and other impurities from the product solution or else it may cause immune responses when inside the human body. Manufacturing bio-therapeutics requires the product to be >99% in purity, which are detailed in drug approval agencies' guidelines. Known types of impurities must be within limits set in these guidelines as well. To purify the product, a variety of unit operations can be used: various types of chromatography to bind then release either the product or impurities; filtration to separate molecules based on size; precipitation and solvent extraction to take selectively either the product or impurities away from its original solution to another. For removing viruses, heat or detergent inactivation is often used. It can be followed by nano-filtration, which is filtration with pore sizes that are nanometres wide, thus only allowing viruses to pass through. The order and combination of these operations depend on product properties such as iso-electric pH and degradation and stability profiles.
6. API Conditioning	This processing block is where the product is prepared for the next manufacturing stage – Stage 2 Drug Formulation. There are different ways in which medicines are supplied to patients. They are pressed powered tablets, liquid capsules, and in general liquid form for either ingestion, sprayed or injection. Typically, the products at the stage are either crystallised or dried, kept in a stabilising solution or freeze-dried to ensure it does not degrade in transit. If the product is required in solid form, crystallisation may be used as part of the previous processing block to differentiate the product and impurities. At the conditioning stage, drying is required. The product can be filtered, basket centrifuged, heated or a combination of these processes to remove water. Milling processes are often to reduce the product into powder form. If the final product is required in solution, it may be transferred to a stabilised solution with the correct pH at a certain concentration. This usually requires an ultrafiltration and/or diafiltration step. If the product is easily degraded even in stabilising solution, then lyophilisation (freeze-dry) is used for better stabilisation.
7. Storage (Optional)	This block is where the product is stored before its transportation. Dry products can be stored in warehouses with low humidity whilst products in solutions, depending on their stability profile, might be kept in cold storage.

3.3.3 Life Cycle Inventory Considerations for Biopharmaceutical Manufacture

Table 3.6 highlights the LCI methodology adopted for this project when conducting a cradle-to-gate LCA on a biopharmaceutical API or product intermediate; and hence supports the goal and scope definitions highlighted in Section 3.3.1 The use of this LCI methodology is demonstrated in Chapter 4, where it presents the LCA on the production of 6-APA. Preference was given to gathering primary data on the use phase, i.e. information on a production facility, to build a model that is reflective of the true process. When primary data was not available on the 6-APA production process, the inventory was extrapolated from literature (see Chapter 4). Mass and energy balancing was conducted to ensure all inputs and outputs for all activities occurring within the plant were accounted. To simplify the data collection process, conventional databases such as EcoInvent (Ecoinvent, 2019) and GaBi (Sphera, 2020a) were used to obtain LCI for the supply of water, steam, electricity, process materials, and equipment, and the treatment of waste that occurs outside of the manufacturing facility. Checks were carried out to ensure that the selected LCIs were relevant and representative of the system being analysed.

Table 3.6: LCI methodology used in this thesis.

LCI Element	Methodology
Data Type / Sources	The preferred data type is primary data on the company's and suppliers' process and plant information. Interviews may be required with suppliers to gather information. Suppliers may also hold environmental product declarations (EPDs) on their products, which are helpful to form the product LCI. For process information that cannot be obtained, benchmarks found in literature can be used as a reference; calculations such as stoichiometry mass and energy balance will assist with attaining adequate information. Engineering rules of thumbs can be used for equipment and facility sizing, auxiliary processes such as water and steam generation and operational process parameters if necessary. When primary data are unavailable, EcoInvent and Gabi databases can be used. As these LCI data sources hold national, regional and global data, this thesis recommends using national LCI where possible to reflect the environmental impact at the site of production.
Data Cut-off	Ensure >95% of the cumulative mass of inputs is collated.
Multi-product Allocations	Substitution Multi-product manufacture is unlikely in biopharmaceuticals unless they are used together to treat a single indication, e.g. polyclonal antibodies, in which case substitution is unnecessary. Substitution should be applied to waste outputs of the manufacturing facility if the final destination of waste materials are recycled or used in energy from waste processes.
Analysis Groups	Analysis Group 1: "Supply", "Use" and "End-of-life" (of equipment and process materials) Analysis Group 2: "Inoculation", "Production Fermentation", "Product Harvest", "Product Conversion", "Purification/Polishing", "Conditioning", "Waste Treatment", "Storage" Analysis Groups 1 and 2 can be integrated to diagnose the life cycle phase of each processing block that contribute highly to each environmental impact.
Units	The units for input and output data should be SI units with the appropriate prefix to indicate the magnitude of the results. Mass of material and substances should be in -grams or -tonnes, and for energy, this should be -joules or –watt-hour. The resulting inventory table may present material input and output data as $kg/kg_{product}$ or kg/year.
Specific Sustainability Metrics	Process Mass Intensity (PMI) E factor Total Water Use (m^3) Total Electricity Use (kWh) Total Steam Use (MJ)

Specific considerations taken for biopharmaceutical manufacture were on the analysis groups to which inventory was allocated. In Table 3.6, Analysis Group 1 reflects the three life cycle phases suggested in Figure 3.2(A) and, Analysis Group 2 reflects the processing blocks defined

in Figure 3.2(B) but includes waste treatment as a separate group. As conveyed in Section 3.2.2, the analysis groups were intended to assist with diagnosing the area of high impact and enabling LCA reporting without disclosing process sensitive information. This is demonstrated as part of presenting LCA results for 6-APA manufacture in Chapter 4 and 5.

An additional consideration for the biopharmaceutical industry is the sustainability metrics that can be obtained from conducting LCI, i.e. LCI-based metrics, which can assist companies with their environmental sustainability reporting (Driver 6 in Table 3.3). As PMI and E factor are metrics already employed by pharmaceutical companies (shown in Chapter 2), extrapolating this information from LCI would align companies with current reporting and check their performances against the norms. Due to water scarcity issues and fossil-fuel related environmental issues in many countries, tracking the use of water and energy to lower their use would satisfy governmental and societal desires.

3.3.4 Life Cycle Impact Assessment Considerations for Biopharmaceutical Manufacturing

Presented in Table 3.7 are the environmental impact categories and the LCIA methodologies that were adopted for this project. The selection of assessment methodologies took inspiration from the ILCD/PEF methodology package (JRC, 2011), where for each impact category, a range of LCIA methodologies were reviewed before one was selected. The choice of methodologies was mainly based on their specificity and accuracy reviews in the literature, particularly Owsianiak, Laurent, Bjørn, & Hauschild, (2014); PE International Sustainability Performance, (2014); and Pennington et al., (2010). The drivers presented in Table 3.3 were also considered when selecting LCIA methodologies. Note that many selected methodologies were designed for European practices, but, according to reports, they are adequate for studying scenarios outside Europe (Laurent et al., 2011; PE International Sustainability Performance, 2014; Renouf, 2015). Due to accuracy levels as compared to mid-point characterisations, damage categories were not used in this project. Global average (annual) impacts per capita were chosen as normalisation factors for this project. It was not possible to use global carrying capacity normalisation factors since they are yet to be developed for all LCIA models (particularly those selected in Table 3.7). Weighting factors were not considered as they are inherently subjective.

To align LCA results with current governmental reporting on emissions and environmental impacts (Driver 3), many LCIA methodologies (or models) adopted for this project used similar methodologies employed by governmental bodies. The methodologies were acidification and eutrophication - terrestrial, developed by Seppälä, Posch, Johansson, & Hettelingh, (2006); global warming potential developed by the IPCC; and ecotoxicity – freshwater and human toxicity – cancer and non-cancer developed by USEtox (Fantke et al., 2017; Rosenbaum et al.,

2008). In addition, units used for eutrophication – freshwater and marine from ReCipe were also found in line with those used in governmental reporting (Acero et al., 2015; Bare et al., 2000; Cosme and Hauschild, 2016; Fantke et al., 2017; Hauschild et al., 2013; Owsianiak et al., 2014; PE International Sustainability Performance, 2014; Renouf, 2015).

Table 3.7: LCIA models adopted for this project and the underlying reasoning behind each choice.

Impact Category	Models / Units	Assessment Level	Comments
Acidification	Accumulated Exceedance (ILCD) (Mole of H+ eq.)	Mid-point	It covers the fewest substances but accounts of the country's policy and critical loads on emission. TRACI covers most substances, but Gabi does not account for all these yet. (PE International AG, 2014)
Ecotoxicity – freshwater	USEtox – (recommended and interim) (CTUe)	Mid-point	UNEP SETAC Life Cycle Initiative recommends USEtox but advises using ReCipe as a consistency check if marine and terrestrial toxicities are of interest. The "recommended" and "interim" USEtox method correlates with the ReCipe method for ecotoxicity due to the number of substances that are considered.
Ecotoxicity – marine	ReCipe (kg 1,4-DB eq.)	Mid-point	It covers the most substances.
Ecotoxicity – terrestrial	ReCipe (kg 1,4-DB eq.)	Mid-point	It covers the most substances.
Eutrophication – freshwater	ReCipe (ILCD) (kg P eq.)	Mid-point	Units in line with governmental usage of information
Eutrophication – marine	ReCipe (kg N eq.)	Mid-point	Units in line with governmental usage of information
Eutrophication – terrestrial	Accumulated Exceedance (ILCD) (Mole of N eq.)	Mid-point	It includes ecosystem sensitivity.
Global warming potential, incl. biogenic carbon	IPCC – GWP100 (kg CO_2 eq.)	Mid-point	Most methods are based on IPCC AR5 version.
Global warming potential, excl. biogenic carbon	IPCC– GWP100 (kg CO_2 eq.)	Mid-point	Most methods are based on IPCC AR5 version.
Human toxicity – cancer	USEtox (recommended and interim) (CTUh)	Mid-point	UNEP SETAC Life Cycle Initiative recommends USEtox, but the use of ReCipe is advised.
Human toxicity – non-cancer	USEtox (recommended and interim) (CTUh)	Mid-point	UNEP SETAC Life Cycle Initiative recommends USEtox, but the use of ReCipe is advised.
Ionizing radiation	Frischknecht et al (2000) (ILCD) (kBq U235 eq.)	Midpoint	Current best practice.
Ozone depletion	ILCD (kg CFC-11 eq.)	Mid-point	ODP values from the WMO are applied to most methods.
Photochemical ozone formation – human health	EDIP (pers*ppm*hours)	Mid-point	EDIP holds country-specific factors, although mainly for European countries, it is adequate for global use.
Photochemical ozone formation – vegetation	EDIP (m^2 UES*ppm*hours)	Mid-point	EDIP holds country-specific factors, although mainly for European countries, it is adequate for global use.
Resource depletion	ILCD (kg Sb eq.)	Mid-point	It takes account of a country's scarcity levels of various minerals and fossil fuels. As the Swiss Eco-Scarcity Method evaluates this for individual resources, it can be used as a consistency check.
Impact on water resources (water scarcity)	Swiss Eco-Scarcity Method (UBP)	Mid-point	It considers each country's scarcity level, which is based on actual and political limits.

To enable environmental process optimisation (Driver 4), LCIA methodologies that factors location and/or local policies as part of their calculations were also chosen where possible. The

LCIA methodologies presented in Table 3.7 that consider location are acidification and terrestrial eutrophication, developed by Seppälä, Posch, Johansson, & Hettelingh, (2006); impact due to water consumption from the Swiss Ecoscarcity Method (FOEN, 2013); photochemical ozone formation (POF) derived from the EDIP method; and resource depletion calculated using the ILCD method (EC JRC, 2011). The primary aim of this was to provide a more reflective indication of the impacts a system may have on the local environment and prompt personalised optimisation activities based on local needs. Secondly, as biopharmaceutical productions occur worldwide, using methodologies that consider location aimed to assist decision-making on the siting of biopharmaceutical plants.

Interestingly, quantifications for both acidification and terrestrial eutrophication use the accumulated exceedance (AE) method, which was the only method that satisfied both Drivers 3 and 4. The European Environment Agency readily reports national accumulated exceedances of critical loads for acidification and eutrophication. This means that this method would align Europe-based biopharmaceuticals with local governmental policies (European Environment Agency, 2018). Moreover, compared to TRACI, Recipe and CML, the AE method was the only method that considers national emission policies and critical loads (PE International AG, 2014). This suggested that results provided by the AE method would highlight more representatively the level of impact a process may have on a region.

Another specific choice made was the Swiss Eco-scarcity Method to take account of the impact on water resources. The method is particularly advantageous because it considers a country's water scarcity level and political targets (FOEN, 2013). It is also the only method that converts water consumption into its potential impact (Thylmann, 2014). Although the Swiss Eco-Scarcity method can also calculate the impact of resource depletion, the ILCD method was chosen. While both methodologies consider the scarcity of resources, the eco-scarcity method provides impact values for fossil depletion, mineral depletion, and metal depletion separately, whilst ILCD considers fossil, metal, mineral and renewables utilisation together. Since ILCD aggregates these environmental impacts into one impact category, it was chosen to help minimise the amount of data handling.

Lastly, the choice to employ the USEtox methodology to calculate ecotoxicity – freshwater and human toxicity – cancer and non-cancer was for the level of recognition it has received worldwide and link to intergovernmental organisations. This toxicity quantification model was developed jointly by the United Nations Environment Programme (UNEP) - Life Cycle Initiative and the Society of Environmental Toxicology and Chemistry (SETAC) (Fantke et al., 2017; Rosenbaum et al., 2008). It is the most updated model and considers the most substances in terms of their toxicity compared to other methods (PE International Sustainability Performance,

2014; Renouf, 2015). However, because some substances are in the “interim” phase, i.e. toxicity models are yet to be defined fully, there could be uncertainties in the LCIA results. It was recommended by PE International AG (2014) to use ReCipe, the next method that considers the most substances, as a consistency/sensitivity check (see Section 3.3.5) for the USETox methodology. The number of substances covered in ReCipe as compared to TRACI and CML methodologies was why it was chosen for ecotoxicity – marine and terrestrial, while no USEtox models are available.

3.3.5 Interpretation Considerations for Biopharmaceutical Industry

Since ecotoxicity – freshwater and human toxicity calculated using the USeTox model have been advised to be checked against other methodologies (PE International Sustainability Performance, 2014), a decision was made that a methodology sensitivity analysis can be conducted on all LCIA calculation methods adopted (Table 3.7). By assessing the system using the ReCipe, CML and TRACI methodology packages and then comparing the results to those generated by the selected models, it can ensure that the LCIA results are robust, particularly when the areas of high environmental impacts are the same. Three methodology packages were considered because not all packages contain an equivalent impact category as those selected for the project, amongst all being well-established packages. Since parameter-based sensitivity analysis is dependent on the uncertainty levels of the assumptions and data collected for an LCA, considerations cannot be generalised for the whole industry. Instead, the sensitivity analysis procedure is demonstrated in Chapter 4.

With considerations for Drivers 4 and 5 (to diagnose hot spots and compare processes for process optimisation), hot-spot analyses, improvement assessments and comparative studies are likely necessary for companies to carry out. Considerations for hot-spot analysis and comparative studies were highlighted in previous sections; for instance, the scope included definitions for the “processing blocks” present in most biopharmaceutical production lines. Once the inventory and impacts generated are allocated to each block for analysis, the use of defined processing blocks would also enable fair comparisons between processes. For improvement assessment and comparative studies, if a full diagnosis of differences is required, beyond understanding the overall percentage differences between process scenarios, it is possible to isolate processing blocks for direct comparison. Note that the specific data analysis methods employed to extrapolate the reasoning behind LCA results would vary case by case as the processes under analysis/comparison would be different. Chapters 4 and 5 demonstrate the procedures and results that can arise from conducting analysis for optimisation purposes.

3.4 CONCLUSION

In this chapter, the likely drivers and barriers for biopharmaceutical companies to adopt life cycle assessment (LCA) were considered into generating guidance for the operationalisation of the LCA methodology to a relevant biopharmaceutical product. The considerations highlighted were made specifically for the production of biopharmaceutical API and product intermediates and satisfied the drivers as set out in Table 3.3. Since the goal and scope definitions for biopharmaceutical manufacture (Section 3.3.2) emulated a company's potential aims for conducting LCA, the methodology adopted for this project should allow companies to produce comprehensive environmental impact results on a product without disclosing sensitive process information.

The project has adopted the consequential product-oriented LCA with a system boundaries approach of cradle-to-gate for the biopharmaceutical product and considers the full life cycle of the manufacturing facility. The guidance includes an extensive list of environmental impact categories and life cycle inventory-based metrics for analysis, both of which can support key drivers for conducting LCA and align with current metrics used by the industry and those by governmental bodies. By generating and reporting metrics that biopharmaceutical companies and the public are familiar with, it can allow the LCA tool to build its repertoire on current sustainability activities that a company may readily carry out.

Using the procedures for conducting LCA on biopharmaceutical processes described in this chapter, the LCA methodology was operationalised to the manufacture of a biopharmaceutical product intermediate, 6-APA. Chapter 4 and 5 describes the case study and presents the results from a series of analyses, including hot-spot analysis, sensitivity analysis, scenario analyses and comparative study.

Chapter 4: 6-APA PRODUCTION IN THE US – A CASE STUDY

4.1 INTRODUCTION

Following the guidance developed for biopharmaceutical manufacture in Chapter 3, the project proceeded to apply the LCA methodology to evaluate the environmental impact of an illustrative product, 6-APA. In this chapter, a background on the product is presented first to give context to why 6-APA was chosen and how analysing the product at a manufacturing plant level and at a global production level will allow the development of an overview of the environmental impacts exhibited by the biopharmaceutical industry. Chapter 4 presents the base-case scenario of producing 6-APA at a 2000 tonnes/year facility in the US. Hot-spot analysis, sensitivity analysis, and scenario analyses were carried out to understand the areas of high environmental impact, the robustness of the model, and how developmental decisions (product titre and production scales) affect the environmental footprint allocated to a biopharmaceutical product.

4.2 CASE STUDY BACKGROUND

As part of this project, the biopharmaceutical industry was analysed to understand the range of products it produces, its scale, and where they are produced (Appendix B). From the analysis, it was clear that the largest biopharmaceutical products by mass (produced via fermentation/cell culture technology for the purposes of this project) are natural and semi-synthetic beta-lactam antibiotics (Tables B.2-3 and Table 4.1).

Antibiotics are an important category of pharmaceuticals (in general) as they are substances that kill or prevent bacterial infections. The global antibiotics market is worth $45 billion (Center for Disease Dynamics, Economics & Policy (CDDEP), 2015; CenterWatch News Online, 2014) and encompasses 12 antibiotic classes. The biggest antibiotic class, beta-lactams, has a market share between 57% and 65% ($25-29 billion) (Elander, 2003; Meštrović and Chow, 2015). These antibiotics are recognisable by the beta-lactam ring within their molecular structures. Penicillin, the first antibiotics to be discovered (in the 1920s by Alexander Fleming), was also the first biopharmaceutical product on the market in the 1940s (Projan and Shlaes, 2004). Research on penicillins was pivotal to the discovery and development of the collection of antibiotics we have today. For instance, the understanding of the beta-lactam ring structure allowed the discovery of other natural beta-lactam antibiotics, cephalosporins, carbapenems, clavams and monobactams; subsequent derivatives of both penicillin and cephalosporin (semi-synthetic beta-lactams); and other synthetic beta-lactams. At present, >60% of all penicillins and >20% of all cephalosporins are reduced first to their beta-lactam rings (Bhattacharyya and Sen, 2006),

most commonly via enzymatic hydrolysis (Figure 4.1), to generate semi-synthetic antibiotics. Penicillin and its derivatives, in particular, are the most annually consumed antibiotics (ECDC, 2018). As shown in Table 4.1, the top antibacterial drugs include four beta-lactam antibiotics, Augmentin, Tazocin, Meropenem and Primaxim. While this illustrated the importance of beta-lactam antibiotics in modern day society, it showed the dominance of penicillin derivatives as Augmentin and Tazocin made up >75% of the top antibacterial drugs by mass.

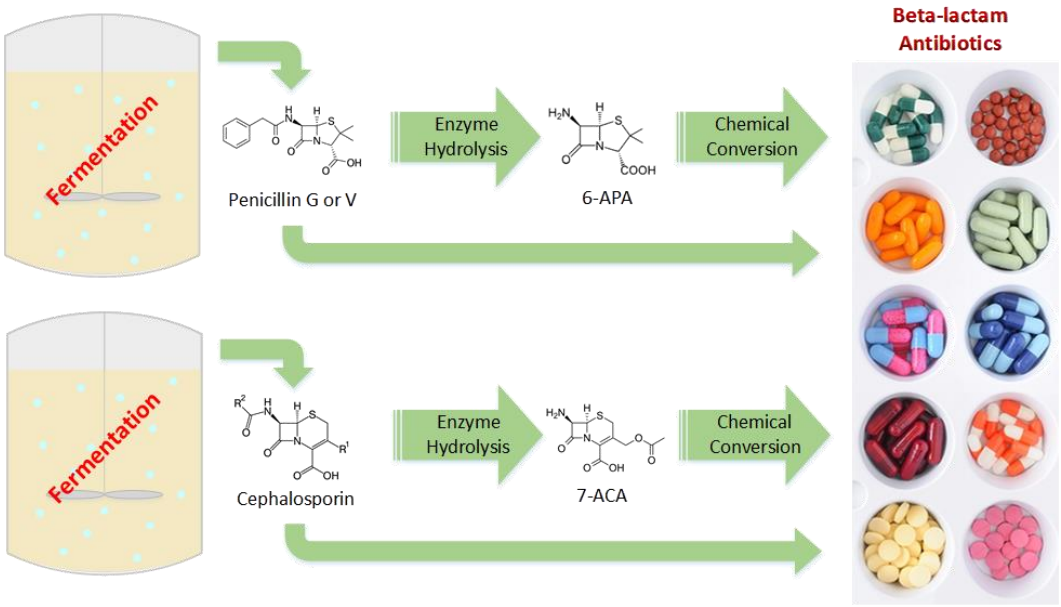

Figure 4.1: The general process of natural and semi-synthetic antibiotics. Though it is possible to convert the original antibiotics to their beta-lactam ring through chemical pathways, it is most common to use enzymes (Carrington, 1971).

Table 4.1: Top-selling antibiotics in 2010 (Business Insights, 2011). *Quantity sold was estimated by dividing global sales by the average selling prices of each product, calculated from prices indicated on the British National Formulary website (https://bnf.nice.org.uk/) and used currency conversion (GBP to USD) provided by XE (https://www.xe.com/) in February 2021. Note that the estimates assume that selling prices do not fluctuate drastically. In bold is the top sold antibiotic based on mass.*

Rank	Brand	API / Molecule	Company	2010 Global Sale ($m)	Estimated Quantity Sold (t) (3s.f.)	Type
1	Levaquin	Levofloxacin	Janssen Pharmaceuticals	1357	223	Synthetic
2	Zyvox	Linezolid	Pharmacia	1176	15.1	Synthetic
3	Avelox	Moxifloxacin	Bayer	984	133	Synthetic
4	**Augmentin**	**Amoxicillin + Clavulanic**	**GSK**	**966**	**2190 1100**	**Semi-Synthetic**
5	Tazocin	Piperacillin + Tazobactam	Pfizer	952	179 22.4	Semi-Synthetic
6	Cravit	Levofloxacin	Daiichi Sankyo	820	135	Synthetic
7	Meropenem	Meropenem	AstraZeneca	817	31.2	Synthetic
8	Cubicin	Daptomycin	Novartis	625	2.60	Natural
9	Primaxin	Imipenem + Cilastin	Merck & Co	610	17.1 17.1	Synthetic
10	Zithromax	Azithromycin	Pfizer	415	216	Semi-Synthetic
			TOTAL	**8722**	**4280**	

Not only that penicillin and its derivatives are significant antibiotics, but they are also significant biopharmaceutical products. The annual global production of penicillin and derivatives is approximately 269,000 tonnes, whilst production mass for cephalosporin and derivatives is approximated to 98,900 tonnes (Table B.2). The next highest outputs, by molecule type, from the biopharmaceutical industry are insulin and monoclonal antibodies (mAbs). They are produced at 35.0 and 25.7 tonnes per year globally (Table B.3). The production mass differences suggested that natural and semisynthetic beta-lactam antibiotics can be assumed to make up at least 80% of all biopharmaceutical annual mass output. The interest with 6-APA lies with the need for this product intermediate to generate penicillin derivatives. By considering the molecular mass ratio of beta-lactam rings to penicillin and cephalosporin derivatives, it was estimated that up to 38,500 tonnes of 7-ACA and 103,000 tonnes of 6-APA are annually produced (Appendix C). Since penicillin conversions to 6-APA typically occur as part of a downstream process (post-fermentation product extraction and purification) and that 6-APA conversions to other antibiotics usually occur at separate facilities, 6-APA can be considered the largest product biopharmaceutical industry.

Since the global production of penicillin and/or 6-APA is by far the greatest amongst all biopharmaceutical products, where a single production site can annually produce up to 10,000 tonnes of penicillin salt (Xiaobo, 2013), an environmental assessment of 6-APA manufacture may be able to highlight the major impacts of the industry. In addition, the manufacturing processes used for this product are the most established and best-documented within the industry because of penicillin being the first biopharmaceutical introduced. Due to the product's market maturity, it was assumed that its production methods should not vary drastically between manufacturers, or else companies cannot maintain price competitiveness. While interviews were conducted to gather information on 6-APA manufacture (Appendix D), where there were missing data, which arose from process non-disclosures issues, there was a degree of confidence in using secondary data (literature figures) as surrogates. However, it must be noted that this project indirectly assumed that 6-APA manufacturing plant energy and materials requirements and production outputs profiles were similar amongst all 6-APA plants.

By applying the LCA methodology set out in Chapter 3, this chapter hopes to gain insights into the environmental impacts of 6-APA manufacture and, by extension, insights into biopharmaceutical API production in general, due to the dominance of this product. The process of operationalising the LCA methodology will assist with evaluating LCA's suitability and robustness in allowing companies to derive recommendations for process optimisation purposes from LCA results. This chapter focuses on the production of 6-APA in a plant situated in the US, which will serve as a base-case scenario going forward. Different production scenarios

(comparing the impacts of producing at different scales and assumed product titre) are also presented in this chapter. A comparative study of producing 6-APA in different countries is presented in Chapter 5. Overall, the intention is to demonstrate how LCA could be used for addressing a broad range of pertinent questions.

4.3 Case Study Methodology

The following sections detail the assumptions made to carry out a life cycle assessment (LCA) on a hypothetical 6-APA production facility, producing 2000 tonnes of 6-APA in the US. This hypothetical 6-APA production process was developed through both literature research and a series of interviews with industry members working, or who have worked, on a penicillin and/or 6-APA production process: Carleysmith (personal communications, 2017) and members of GSK (personal communications, 2016). Personal communications and their outcomes can be found in Appendix D. Moreover, the process developed was assumed to represent an industry the average process and was used to model the environmental consequences of producing 6-APA in other countries besides the US (see Chapter 5).

4.3.1 Goal and Scope

The goal of this case study was to evaluate the environmental performance of producing 6-APA at an average plant. The production scale of this average plant was assumed to be 2000 tonnes per annum based on information as provided by Carleysmith (personal communications, 2017) and GSK (personal communications, 2016) (Appendix D). As large-scale production of penicillin initiated in the US, and since the US has the greatest number of biopharmaceutical manufacturing facilities (565, 31.2% of the global total) (BioPlan Associates Inc., 2020), it was deemed appropriate to assume production in the US as the base-case scenario to reflect the current impact profile of the biopharmaceutical industry. The plant was specifically assumed to be situated in New Jersey, USA, where manufacturers of beta-lactam antibiotics, such as Merck, Wyeth LCC, Sun Pharmaceutical Industries and Teva Pharmaceuticals, hold one or more of their production facilities (Kurmann Partners, 2017).

As outlined in Chapter 3, the LCA approach taken for this study is a **cradle-to-gate, consequential, product-oriented LCA,** designed to allow the answering of the following questions:

1. What are the net environmental impacts of the production* of 6-APA produced in a manufacturing facility in the US?
    a. How significant are the net impacts attributed to 6-APA production?
    b. What are the major impacts that should be considered when developing new therapeutics in this region? (This assumed that the impacts generated by 6-APA

production would indicate the impacts associated with biopharmaceutical products).

2. How can we decrease the net environment impacts associated with production* of 6-APA produced in a manufacturing facility in the US?
    a. What process(es) account for generating the highest net impact?
    b. Which production parameters affect most the overall environmental impacts associated with the production process?

(*As stated in Chapter 3, it is possible to use the term "production" when the product, 6-APA, is assumed consumed immediately after its production, by the next product life cycles stage).

Following the guidance in Chapter 3, the functional units (FUs) employed are "**the production of 1 kg of 6-APA**" and "**one year of producing 6-APA**" in a manufacturing facility in the US.

4.3.1.1 The 6-APA manufacturing process and the scope

As part of taking the cradle-to-gate approach, considerations were made for the source of materials for 6-APA production and the manufacturing facility and the subsequent end-of-life of waste generated by the facility (operational waste and plant renewal). Figure 4.2 highlights the processes in the supply (grey box), use (red box) and end-of-life phases (grey box) that were considered as part of the analysis. Figure 4.3 presents the process flow diagram of the theoretical 6-APA production process that the LCA was conducted on, i.e. the process that sits within the use phase in Figure 4.2.

The theoretical 6-APA production process that was developed for this project is also deemed the "average" 6-APA process. Due to the maturity of producing this product intermediate, it was assumed that the production process does not vary much between sites. This average process is loosely based on the 6-APA production plant in Irvine, UK (a GlaxoSmithKline production facility) and literature sources highlighting the production of penicillin and 6-APA (Carrington, 1971; Goldrick et al., 2015; Harding, 2008; Heinzle et al., 2006; Nandi et al., 2014) and, developed in consultation with Carleysmith (personal communication, 2017) (Reo Process improvement Ltd.). As discussed in Chapter 3, unit operations directly involved in 6-APA manufacture in the use phase are split into the six processing blocks: Inoculation, Stirred Tank Fermentation, Product Harvest, Product Conversion, Purification and Conditioning (purple boxes in Figure 4.2); waste treatment is also treated as an individual processing block (black box). While plant utilities and materials preparation are depicted separately to the main production process in Figures 4.2, the inventory associated with these processes was allocated to each processing block based on their use levels.

As a whole, in accordance to interviews conducted with industry contacts (Carleysmith, personal communications, 2017; GSK, personal communications, 2016) (Appendix D), it was assumed that

the production plant had a mixture of 100m^3 and 200m^3 size production bioreactors operated either in parallel or a staggered configuration to supply the average demand of 2000 tonnes of 6-APA per annum. Assuming an equal number of batches were carried out in each production bioreactor size and a failure rate of 5% (Heinzle et al., 2006), 195 batches are required to be produced from each production bioreactor size per year to meet the demand (total of 2007 tonnes).

Hillier from GSK (personal communications, 2016) and Carleysmith (personal communications, 2016), both experts in the fermentation of penicillin, confirmed the upstream processes involved. Both advised the typical durations of each fermentation stage and the approximate cell and penicillin concentrations at the end of production fermentation, which was used to calculate input and output requirements at each cell culture stage. In Appendix E, Table E.1 summarises the growth rate of *Penicillium chrysogenum* at different growth phases; they were subsequently used in Table E.2, which presents the growth assumptions (scale, duration and growth phases) and the calculated biomass at each cell culture stage (rice, flask, can, N-2, N-1 and production). The validity of the resulting cell concentration at the end of production fermentation, 48g/L, was used to justify the growth assumptions employed. By using the product to biomass ratio – 1.2 (employed by Harding et al. (2007), it was found that the final cell concentration gave a product concentration of 57.6g/L, which is close to the product titre 57g/L adopted, from Heinzle et al. (2006). When asked, Carleysmith (personal communications, 2017) also validated that the product titre assumed was reasonable.

The downstream (post-fermentation) unit operations designed for this project considered the process flow presented by Heinzle et al. (2006), Harding et al. (2007) and confirmed during a tour at GSK (personal communications, 2016). The processes that the three studies shared: rotary vacuum filtration, in-line acid addition, solvent and back extraction stages, crystallisation, basket centrifugation and drying; were incorporated into this average 6-APA production process. During the GSK tour, it was noted that the cooling of fermentation broth post-production was critical to preserving the product and that the use of phosphate buffer and the recycling of solvent and phenylacetic acid were common practice. The tour also prompted research further on purified water, water for injection, process steam and pure steam generation and, waste treatment within pharmaceuticals. Generic processes obtained from Veolia Water (2007) and the US Department of Energy (2015) were assumed for this project. The CIP and SIP protocol was adopted from McNulty (2016) but also considered Chisti and Moo-Young (1994), Junker et al. (2006), SPX (2013), and Vincent (2008).

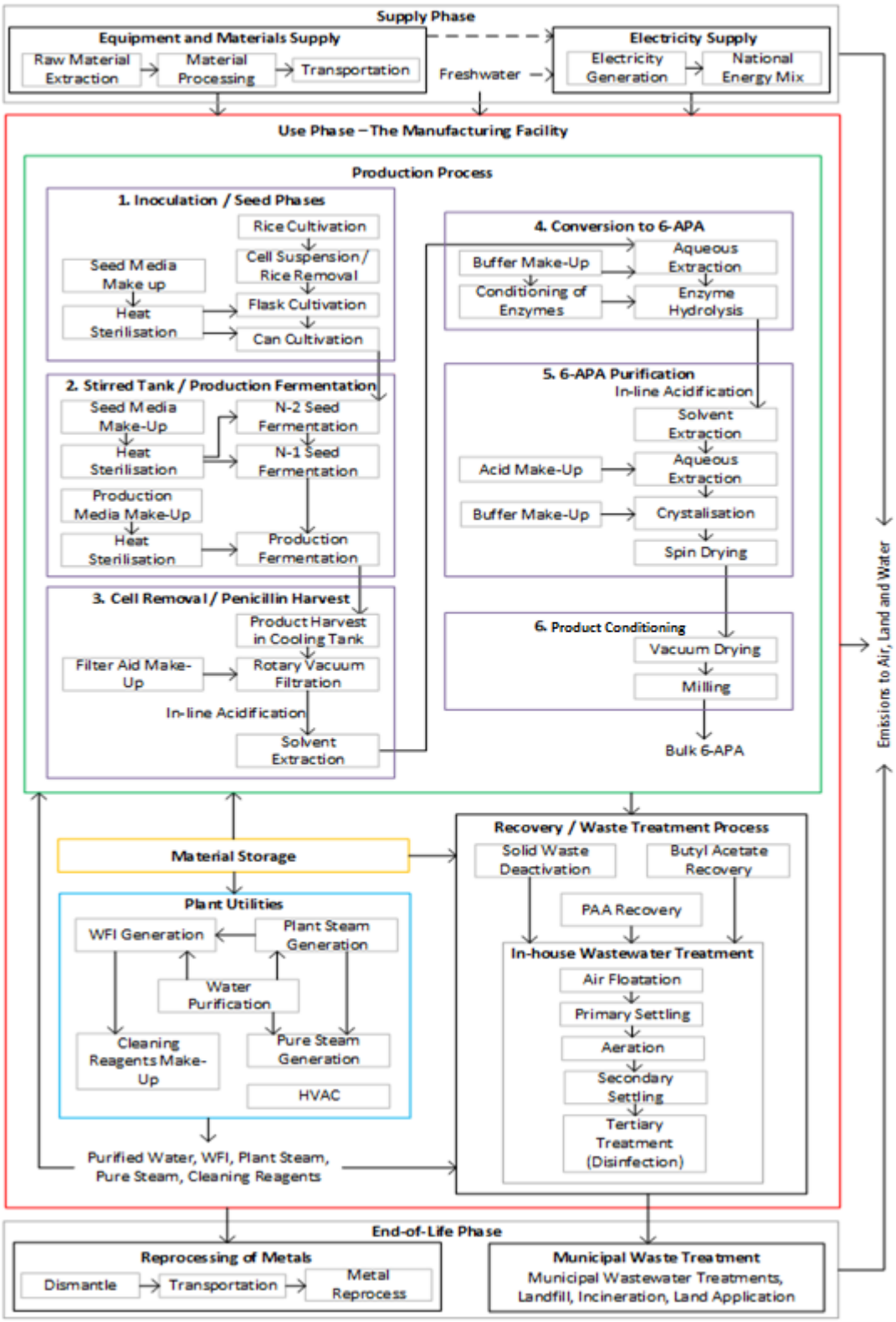

Figure 4.2: The cradle-to-grave system boundaries for a 6-APA production plant used in this case study. The processes involved in producing 6-APA are split into the supply, use and end-of-life phases, and the processing blocks according to the LCA guidance set out in Chapter 3. The use phase of the LCA also considers the plant utilities and waste treatment processes within the manufacturing facility.

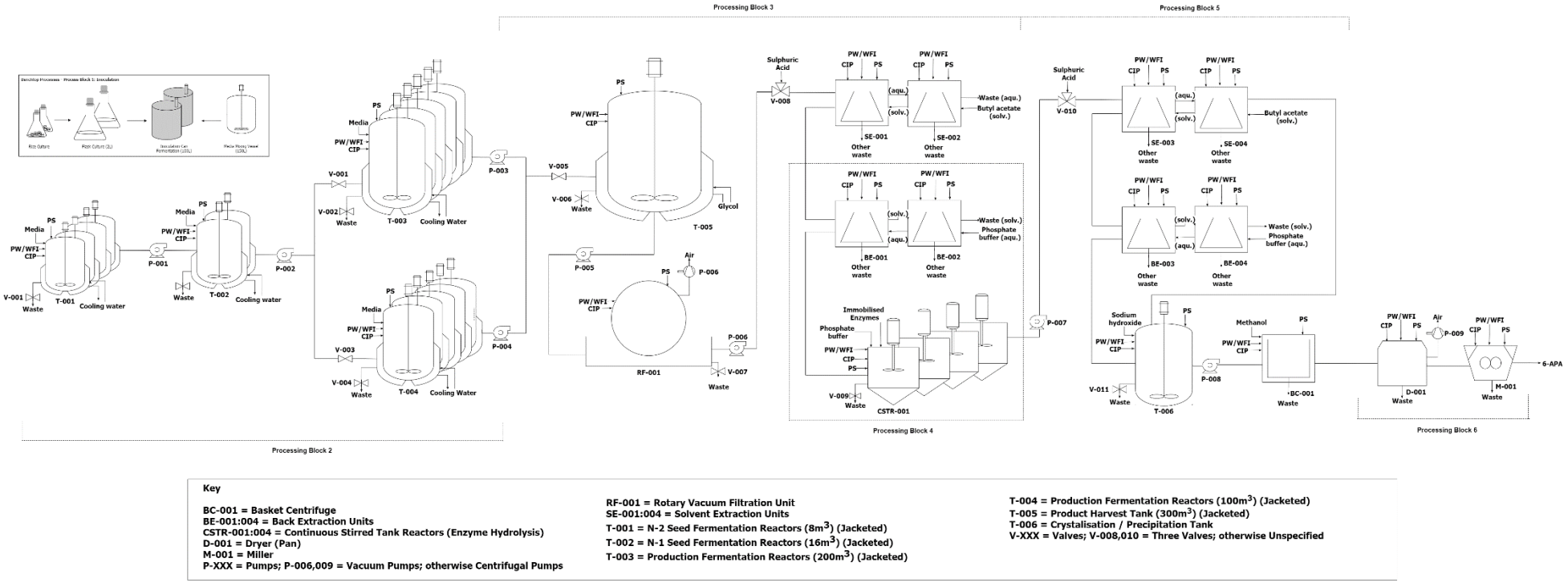

Figure 4.3: The "average" 6-APA production process flowsheet developed and used for this project. Not all process equipment (namely piping, pumps and valves) are depicted in this process diagram. PS = pure steam, PW = purified water, WFI = water for injection, CIP = cleaning-in-place.

4.3.2 Life Cycle Inventory

The life cycle inventory (LCI) approach taken for this project prioritised gathering information from industry contacts to understand the magnitude of all the elementary flows of the main production process. Due to the limited amount of process data that can be disclosed through interviews, a mixture of literature-based values and assumptions were used to calculate process mass and energy inputs and outputs requirements (see 4.3.2.1 Process Assumptions and Calculations, Appendix D – personal communication outcomes, Appendix E – process assumptions and inventory calculations). Discussions with Carleysmith (personal communications, 2017) and details disclosed by GSK (personal communications, 2016) were then used to quality check values calculated.

For calculating the inventory for one year of 6-APA production, calculations were made for the production of one batch first. Values were then scaled to per year of production (and per 1 kg or 6-APA produced). This approach was based on the assumption that the overall product mass output would be a multiple of the mass output per batch manufacture. There are a finite number of batches, which can run in an operational year. The risk of batch failure was assumed constant (5%). It was also assumed that the number of batches carried out per year would be constant. An advantage of modelling the average number of batches is that it enables us to recalculate the inventory if the failure-rate assumption changes, i.e. higher batch failures will result in higher environmental impact per kilogram of 6-APA produced. However, when comparing the annual environmental impact of two manufacturing facilities, the annual production mass should be equal for a fair comparison. Benchmarking in this form will provide the average impacts of production facilities working at a similar scale.

4.3.2.1 Process Assumptions and Calculations (Mass Balancing, Equipment Sizing and Process Energy Requirements)

4.3.2.1.1 Upstream Processing Calculations

Carleysmith (personal communications, 2016) and Hillier (personal communications, 2016) provided advice on the fermentation production scales (reactor size), duration and overall production titre. Literature-based assumptions on cell growth rates were then used amongst fermentation duration assumptions to calculate biomass inputs and outputs at each fermentation stage (Eq. 4.1). The final biomass concentration was checked using the product to biomass ratio as suggested by Harding et al. (2007) and was shown to be in the correct range to provide the assumed product titre (penicillin) (57 g/L).

(Eq. 4.1) $x = x_0 * \exp^{ut},$

where x= cell mass (kg), x_0 = initial cell mass (kg), u= growth rate (hr^{-1}) and t= time (hr)

As the biological processes for producing biomass and penicillin are complex and involve many substances, the project took to balancing simplified stoichiometry for cell growth and product formation. Only main substances were considered to calculate the input requirements and the associated outputs of fermentation. In Appendix E, Table E.3 presents production assumptions and ratios used to define the stoichiometric equations. $Y_{x/glu}$, the yield of biomass on glucose, and M_{glu}, the glucose requirements required for cell maintenance, were used to calculate the amount of glucose required at each fermentation stage by considering the starting and overall increased amounts of biomass and the stage duration. With the glucose requirements and biomass known, it was possible to solve the stoichiometry for each fermentation stage; Eq. 4.2 presents the average inputs and outputs for generating 1 mol of biomass. Similarly, $Y_{pen/glu}$, the yield of penicillin on glucose, expressed in g_{pen}/g_{glu}, was used to calculate the amount of glucose required at the production fermentation stage to produce penicillin G.

Knowing both the amount of glucose required for the assumed product titre, it was possible to solve the stoichiometry for penicillin G production. Eq. 4.3 presents the input requirements and associated outputs for producing 1 mol penicillin G; see Table E.5 for individual inputs and outputs for each fermentation stage. These inputs were used to assume the production fermentation media composition shown in Table E.6. Due to penicillin fermentation cultures conventionally use corn steep liquor as the carbon source, a standard media composition (Nielsen et al., 1995) was assumed for all fermentation stages up until production fermentation, where the standard media was modified to the production media by including calculated values of glucose, ammonium sulphate and phenylacetic acid.

(Eq. 4.2) $$0.311\ Glucose + 0.100\ Ammonium\ Sulphate + 0.617\ Oxygen \rightarrow 1\ Biomass + 0.867\ Carbon\ Dioxide + 1.27\ Water$$

(Eq. 4.3) $$2.39\ Glucose + 1\ Ammonium\ Sulphate + 0.538\ Phenylacetic\ Acid \rightarrow 1\ Penicllin\ G + 2.42\ Carbon\ Dioxide + 10.1\ Water$$

In addition to cell culture vessels, other equipment required for upstream processing included incubators for rice and flask cultures, media preparation/hold tanks and the media sterilisation unit. According to advice from the fermentation experts and engineering principles, the vessels were sized using tank aspect ratios and impeller sizing from Doran (1995) and Mudde et al. (2016). Wall thickness was also adopted from Doran (1995) to calculate the amount of steel required to fabricate the equipment. Energy requirements for mixing and pumping were determined use using engineering principles from Doran (1995) and Mudde et al. (2016) (Table E.7); calculations considered density of liquids being manoeuvred, the desired tip speed of

impellers (adopted from (Mudde et al., 2016) and resistance during pumping (average values taken from pump operation guides (Aliasso and Corporation, 1999; KSB, 2005)). Note that energy requirements related to pumping were allocated to the unit operation and processing block that the material is being pumped into. Where materials from one vessel were assumed to be distributed to more than one tank (for instance, fermentation media being sent to fermenters working in parallel), energy arising from mixing was allocated to each unit operation according to mass input ratios next vessels.

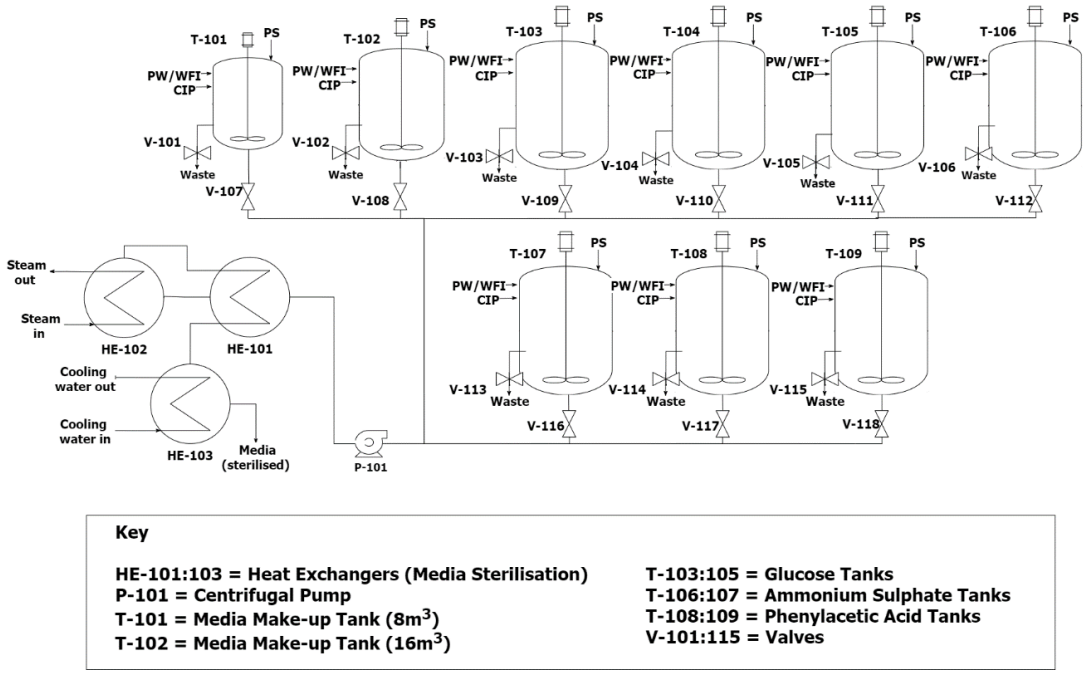

Figure 4.4: Fermentation media make-up and sterilisation process. PS = pure steam, PW = purified water, WFI = water for injection, CIP = cleaning-in-place.

The media sterilisation process was assumed to involve three heat exchangers (HEs), as shown in Figure 4.4. The three-HE configuration requires new (un-sterilised) media to enter HE-101 where it is heated to 80°C (by heated media), which then enters HE-102 to be heated to 145°C (by process steam), re-enters HE-101 to be cooled to 85°C (by new media), and then finally cooled to 35°C in HE-103 (by cooling water). Post cooling, the media is assumed sterile and ready for use in cell culture. It was assumed that heat transfer resistance was negligible. Eq. 4.4 and Eq. 4.5 were used to determine the mass flow rate for cooling water and process steam. The overarching assumption was that fermentation media was pumped at 20,000 L/hr, which, depending on which media is being sterilised (seed or production media), yielded different mass flow rates (presented in Table 4.2). Table 4.2 highlight further the specific heat capacities and enthalpy, amongst other assumptions, used to calculate the mass flow rate.

(Eq. 4.4) $Q=m_c*C_{pc}(T_{out}-T_{in})_c=m_h*Cp_h(T_{in}-T_{out})_h$

(Eq. 4.5) $Q=m_c*C_{pc}(T_{out}-T_{in})_c=m_hH_{fgh}$

Where m = mass flow rate (kg/hr); C_p = specific heat capacity (kJ/kgK); (T_{out}-T_{in}) and (T_{in}-T_{out}) = the change in temperature (K or °C); H_{fg} = specific enthalpy of evaporation (kJ/kg); c refers to the cold medium; and h refers to the hold medium

Table 4.2: The specific heat capacities and specific enthalpy of evaporation used to calculate and sense-check the mass flow rate of steam and cooling water.

	Specific heat capacity (C_p) ($kJkg^{-1}K^{-1}$)	Specific enthalpy of evaporation (h_{fg}) (kJ/kg)	Mass Ffow rate (kg/hr)
Fermentation media			
Seed fermentation	3.94 (20°C) – 3.99 (60°C)*		21000**
Production fermentation	3.76 (25°C) – 3.83 (53°C)*		25600**
Process steam		2015 (180°C)***	
Seed fermentation			2680
Production fermentation			3140
Cooling Water	4.19 (15°C)		
Seed fermentation			66100†
Production fermentation			77600†

* Darros-Barbosa et al. (2003) presented C_p for liquids containing various sugar concentrations at temperatures up to 60°C. Values for 10%w/v sucrose was assumed for seed fermentation media, and 16%w/v glucose was assumed for production fermentation media. The starting temperature for the media was assumed to be 20°C, the C_p at 25°C was used for production fermentation media as there was no value available for 20°C. The C_p for the highest temperature available was used as the C_p for fermentation media at 80°C and 85°C.
** Calculated using media composition in Appendix E - Table E.6.
***Steam table value (Rogers and Mayhew, 1995)
†Outlet temperature of 30°C was assumed.

The cooling water system required for cooling bioreactors during fermentation, amongst other heat exchanging (i.e. media sterilisation) and condensing activities in the facility, was assumed to be open-loop. Water was assumed drawn from a nearby freshwater source but was returned after use. The mass of freshwater consumed, i.e. water the did not return to the source by the facility, was assumed to be 5% of the total cooling water withdrawn. Water withdrawn was calculated based on the assumed heat energy equivalency shown in Eq. 4.6, where negligible heat transfer resistance was also assumed. The heat generated through fermentation considered heat energy generated through mechanical agitation of cell culture broth and oxygen consumption by cells. Table E.26 presents the assumptions and calculation for the mass flow rate of cooling water and the assumed water consumption due to the cooling of each bioreactor.

(Eq. 4.6) $Q = \text{Heat generated through fermentation} = m_c * C_{pc}(T_{out}-T_{in})_c$

4.3.2.1.2 Downstream Processing

As advised by Carleysmith (personal communications, 2016) and Hillier (personal communications, 2016), the downstream unit operations were assumed to work in a continuous mode. This means those process parameters (such as flow rate) for one process may determine process parameters for the following process. The mass and energy balancing and equipment sizing of post-fermentation operations were mainly calculated based on mass and volume inputs and yield assumptions, amongst other process parameters such as flow rate requirements (for both product stream and process materials) (Table E.8 to E.25). These assumptions were based on literature that considered penicillin and 6-APA manufacture and off-the-shelf equipment operation guides. The sizing of equipment was mostly determined by selecting available off-the-

shelf equipment capable of processing the required process load and/or providing the correct process conditions. This was the case for rotary vacuum filtration, solvent extraction and back extraction (of penicillin and 6-APA), basket centrifugation, pan drying of precipitate and milling of 6-APA. For product harvest (Table E.8), enzyme hydrolysis (Table E.14) and crystallisation (Table E.18), reactor-sizing calculations were carried out, using similar engineering principles to fermenters and media preparation tanks, by considering the aspect ratios and mixing requirements of the process (Table E.7).

The fermentation broth cooling post-harvest considered only the potential penicillin degradation and line-lost during the transfer from fermenters to the harvest tank and the cooling process itself. The coolant, polyethylene glycol, system was not modelled due to the major assumptions involved. In a conventional pharmaceutical plant, glycol is used to cool various pipes and reactors where high amounts of heat are generated, such as the onsite water purification system and steam generation system; glycol may also be used in air handling units as a cooling and dehumidification agent. To model the full glycol system, assumptions must be made on the amount of equipment and piping that requires cooling by accounting for all the auxiliary operations. However, life cycle inventories (LCIs) on water and steam generation, taken from GaBi (Sphera, 2020a) and EcoInvent (Ecoinvent, 2019) databases, may have readily considered the use of a chilling system, which then adds complexity to designing an addition system for the 6-APA process. Since assuming a smaller off-the-shelf glycol unit for fermentation broth cooling would not be representative of actual manufacturing practice, it was omitted from the study. In addition, it was also partly assumed that glycol is a closed loop recycled without line-lost. Hence, the impact of sourcing glycol could be low per functional unit. However, it was understood that the energy requirements for 6-APA manufacture would increase from the overall calculated value if the recirculation of glycol were included.

Similar to the upstream processes, equipment required for downstream processing include buffer preparation and hold tanks. Accompanying operations and materials were required (see Table 4.3) to support the protocols adopted for this average 6-APA production process. The sizing for each tank was calculated based on the volume required and according to engineering principles as set out in Table E.7, like media preparation and fermentation tanks. When the same materials were involved in more than one unit operations, the environmental impacts associated with their sourcing, mixing in their preparation tanks and pumping were allocated based on the mass proportion of each unit operation and processing block use.

Table 4.3: A summary of the functions of each downstream unit operation and input requirements.

Downstream Unit Operation	Function	Process Materials – Notes
Product harvest to tank	To pool fermentation broths from the bioreactor and cool broth to prevent product degradation.	Polyethylene glycol - required in the cooling jacking around the pipes and reactor to assist cooling.
Rotary vacuum filtration	To separate cellular biomass and penicillin	Filter aid – required to coat rotary filter and forms filter cake with biomass.
pH adjust	To pH adjust the fermentation broth prior to solvent extraction, i.e. optimising the process condition of the next step.	Sulphuric acid – as acid addition
Solvent extraction (1)	To extract penicillin from the original fermentation broth to a solvent. The original broth is directed to waste.	Butyl acetate – as the solvent
Back extraction (1)	Extract penicillin back into an aqueous solution, ready for enzyme hydrolysis step.	Phosphate buffer – increases the stability of penicillin G acylase and therefore provide an optimal operating condition for the next operation.
Enzyme hydrolysis	Using immobilised enzyme to cleave penicillin into 6-APA and PAA.	Immobilised enzymes and phosphate buffer
pH adjust	To pH adjust the product stream the prior solvent extraction; i.e. optimising the process condition of the next step.	Sulphuric acid – as acid addition
Solvent extraction (2)	To extract penicillin that did not get hydrolysed into a solvent. 6-APA remaining in the aqueous solution is directed towards the precipitation tank.	Butyl acetate – as the solvent
Back extraction (2)	To extract any 6-APA in the solvent phase back into an aqueous solution.	Sulphuric acid – as the aqueous solution
Precipitation	To precipitate 6-APA out of solution requires adjusting pH to 4.3 and mix for three hours.	Sodium hydroxide – as base addition
Basket centrifugation	To separate the crystallised 6-APA from the process liquid. The spinning motion of the centrifuge will force liquid through the filter whilst trapping the solids forming a filter cake layer.	Methanol and water mixture – use to rinse the wash the filter cake later before a further spin cycle is conducted to dewater the product compartment.
Vacuum Drying	To dewater further the product by heating it inside an agitated pan compartment where a vacuum pump is employed to remove moisture.	N/A (Requires heat energy)
Milling	To make uniform 6-APA crystals.	N/A

4.3.2.2 Utility Requirements (Steam, Water, CIP and HVAC)

As part of the manufacturing process, the production of purified water, water for injection (WFI), process steam, pure steam, cleaning buffer, and the running of the HVAC system in the manufacturing facility were all assumed as necessary. Table 4.4 summarises the purpose of each entity in manufacturing. Both purified water and process steam were involved in the upstream processing blocks (Inoculation and Stirred Tank Fermentation) to prepare and sterilise fermentation media. Their mass requirements were calculated based on the overall amount of media required for each cell culture stage (Table E.6 and Table 4.2). They were also involved with the generation of WFI and pure steam, which were required for cleaning equipment (cleaning in place (CIP) and steaming in place (SIP) processes). The amount of WFI included the amount necessary to generate the correct concentrations of sodium hydroxide and nitric acid, which were assumed as the caustic and the acid cleaning buffers, respectively. The cleaning procedures were adapted from SPX (2013), McNulty (2016) and Vincent (2008), where they discussed the rule of thumbs of cleaning bioprocess equipment. Volumes of WFI and cleaning

buffers were calculated based on the assumed pre-rinse and rinse durations, flow rates that would allow turbulent flow through pipes and tank spray balls, and recirculation volumes (Table E.29). Pure steam requirements were calculated based on the overall vessel volume that required sterilisation, which included all piping. A simplifying approach was taken such that the volume of steam required was 1.5 times the capacity of each piece of equipment. As stated in Table 4.4, HVAC only considered the energy input that allowed air handling units to provide the necessary air changes and ventilation for the manufacturing facility. The calculation took account of equipment size, floor space requirement by employees and the assumed ISO room classification for each processing block (Table E.30).

Table 4.4: Functionality of each utility component and the assumed process modelled in this life cycle assessment study.

Utilities	Function	Assumed Process
Purified water	Used for fermentation media preparation and WFI generation.	Generic process for reverse osmosis and deionised water production from the GaBi database (Sphera, 2020a).
Water for injection (WFI)	For buffer preparation (process and cleaning buffers) and as part of the cleaning in place (CIP) of equipment.	As suggested by Veolia Water (2007), WFI is produced via the multi-effect water distillation process. The input requirements for such a process, which would meet the WFI demand, were taken from a technical data sheet by Steris® (2010a).
Process steam	For heat sterilisation of fermentation media and pure steam generation.	Generic process for process steam generation from 95% natural gas from the GaBi database (Sphera, 2020a)*.
Pure steam	For the steaming in place (SIP) of equipment.	Guidance from Veolia Water (2007) also showed that pure steam is generated in conjunction with WFI using the multi-effect process. The input requirements for the pure steam production process were taken from a technical data sheet by Steris® (2010b).
Cleaning buffers (caustic and acid)	For the CIP of equipment.	Cradle-to-gate LCI for sodium hydroxide and nitric acid used from the GaBi database (Sphera, 2020a).
HVAC system	To ensure health and safety and containment requirements are met when producing 6-APA.	Only the amount of energy required to provide the appropriate air handling and ventilation was calculated. The calculation involved the sizing and assuming the average air changes per hour of the production spaces (see Appendix E -Table E.30).
* The process steam operation was initially assumed based on steam temperature, pressure and flow rate requirements for sterilisation and pure steam production; the values were inputted into the boiler and deaerator calculator provided by the US Department of Energy (2015), which provided the energy requirements for this process (Appendix E - Figures E.2 and E.3). This simplified process was modelled on GaBi Software and was compared with process steam generation processes provided within the GaBi database, and the environmental impacts were found to be similar.		

4.3.2.3 Waste Treatment

Figure 4.5 shows the waste treatment processes. It includes the deactivation of solid waste from the rotary vacuum filtration unit operations, solvent (butyl acetate) recovery, phenylacetic acid (PAA) recovery and wastewater treatment. Solid waste from the filtration step comprises biomass and filter aid. They were assumed heat deactivated in drum dryers (Table E.31) before being directed off-site to anaerobic digestion. The flow rate of the solvent stream towards the vacuum stripping column for butyl acetate recovery was assumed to be the same as the operating flow rates used in solvent extraction. The flow rates of process steam and cooling

water that would allow the evaporation and condensing of butyl acetate were assumed based on typical piping sizing and linear flow rate for both materials. This allowed the calculation of the quantity of water and steam required for solvent recovery (Table E.32). The PAA recovery process followed the protocol provided by Zou and Tao (2011), which require the settling and purging of known materials within the mixture (Table E.33). Lastly, a typical wastewater treatment procedure was adopted from Singh et al. (2016). Equipment was sized according to the daily throughput of waste, which considered the retention times in each settling and aeration tank (Table E.34).

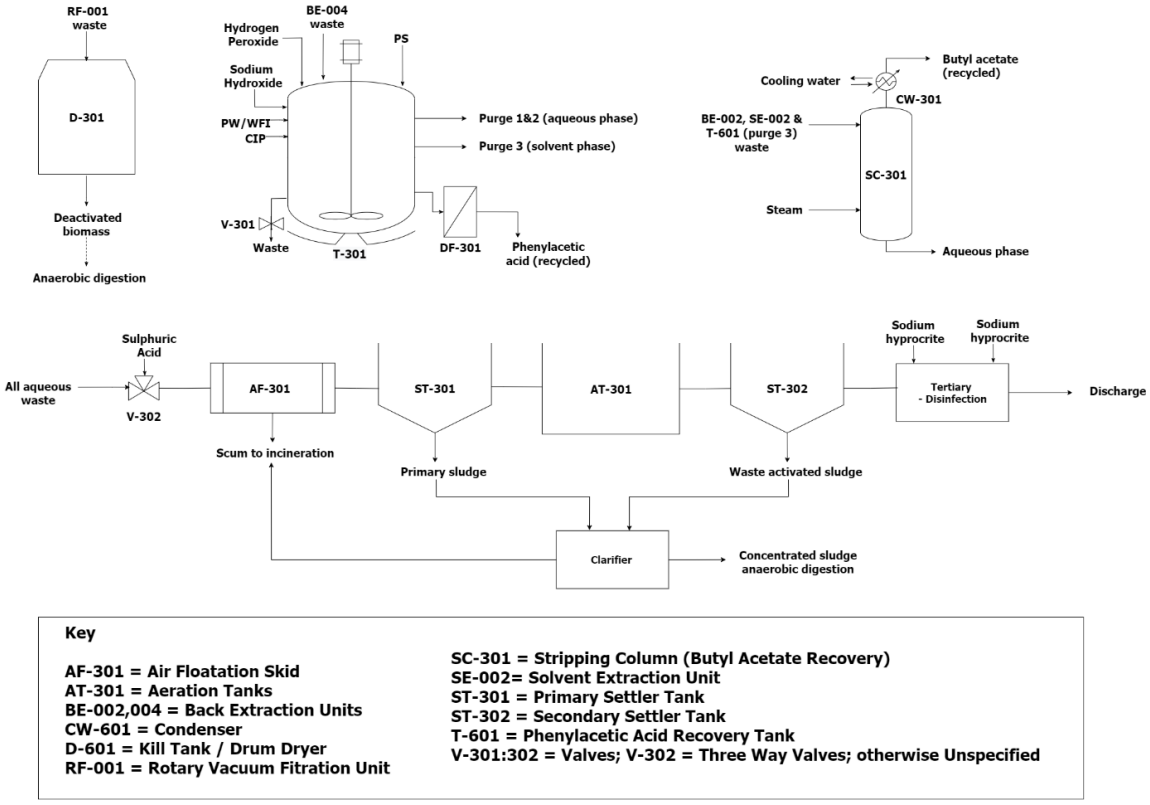

Figure 4.5: Waste treatment processes assumed for the 6-APA manufacturing facility.

4.3.2.4 Inventory Databases and Material Sourcing Assumptions

Life cycle inventories (LCI) of materials associated with the manufacture of 6-APA were obtained from GaBi (Sphera, 2020b) and Ecoinvent (Ecoinvent, 2019) databases to formulate the cradle-to-gate LCA model. Where LCIs of materials were not present in the databases, for instance, phenylacetic acid (PAA) and pluronic (surfactant), generic inventories “market for chemicals, organic” and “market for chemicals, inorganic” were used as substitutes, respectively. Where possible, the LCIs employed were location-specific (national LCIs); otherwise, regional or global LCIs were used. This means that LCIs that represented the average emissions in the specific country were primarily chosen (based on the geographic code, in this case [US], noted as part of the LCI within the database. If national LCIs were unavailable, LCIs with regional geographic

code, in this case [RNA] (denoting North America) were chosen, before LCIs with the global code, [GLO] (denoting global) were used.

The supply phase and end-of-life phase of the LCA study were modelled around the theoretical location of the manufacturing plant (the US), and the national practices in the supply of resources were assumed. It was assumed that materials required for product manufacture were supplied and disposed of locally, but the supply of equipment was supplied from China. The closest supplier for specific materials and equipment were chosen to obtain transport distances (See Appendix F).

4.3.2.5 Additional Life Cycle Inventory Metrics

As per Chapter 3, PMI and E-factor were particularly calculated and compared to literature values to sense-check the LCA carried out.

4.3.3 Life Cycle Impact Assessment (LCIA)

4.3.3.1 Software and LCIA methodologies

The LCA software, which contained the life cycle impact assessment (LCIA) calculation methods used for this analysis, was GaBi (Sphera, 2020b). A series of processes were made to represent each unit operation; input and output flows were parameterised to allow the overall LCA model to recalculate the mass and energy flows and allow input parameters to be modified. Incorporating the relationships between variables (i.e. equations used for their calculations) allowed the verification of the calculations made in Microsoft Excel (Microsoft Corporation, 2021) and enabled sensitivity analysis of the whole LCA model.

The LCIA methods used to analyse the 6-APA process were as described in Chapter 3. This included the recommended impact analyses and the methods required for sensitivity analysis (Section 3.3.4).

4.3.3.2 Normalisation

For understanding whether the environmental impacts associated with 6-APA production were significant, the values per year (function unit (FU)) were normalised by the global per capita emissions values per year (Table 4.5). Global per capita emissions are total anthropogenic emissions divided amongst the global population. These values were recommended normalisation factors provided by the Joint Research Centre (JRC) who compile emissions data from various sources to estimate the total environmental impacts within the referenced year (Sala and Crenna, 2017). The compiled data were reported originally by environmental agencies, who routinely collect environmental data from all sectors. Due to activity differences in each region, emissions allocable to each person will differ around the world if regional estimates are

used. Country-specific per capita emissions can also be used to normalise LCIA environmental impact results, but since this project study aimed to assess the global impacts (Chapter 5), global per capita emissions were chosen as the reference values for normalisation. This choice allowed a consistent normalisation method to be used for each production location assessed in Chapter 5. Global carrying capacities were considered, but the normalisation factors are yet to be developed for a few of the LCIA calculation methods employed in this study.

*Table 4.5: Normalisation factors used in this case study. Reference values were obtained from literature, mainly from Sala and Crenna (2017). Otherwise, *Goedkoop et al. (2015) and **Laurent et al. (2011) were used. Both Sala and Crenna (2017) and Goedkoop et al. (2015) provided global emission values; however, Laurent et al. (2011) provided European emission values. Normalisation factors were not available for total freshwater consumption and impact on water resources due to water consumption.*

Environmental Impact Category [Units]	Global Per Capita Emission Per Year
Acidification - ILCD/PEF (v1.09) [Mole of H+ eq.]	5.55E+01
Ecotoxicity (Freshwater) - USEtox 2.1 [CTUe]	8.74E+03
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	2.46E+00*
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	5.93E+00*
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H) [kg P eq.]	7.34E-01
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H) [kg N eq.]	2.83E+01*
Eutrophication (Terrestrial) - ILCD/PEF (v1.09) [Mole of N eq.]	1.77E+02*
Global Warming Potential, excl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	-
Global Warming Potential, incl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	8.40E+03
Human Toxicity, cancer - USEtox 2.1 [CTUh]	3.85E-05
Human Toxicity, non-cancer - USEtox 2.1 [CTUh]	4.75E-04
Ionising Radiation, human health -ILCD PEF (v1.09) [kBq U235 eq.]	4.22E+03
Ozone Depletion - ILCD PEF (v1.09) [kg CFC-11 eq.]	2.34E-02
Photochemical Ozone Formation, human health - EDIP 2003 [pers*ppm*hours]	2.84E+00**
Photochemical Ozone Formation, vegetation - EDIP 2003 [m2 UES*ppm*hours]	5.97E+04**
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09) [kg Sb eq.]	6.36E-02
Total Freshwater Consumption (including rainwater) [kg]	-
Water resources - UBP 2013 [UBP]	-

By normalising the annual LCIA results, environmental impact values were presented as people equivalence. This highlighted the magnitude by which the 6-APA production process contributes to each environmental impact category. Where people equivalences were found significant, the focus was placed on diagnosing the areas of manufacturing that contribute most to those specific impact categories. This was carried out by hot-spot analysis detailed in the next section.

4.3.4 Analysis of Results - Interpretation

4.3.4.1 Hot-spot analysis

A hot-spot analysis is intended to find areas of high environmental impact. Every impact category, especially those found to be significant, was analysed to find the highest contributors. To obtain an overview of the environmental hot-spots of the 6-APA manufacturing system,

environmental impact values were first allocated to their respective life cycle phases – supply, use and end-of-life (EoL) phases. By calculating the contribution of each phase towards each impact category, results showed which phase had the highest environmental burden. The values were then reallocated to the processing blocks that they were associated with, either Inoculation, Stirred Tank Fermentation, Harvest, Conversion, Purification, Conditioning or Waste Treatment. Once impact values were allocated, the percentage impact contributions of each phase and processing block were calculated and revealed which processing block contributed most towards each category.

Hot-spot analysis on the LCI results was carried out also to understand the resource intensity (i.e. the use of process materials, water, electricity and steam) of each processing block. Summarising the input requirements of each resource within each block allowed a more specific diagnosis of the areas associated with high environmental impact. It helped explain the reasoning behind the order of contribution (ranking) of the processing blocks towards each impact category.

4.3.4.2 Sensitivity Analysis

To check the reliability and robustness of this LCA on 6-APA production, sensitivity analysis was carried out by varying parameters by either their uncertainty range or 1% (arbitrary). Since parameters used to model 6-APA production were obtained by analysing literature, where there was more than one piece of literature, it presented a range of values for various parameters. For example, HVAC requirements were calculated based on room sizing, which was dependant on the minimum floor space required per full-time employee (FTE) and the number of FTE (Table E.30). The uncertainty range for room sizing was achieved by increasing and decreasing the number of employees required for each process, which was approximately ± 25%. Equally, flow rates and equipment lifespan appears to vary depending on the literature; uncertainty ranges of ±5% and ±10% were approximated, respectively. Table 4.6 further highlight the uncertainty ranges tested for each cleaning parameter. It was noted that upstream input calculations were determined by the overall fermentation product titre, which subsequently determined the manufacturing configuration, batch number and downstream processing needs, and for this reason, sensitivity analysis was not carried out on upstream parameters. Instead, the effect of changing product titres was explored as part of scenario analysis (below). Sensitivity analysis focused mainly on downstream processing yields and the input ratios of buffer and solvents, which vary in accordance with the product concentration in the feed. The confidence level of parameters was classed as adequate as they were obtained mainly from the literature. The percentage variation tested on downstream processing yields and input ratios were ±1%. This

assisted with checking for the model's completeness and enabled an assessment of how the change of each parameter affects each environmental impact category.

Table 4.6: Sensitivity test parameters for cleaning-in-place and steaming-in-place.

	Percentage Variation	
CIP Requirements	**Lower Bound**	**Upper Bound**
Acid Volume	-20%	5%
Caustic Volume	-20%	5%
Pre-Rinse Volume	-12%	25%
Steam Mass	-20%	5%
WFI Volume	-12%	25%

As described in Chapter 3, LCIA results deriving from alternative methods, CML, ReCipe and TRACI, should be used to compare against results derived from the chosen LCIA methodologies. The comparison was carried out by first analysing the percentage contribution of each processing block toward each environmental impact category for each LCIA method individually before comparing the rankings (from highest contributing block to lowest) derived from the different methods. This assisted with checking whether the areas of high environmental impact diagnosed with the chosen impact assessment methodologies were independent of LCIA methodology packages.

4.3.4.3 Scenario Analyses

Scenario analyses were carried out by allowing deviations in the product titre and production scale to understand the environmental effects of these process design parameters. Conventionally, companies must design a production process that can support the demand for the product and find the right balance between the product titre provided by the chosen cell line and the scale at which the product is produced to ensure it is technically and economically viable. Hence, the two design parameters are indicative of the market share that a company may aim to penetrate. It was hypothesised that assessing the change in impacts would, by extension, enable insights into how company decisions can affect the environmental impacts associated with a product. See Table 4.7 for the scenarios that were compared to the base-case scenario.

First, two scenarios were formulated and compared with the base case scenario, where 57 g/L product titre was assumed; these were 35g/L (Scenario 1) and 100g/L (Scenario 2). It was assumed that each batch would continue to be produced using the 200m^3 and 100m^3 fermenters as with the base case scenario. Since the base case scenario, the media composition took account of stoichiometry calculations for how much glucose, ammonium sulphate and phenylacetic acid (PAA) were required to generate the assumed product titre; media compositions were recalculated to meet the new demands (See Table 4.8). Note that some

components were left unchanged because they were the base requirements for sustaining the viability of cells and did not contribute to the production of 6-APA.

Table 4.7: A summary of scenarios analysed and compared to the base-case. The base-case scenario was compared with Analysis 1 and then Analysis 2 scenarios separately to under the effect of each design parameter, product tire and production scale on a standalone basis.

	Scenario	Product Titre (g/L)	Production Fermentation Capacity (m^3)	Batches per year
	Base-case	**57**	**300**	**195**
Analysis 1	**1**	35	300	317
	2	100	300	111
Analysis 2	**3**	57	150	195
	4	57	75	195
	5	57	15	195
	6	57	3	195

Table 4.8: Fermentation media composition of the assumed product titre.

Fermentation Parameters	Base Case Scenario	Scenario 1	Scenario 2
Product Titre (g/L)	57	35	100
Glucose Concentration in Media (g/L)	214	185	271
PAA Concentration in Media (g/L)	14.7	9	25.6
Ammonium Sulphate Concentration in Media (g/L)	32.4	23	50.7
Fermentation Media Density (kg/L)	1.27	1.23	1.36
Corn Steep Liquor Concentration in Media (g/L)	25	25	25
Calcium Source Concentration in Media (g/L)	0.12	0.12	0.12
Pluronic Concentration in Media (g/L)	0.4	0.4	0.4

Changes in the fermentation media composition meant that the density of the media into the fermentation process was changed, which brought subsequent changes to the manufacturing process. This included pumping requirements and power consumptions due to mixing within fermentation bioreactors. Since the GaBi-built LCA model on 6-APA production was created such that inputs and outputs were parameterised, by manually updating parameters that contributed to the overall media density, GaBi automatically updated other parameters as a consequence of these changes. Note that pumping and power consumption requirements were built as a function of process liquid density and other parameters; fermentation media density was also built as a function of the different media components. See Appendix G for extracts of the LCA model built in the GaBi software.

Aside from stirring and pumping power requirements, downstream processing was assumed to remain the same across all scenarios. This assumption was permitted because product concentration was not typically used to calculate input materials for these processes. For example, within the production harvest processing block, filter aid and buffer requirements for

the rotary vacuum filtration unit operation depended on cell mass which was assumed the same. In addition, in the solvent extraction unit operation, butyl acetate requirements were calculated by applying a volumetric ratio between the fermentation broth and the solvent, which was also assumed to remain the same for both scenarios. It was noted that enzyme requirement might change due to changes in the total mass of penicillin produced. However, because enzymes were assumed immobilised and have a lifespan between 2000 to 4000 hours, their inventory and impacts allocable to both functional units (1 kg produced and one year of production) become negligible. For this reason, enzyme quantities were not recalculated for scenario analysis.

The analysis on the effect of product titre was first carried out by comparing per batch emissions. This allowed the evaluation of how sensitive the LCA model was to this design parameter. Secondly, the emissions associated with the production of 2000 tonnes of 6-APA per year were compared to the base case scenario. The number of batches required within the plant was recalculated to model the production to this functional unit (2000 tonnes/year). Since equipment supply was not a high contributor to any environmental impact categories, it was assumed that the effects of changing equipment requirements on the overall impact scores would be negligible. The number of batches that were assumed was 195 (base case), 317 (Scenario 1) and 111 (Scenario 2).

A separate scenario analysis was carried out next for a range of production scale. The production per year scenarios that were explored were 2000 tonnes (base case), 1000 tonnes (Scenario 3), 500 tonnes (Scenario 4), 100 tonnes (Scenario 5) and 20 tonnes (Scenario 6). The number of batches was assumed the same across the scenarios, and equipment was resized to reflect the changes. For instance, 300m^3 total fermentation bioreactor capacity was used for the base case scenario; this translated to using 150m^3, 75m^3, 15m^3 and 3m^3 fermentation bioreactor capacity for each of the other scenarios (Scenarios 4 to 6), respectively.

A complete list of parameters changed due to the change of production scale is listed in Appendix J – Table J.1. The change in production scales meant that all equipment sizes were altered, and power consumption levels were changed as a result. The CIP requirements and total processing volumes were also altered. Equipment sizing for downstream processing unit operations was sized based on off-the-shelf equipment presented in equipment specification catalogues. As equipment was specified to operate under a range of throughputs, the same sizing of equipment was used across two scenarios depending on the unit operation.

4.4 RESULTS

4.4.1 Life Cycle Inventory Results

Table 4.9 summarises the life cycle inventory (LCI) results for each processing block in the format as suggested in Chapter 3. It highlights that fermentation in bioreactors required the most resources. This was attributed to the number of fermentation stages, the running time of the reactors, which determined the energy usage of each step; and, feeding requirements that allow cells to produce the about of penicillin required. Conversely, due to the small scales at which the Inoculation processing block operates, it required the least resources over one year. The E factor excluding water generated, 65.2 $kg_{waste}/kg_{product}$ sits within the range suggested for pharmaceuticals (Sheldon, 2017). On the other hand, the PMI (incl. process water) value generated, 617 $kg_{inputs}/kg_{product}$, is three times higher than the quoted median PMI for a pharmaceutical (168 kg/kg) (Roschangar et al., 2015). However, when compared to another fermentation-derived pharmaceutical, monoclonal antibodies - PMI (incl. water and consumerables) ranged 3000 to > 20,000 $kg_{inputs}/kg_{product}$ (Budzinski et al., 2019)), the value generated for 6-APA is significantly lower.

Table 4.9: Material and utility usage per processing block. Values are expressed per year of production and per kilogram of 6-APA produced. Note: Only direct usage is summarised in the table; electricity, water and steam usage due to HVAC requirements, water purification and pure steam generation are not allocated. Green = the lowest unit use of resources; red = the highest unit use of resources. Grey = not applicable.

	Processing Blocks							
Process Parameters	**Inoculation**	**Production Fermentation**	**Product Harvest**	**Product Conversion**	**Purification /Polishing**	**Product Conditioning**	**Waste Treatment**	**Total**
Total Input Materials								
(kt/yr.)	4.09	26.9	13.7	3.27	4.76	0.145	0.152	49
PMI* (kg/kg)	0.0254	13.4	6.83	1.63	2.37	0.07222	0.0757	24.4 (617)**
Total Water Usage								
(kt/yr.)	3.50	453	348	243	127	10.6	9.01	1,190
(kg/kg)	1.74	226	173	121	63.3	5.28	4.49	593
Total Electricity Usage								
(GWh/yr.)	0.163	21.1	52.0	5.10	3.26	0.758	1.49	83.9
(kWh/kg)	0.0812	10.5	25.9	2.54	1.62	0.378	0.742	41.8
Total Steam Usage								
(TJ/yr.)	3.57	388	232	139	83.4	5.89	54.9	907
(MJ/kg)	1.78	193	116	69.3	41.6	2.93	27.4	452
Waste Generation†								
(kt/yr.)								1210 / 131
E factor (kg/kg)								602 / 65.2

*PMI = process mass intensity - excludes water use, ** includes water use
† including water released post-wastewater treatment/excluding water released post-wastewater treatment.

4.4.2 Life Cycle Impact Assessment Results

The overall environmental impact results and their emission equivalence for producing 6-APA in a US manufacturing site are presented in Table 4.10. Results presented in this table form a benchmark for subsequent analyses (namely scenario analyses (Section 4.4.4) and the comparative study in Chapter 5. The normalised results suggested the significance of impacts attributed to 6-APA production in relation to the emissions generated annually and globally,

which were allocated amongst the global population. Ultimately, people equivalence indicated the proportion of environmental impact that our global society annually emits that can be attributed to 6-APA production. The results showed that the most significant results were ecotoxicity – terrestrial and ecotoxicity – freshwater, where emissions represented > 0.03% of global emissions (equivalent to emissions by over 2.5 million people). The next highest results were human toxicity – cancer and ecotoxicity – marine, where emissions represented > 0.002% and equated to over 120,000 people emissions. All other impacts represented <0.001%, with ozone depletion generating the lowest result. See Section 4.5 Discussion for the significance of each environmental impact results.

*Table 4.10: Life cycle impact assessment (LCIA) results for the production of 6-APA in the US. The table shows the environmental impact per functional unit – per $kg_{product}$, per year of production (2000 tonnes/yr.) and per year values normalised by factors highlighted in Table 4.5. *Global population assumed: 7.63 billion – 2018 value provided by* (Worldometer, 2020).

Environmental Impact Category	Per $kg_{product}$	Per Year	People Equivalencepe q./yr.	Percent of Global Population * (%)
Acidification ILCD/PEF (v1.09) [Mole of H+ eq.]	1.65E-01	3.31E+05	5.96E+3	<0.001
Ecotoxicity – freshwater USEtox 2.1 [CTUe]	1.14E+04	2.29E+10	2.62E+6	0.034
Ecotoxicity – marine ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	1.49E-01	2.98E+05	1.21E+5	0.002
Ecotoxicity – terrestrial ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	1.15E+01	2.32E+07	3.91E+6	0.051
Eutrophication – freshwater ReCiPe 2016 v1.1 (H) [kg P eq.]	2.51E-03	5.04E+03	6.87E+3	<0.001
Eutrophication – marine ReCiPe 2016 v1.1 (H) [kg N eq.]	5.88E-03	1.18E+04	4.16E+2	<0.001
Eutrophication – terrestrial ILCD/PEF (v1.09) [Mole of N eq.]	3.46E-01	6.94E+05	3.92E+3	<0.001
Global warming potential, excl. biogenic carbon IPCC AR5 [kg CO2 eq.]	8.01E+01	1.61E+08	-	-
Global warming potential, incl. biogenic carbon IPCC AR5 [kg CO2 eq.]	7.39E+01	1.48E+08	1.76E+4	<0.001
Human toxicity – cancer USEtox 2.1 [CTUh]	6.89E-06	1.38E+01	3.58E+5	0.005
Human toxicity – non-cancer USEtox 2.1 [CTUh]	3.95E-06	7.93E+00	1.67E+4	<0.001
Ionising radiation – human health ILCD PEF (v1.09) [kBq U235 eq.]	3.00E+00	6.02E+06	1.43E+3	<0.001
Ozone depletion ILCD PEF (v1.09) [kg CFC-11 eq.]	9.24E-07	1.85E+00	7.91E+1	<0.001
Photochemical ozone formation – human health EDIP 2003 [pers*ppm*hours]	1.83E-02	3.67E+04	1.29E+4	<0.001
Photochemical Ozone Formation – vegetation EDIP 2003 [m2 UES*ppm*hours]	2.50E+02	5.02E+08	8.41E+3	<0.001
Resource Depletion, mineral, fossils and renewables ILCD PEF (v1.09) [kg Sb eq.]	2.61E-04	5.25E+02	8.25E+3	<0.001
Total freshwater consumption (including rainwater) [kg]	3.99E+03	8.01E+09	-	-
Impact on water resources (water scarcity) UBP 2013 [UBP]	3.24E+02	6.51E+08	-	-

4.4.3 Hot-spot Analysis Results

Hot-spot analysis was conducted on each environmental impact category by first allocating impact values to individual analysis groups as specified in Chapter 3. Figures 4.6 and 4.7 illustrates the impact contributions of each life cycle phase and each processing block, respectively. Results showed that environmental impacts derived mainly from the supply phase, which comprised raw material and equipment sourcing activities, for most impact categories. Only the results for two impact categories, ecotoxicity – marine and global warming potential (incl. biogenic carbon), were derived mainly from the use phase (63.9% and 50.7%, respectively). The end-of-life phase, which considered only the decommissioning of equipment and waste-treatment processes outside the manufacturing facility, contributed <1.5% of total emissions.

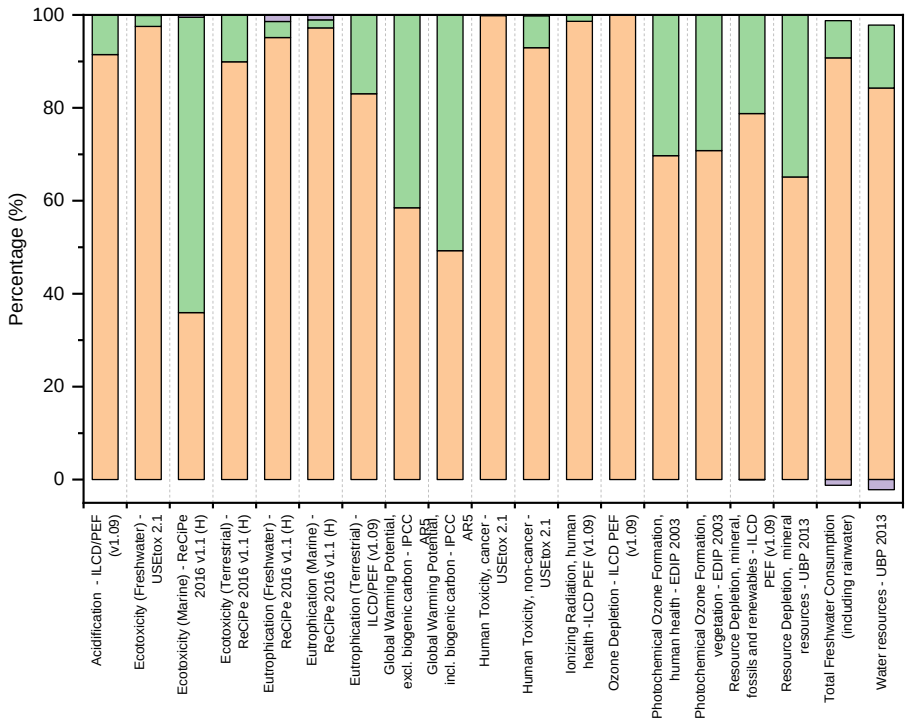

Figure 4.6: Hot-spot analysis of 6-APA production in the US - phase contribution. The figure illustrates the percentage contribution each life cycle stage has towards each environmental impact category. *Supply Phase.* *Use Phase.* *End-of-Life Phase*

Figure 4.7 shows that the Stirred Tank Fermentation and Product Harvest stages were the processing blocks that contributed the most towards each environmental impact category. These two blocks combined generated >67% of all impacts. Inoculation and Product Conditioning formed the least impactful processing blocks where their contributions toward each impact category were <1.6%. These results coincided with LCI results, where the order in which the blocks ranked, in terms of their resource requirement levels, was also the order in which they ranked in their contribution towards most impact categories. Stirred Tank Fermentation required the most resources, i.e. raw materials, steam and water, and generated the highest impact in 14 out of the 19 impact categories analysed. It can be inferred that due to

the large amounts of resources necessary for fermentation compared to other blocks (Table 4.8), it has generated the most impact. The Product Harvest processing block, which considered the holding of fermentation broth in the product harvest tank, rotary vacuum filtration and the first solvent extraction process, generated the highest impact in the other five impact categories. The five impact categories were ecotoxicity – freshwater, ecotoxicity – terrestrial, ionising radiation, ozone depletion and resource depletion – mineral, fossils and renewables. With further analysis, this was found to be attributed to the use of butyl acetate in solvent extraction and the block's overall energy requirements.

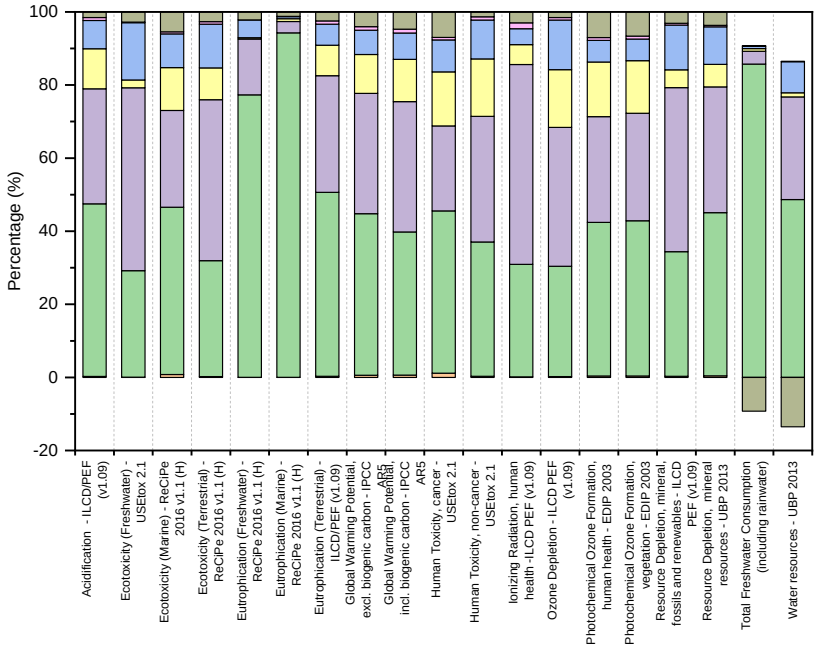

Figure 4.7: Hot-spot Analysis of 6-APA Production in the US - process block contribution. The figure illustrates the percentage contribution of each processing block has towards each environmental impact category. Inoculation. Stirred Tank Fermentation. Product Harvest. Product Conversion. Product Purification. Product Conditioning.

Further hot-spot analyses were conducted separately on each impact category by allocating impacts into the processing blocks first. Impacts were then allocated into the process inputs that they derived from. By doing so, the hot spots were narrowed further from understanding which block generated the highest impacts. Table 4.11 presents the contribution breakdown (%) for global warming potential (GWP), including biogenic carbon, as an example of the analyses carried out. For the breakdown on contribution for all other impacts, see Appendix H. In the table, row total presents the percentage contributions of each processing block toward the particular impact category, in this case, GWP, including biogenic carbon. Similarly, column total presents the percentage contributions of the impacts associated with each input category.

Table 4.11 shows that the highest contributing factors towards GWP were steam and electricity generation in general. Since steam was required for the sterilisation of equipment, all processing

blocks has contributed to this impact. However, steam was also required for media sterilisation in the Stirred Tank Fermentation block, and hence, carbon emissions associated with steam, which mainly derived from the burning of natural gas, were attributed mostly to this block. All unit operations also required electricity to function, but most impacts were allocated to the Product Harvest block. This was mainly due to the harvesting of fermentation broth required to be carried out over a longer period than all other unit operations and therefore required a higher amount of electricity to operate.

Table 4.11: Contribution breakdown within each processing block towards global warming potential, including biogenic carbon. Red = highest contributing elements towards this impact category. (Appendix I for the breakdowns for all other environmental impact categories).

	Equipment	Materials - Production	Water	Steam	Material - Cleaning	Electricity	Row Total
Initial Inoculation/Seed	0.00%	0.00%	0.00%	0.53%	0.04%	0.06%	**0.63%**
Stirred Tank Fermentation	0.00%	0.43%	5.91%	19.8%	4.57%	8.47%	**39.2%**
Product Harvest & Cell Removal	0.00%	1.74%	0.05%	10.0%	4.17%	19.7%	**35.7%**
Product Conversion	0.00%	0.01%	0.03%	6.69%	2.79%	2.02%	**11.5%**
Product Purification	0.00%	0.57%	0.02%	3.81%	1.57%	1.25%	**7.22%**
Product Conditioning	0.00%	0.00%	0.00%	0.30%	0.12%	0.61%	**1.04%**
WASTE	0.00%	0.11%	0.42%	3.15%	0.00%	1.05%	**4.73%**
Column Total	**0.00%**	**2.86%**	**6.42%**	**44.3%**	**13.27%**	**33.2%**	**100%**

From analysing all other environmental impact categories, it was found that five key inputs consistently generated high impacts across the different type of impacts. They were glucose, used as part of the media makeup in fermentation; sodium hydroxide, used as the caustic cleaning agent; electricity from fossil fuels; butyl acetate, required for solvent extract processes; and steam produced from natural gas. Table 4.12 summarises the impact contributions that exceeded 15% of the total impact scores.

The utilisation of steam was found to be most impactful in five impact categories: ecotoxicity – marine, GWP (excl and incl. biogenic carbon), human toxicity – cancer and resource depletion. It has also generated non-negligible impacts towards four additional categories: acidification, eutrophication – terrestrial and photochemical ozone formation (POF) (human health and vegetation). As previously stated, the overall GWP score was derived mainly from the carbon emissions arising from the burning of fossil fuels to generate electrical and steam energy. For other impacts, the analysis showed that the high contribution of steam could be attributed to the supply of the natural gas mix, which included natural gas production (from different countries) and transport to distribution points where the different supplies were mixed. These activities could be presumed areas where emissions were generated. Similarly, the other impacts that the supply of electricity was associated most with were eutrophication – terrestrial and POF (human health and vegetation), which could be attributed to the extraction and/or refining of hard coal and natural gas.

Sodium hydroxide, a cleaning agent used across all processing blocks (mostly in the Stirred Tank Fermentation block), was found to generate the highest impact in four impact categories whilst also generating non-negligible impacts towards an additional three categories (Table 4.12). Sodium hydroxide is conventionally co-produced with chlorine by the electrolysis of sodium chloride from rock salt or saturated brine; the process can emit chlorine gas, contributing to ozone depletion. This could explain the material's high contribution to this impact category. The gas could also mix with water to form a weak acid and induce an acidification effect. The purification of saturated brine before electrolysis and metals in the electrolysis is also linked to the release of substances with ecotoxicity and human toxicity effects.

*Table 4.12: Top contributors toward each environmental impact categories. ✓(%) indicates that the input generated >=20% of the total impact. (%) are the next highest contributors that bring the percentage contributions of top contributors to >60%. Red = the highest contributing element. *Organic chemicals refer to materials used in fermentation, which includes phenylacetic acid and pluronic.*

Impact Category	Butyl Acetate	Electricity (from fossil fuels)	Glucose	Sodium Hydroxide	Steam (from natural gas)	Other
Acidification			✓24.8%	✓39.4%	✓ 20.9%	
Ecotoxicity (Freshwater)	✓49.7%					(12.3% organic chemicals*)
Ecotoxicity (Marine)					✓61.1%	
Ecotoxicity (Terrestrial)	✓22.6%	18.8%		✓29.1%		
Eutrophication (Freshwater)	14.7%		✓65.9%			
Eutrophication (Marine)			✓91.3%			
Eutrophication (Terrestrial)		✓32.5%	✓20.7%	18.1%	15.9%	
Global Warming Potential, excl. biogenic carbon		✓30.6%			✓40.9%	
Global Warming Potential, incl. biogenic carbon		✓33.2%			✓44.3%	
Human Toxicity, cancer					✓98.6%	
Human Toxicity, non-cancer				✓65.6%		
Ionising Radiation, human health						✓85.7% electricity from nuclear
Ozone Depletion	16.6%			✓74.9%		
Photochemical Ozone Formation, human health		✓26.1%	14.9%	19.2%	✓25.3%	
Photochemical Ozone Formation, vegetation		✓26.8%	15.1%	18.5%	✓25.0%	
Resource Depletion, mineral, fossils and renewables	19.5%				✓21.4%	15.8% organic chemicals* (11.3% (electricity from nuclear)
Total Freshwater Consumption			✓85.9%			
Water Scarcity	✓43.0%		✓35.9%			

Unlike the previous three inputs, glucose and butyl acetate were specifically used for a specific process – fermentation (Stirred Tank Fermentation) and solvent extraction (Product Harvest and Product Purification), respectively. The starch hydrolysis process used to manufacture glucose

required a large amount of water; as shown in Table 4.12, glucose contributed highly to total freshwater consumption. Through the glucose hydrolysis process, other products that are typically produced include oligomers, maltose, fructose, 5-hydroxymethylfurfural and furfural. These nutrients can be emitted as a result of their production process and have eutrophication effects (impacts that glucose contribute most towards). Butyl acetate is conventionally produced via the esterification and reactive distillation of butanol and acetic acid. The removal of water by vaporisation during the distillation process can explain the input's high contribution towards the water scarcity impact category, i.e. impact on water resources. However, since water scarcity considers both the consumption of water and the scarcity of water at the source location, it could suggest that the extraction and generation of butanol and acetic acid require water consumption in a scarce location. Butyl acetate was also the highest contributor of ecotoxicity – freshwater, the chemical itself, butanol and acetic acid are substances with some levels of ecotoxicity effect when released to the environment. During the production process, these substances are likely to be emitted as residues in the waste stream, contributing to the impact score.

4.4.4 Sensitivity Analyses Results

4.4.4.1 Model Sensitivity to Parameters

Tables 4.13 and 4.14 summarised the largest deviations from the base-case impact values when parameters were varied by their assumed uncertainty ranges. The sensitivity analysis showed that varying CIP and SIP parameters had the greatest effect across all environmental impacts. While overall steam mass and pre-rinse volume resulted in only small changes to each of the environmental impact categories (up to ±0.09% change), varying WFI volume resulted in changes of up to ±2.92% (human toxicity – cancer). When acid and caustic volumes were varied, human toxicity - cancer varied ±6.48%, and ozone depletion changed up to ±14.1%, respectively (Appendix I – Tables I.2 and I.3).

Changing the caustic requirements for cleaning had a notable effect across all of the environmental impact values. The highest potential percentage changes were shown to be ozone depletion, human toxicity - non-cancer (±9.68%) and acidification (±7.50%). All other impacts gave potential changes of up to ±6% (average change ±4.49%). However, since the variation of caustic volume was -20% and +5%, the percentage changes in impact scores were expected. Nevertheless, the overall magnitude of each environmental impact category remained the same.

Similarly, the use of nitric acid as acid wash within CIP was varied by ±20%. This resulted in a change of up to ±5% to each impact category. Since the parameter used to calculate the overall volume of caustic and acid solutions was the same (recirculation volume to vessel volume ratio), the potential environmental impact deviations for them should be summated to reflect the potential changes to environmental impacts arising from a single decision. With this, changes between ±0.93% and ±14.2% to each impact scores were seen. Although a maximum change of -14.2% would see environmental impacts remain in the same order of magnitude, it is arguably a significant change. However, the -20% volume uncertainty was set base on a small scale CIP protocol (Verghese, 2003). It was deemed unlikely to use 20% less caustic during CIP. Hence, maximum change ought to be lower.

By varying each downstream process yield and input ratio by ± 1%, the highest environmental impact change observed was ±0.55% towards freshwater ecotoxicity. A thorough analysis of results was conducted to understand how certain parameters affect the overall process' environmental profile. See Appendix I – for all sensitivity results.

The impact categories shown to be the most sensitive to downstream processing parameters were freshwater ecotoxicity and resource depletion. It was found that they were most sensitive to the input ratio between solvent and aqueous phase, assumed for the solvent extraction process; and, the liquid yield (the percentage of liquid (fermentation broth) that proceeds to the next unit operation), assumed for the rotary vacuum filtration process. Both processes occurred in the Product Harvest block; the rotary vacuum filtration stage removed cells from the broth before it is passed to the solvent extraction units where penicillin was transferred into the solvent phase (butyl acetate). Both parameters directly affected the amount of solvent required in the solvent extraction process. As the cells formed a cake on the rotary filter, a small percentage of liquid was assumed trapped in filter cake – using the liquid yield parameter. Changes in this percentage altered the volume proceeding to solvent extraction and, since the amount of solvent required was calculated based on an input ratio, the amount of solvent required for penicillin extraction was also altered. As shown in the hot-spot analysis, butyl acetate contributed greatly to multiple environmental impact categories, particularly ecotoxicity – freshwater; sensitivity analysis results reinforced the correlation between the use of this solvent and the ecotoxicity impact scores.

It must be noted that Rai (2012) did present that the solvent to aqueous ratio can be between 5 and 7:1. This study assumed the latter ratio, which means that the LCA of 6-APA evaluates the worst-case scenario for butyl acetate use. If the assumed ratio is reduced to 5:1 (by 28.6%), the impact of ecotoxicity – freshwater would decrease by 15.4%. Equally, all other impacts would

decrease 28.6 times the percentage deviation presented in Table I.1, which resulted in < 11% reductions.

Changing the liquid yield of rotary vacuum filtration and solvent to aqueous phase ratio was analysed to have a cascading effect on subsequent process inputs. The parameters had indirect effects on the total power requirements of the production system. By determining the volume of butyl acetate and fermentation broth, it determined the power consumptions required for pumping liquids to and from all subsequent unit operations. Hence, minor impact changes were observed for categories associated with energy, such as GWP, photochemical ozone formation (POF) and resource depletion. Most other variations of the downstream input parameters caused changes to environmental impact values of less than 0.1%, which were deemed undetectable (See Table I.1).

In addition, all other parameters tested, flow rates, pump efficiency, HVAC requirements, and equipment life-span generated <1% changes to all environmental impact categories. The most sensitive impact category to pumping was freshwater ecotoxicity (±0.75%), impact on water resources (±0.64%) and total water consumption (±0.49%). These impact categories were most sensitive because they were associated with input materials where flow rates assumptions determined their mass flow. For instance, the total volumes of cooling water and water rinses for CIP were calculated based on flow rates and the duration of the operations. As a whole, the environmental impact results have low sensitivity to flow rates and pump efficiency changes, like most downstream process parameters. For HVAC electrical requirements, the maximum deviation across all impact categories was below 0.6%, comparatively minuscular to the potential deviations for electrical requirements (25%). This was because the amount of electricity required to support the HVAC system makes up a small fraction (only 2.9%) of the total amount required for 6-APA production. Changes to room sizing meant changes to the number of air handling units installed, but this was also reviewed to have generated negligible changes to impacts scores. Varying the total mass of equipment required for fabrication and the life spans of the equipment by ±10% resulted in negligible changes to all impact categories, with the greatest possible change as 0.000654% to ecotoxicity - terrestrial.

Table 4.13: Summary table of sensitivity analysis on manufacturing parameters (part 1). The parameters were varied by their uncertainty levels that were based on the range of values and assumptions found in the literature. The results show the maximum percentage deviation from the base case value. Blue = values ≥ 1% and < 2%. Green = values ≥ 2% and < 3%. Yellow = values ≥ 3% and < 4%. Orange = values ≥ 4% and < 5%. Red = values ≥ 5%.

		Maximum ± % Change								
Parameters Tested	**Variation**	**Acidification**	**Ecotoxicity (Freshwater)**	**Ecotoxicity (Marine)**	**Ecotoxicity (Terrestrial)**	**Eutrophication (Freshwater)**	**Eutrophication (Marine)**	**Eutrophication (Terrestrial)**	**GWP100 (excl. biogenic carbon)**	**GWP100 (incl. biogenic carbon)**
Process Yields & Ratios	**± 1%**	0.06	0.55	0.12	0.28	0.18	0.01	0.05	0.15	0.14
Flow rates & Pump Efficiencies	**± 5%**	0.11	0.75	0.21	0.48	0.27	0.01	0.09	0.16	0.15
CIP Requirements										
Caustic, Acid & Steam	**-20% / 5%**	7.50	1.29	4.45	5.80	0.45	0.61	3.88	4.23	4.00
Pre-Rinse & WFI	**-12% / 25%**	0.48	0.11	1.91	0.49	0.12	0.08	0.83	1.53	1.44
HVAC Requirements	**± 25%**	0.15	0.03	0.02	0.13	0.00	0.02	0.22	0.21	0.21
Equipment Life	**± 10%**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.14: Summary table of sensitivity analysis on manufacturing parameters (part 2). The parameters were varied by their uncertainty levels that were based on the range of values and assumptions found in the literature. The results show the maximum percentage deviation from the base case value. Blue = values ≥ 1% and < 2%. Green = values ≥ 2% and < 3%. Yellow = values ≥ 3% and < 4%. Orange = values ≥ 4% and < 5%. Red = values ≥ 5%.

		Maximum ± Change								
Parameters Tested	**Variation**	**Human Toxicity (Cancer)**	**Human Toxicity (Non-Cancer)**	**Ionising Radiation**	**Ozone Depletion**	**POF (Human Health)**	**POF (Vegetation)**	**Resource Depletion**	**Water Consumption Footprint**	**Total Water Consumption**
Process Yields	**± 1%**	0.33	0.08	0.04	0.21	0.16	0.14	0.39	0.07	0.16
Flow rates & Pump Efficiencies	**± 5%**	0.33	0.11	0.19	0.28	0.11	0.10	0.29	0.64	0.49
CIP Requirements										
Caustic/Acid/ Steam	**-20% / 5%**	6.48	9.68	2.17	14.1	3.60	3.65	1.87	2.16	1.77
Pre-Rinse/ WFI	**-12% / 25%**	2.92	0.34	0.90	0.03	1.02	1.01	0.75	0.63	0.64
HVAC Requirements	**± 25%**	0.00	0.08	0.57	0.00	0.18	0.18	0.11	0.49	0.38
Equipment Life	**± 10%**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00

As a whole, based on the level of uncertainty assumed for each process parameter, the highest deviation observed was 15%. This, in turn, suggested that the environmental impacts generated for the production of 6-APA were 85% accurate (or robust). As LCA aims to be as precise as possible, steps could be carried out to improve the model's robustness. As the primary sources of uncertainty derived from using literature values in building the LCI for this study, steps to obtain industry data to replace current input values could reduce uncertainties. However, industry data was previously unavailable.

4.4.4.2 Sensitivity to LCIA Methodologies

As suggested in Chapter 3, a methodology sensitivity would be beneficial in ensuring the conclusions that could be drawn from the environmental impact results were robust. Figure 4.8 illustrates the process contributions toward each environmental impact category generated by using different life cycle impact assessment (LCIA) methodologies. The process contributions derived from using the LCIA methodologies designed for this case study were compared with the corresponding process contributions derived from using CML (Acero et al., 2015), ReCipe (Goedkoop et al., 2008) and TRACI (Bare, 2011) methodology packages, where possible. All three methods were found to not calculate all impact categories, as stated in Chapter 3. As a result, it was not possible to compare marine eutrophication and terrestrial eutrophication. In addition, unlike CML and ReCipe, the TRACI methodology did not sub-categorise the ecotoxicity and eutrophication impact categories to freshwater, marine and terrestrial. LCA guidance reports by Klöpffer and Grahl (2014) and PE International Sustainability Performance (2014) have reviewed the methodologies and suggested the TRACI methods for calculating ecotoxicity and eutrophication are more suited for measuring aquatic destinations. Hence, TRACI was compared only to freshwater and marine sub-categories for the two impact categories.

For most impact categories, the ranking of processing blocks, from highest contributing to lowest, towards a specific environmental impact was independent of the LCIA methodology. This was true for acidification, ecotoxicity – marine and terrestrial, global warming potential (incl. and excl. biogenic carbon), human toxicity – non-cancer, ionising radiation, ozone depletion, photochemical ozone formation (impact on vegetation), and impact on water resources (water scarcity) (Figure 4.8). The following paragraphs highlight the main differences in the hotspots determined using the different methodologies.

Slight differences were observed when comparing the methodologies used to estimate ecotoxicity – freshwater. This was attributed to the level of impact placed onto the supply for butyl acetate compared to other materials. The USEtox methodology appeared to estimate a relatively greater impact on the supply of this material than the supply of others. While other methods also recognised that the supply of butyl acetate was a high contributor, the level of

impact was more relative to the supply of other materials. Since the material was used in the Product Harvest processing block, the block had a higher percentage contribution towards this impact category (Figure 4.8). For LCIA methodologies that used a relatively lower characterisation factor for butyl acetate, the process contribution of the Stirred Tank Fermentation processing block was significantly higher than the block's contribution derived from the USEtox methodology. However, it was believed that USEtox is the most up-to-date methodology in characterising the impact of different materials and substances, and therefore, most reflective of actual environmental impact. The ranking (highest to lowest impacts) and percentage contributions of process materials derived from the USEtox methodology were assumed to have suggested the order and the magnitude of focus for reducing impacts. For instance, when those materials are reduced, then the overall impact would also reduce. In this case, the focus should be placed on reducing impacts stemming from butyl acetate first.

The USEtox methodology was also used to calculate human toxicity (cancer) and the hot-spot analysis revealed that the supply of natural gas contributed greatly to this impact (>95%). CML, Recipe and TRACI did not consider the supply of natural gas as a high contributor and instead suggested that the supply and use of sodium hydroxide, glucose, hard coal and butyl acetate were the main contributing factors. USEtox characterises the emissions associated with the four materials to have human toxicity potential but not as high as those associated with natural gas. A criticism of using USEtox over the other three methods was that it might mask potential emissions that contribute to human toxicity. On the contrary, emissions stemming from the use of natural gas were not recognised at all by the three other methods. The differences between USEtox and other methodologies is that it characterises both "recommended" and "interim" substances to the human toxicity impact category, whilst CML, ReCipe and TRACI consider only the "recommended" substances. "Recommended" are those known to have toxic human effects and where calculations on their effects are said to be robust. "Interim" are those that have potential human toxic effects, and where calculations on their effects are not yet mature—emissions from the supply of natural gas fall in the "interim" category. Using the USEtox methodology would provide an over-cautious result, but using either of the three other methodologies would miss potential hazards.

In the case of freshwater eutrophication, the use of ReCipe and TRACI resulted in similar trends, and the order of contributions remained the same (1. Fermentation. 2. Product Harvest.3. Production Purification. 4. Wastewater Treatment. 5. Production Conversion. 6. Production Conditioning. 7. Inoculation). However, when the CML method was used, the ranking for Wastewater Treatment and Production Conversion were switched. It was found that generating electricity from coal combustion gave a higher contribution towards this impact category when

using the CML method than for either the ReCipe or TRACI methods. This meant that the CML method weighed one or more emissions from coal combustion, relatively higher than the other methods. Since Product Conversion utilised more electricity than other processing blocks, this block was perceived to be more impactful when using the CML method.

The LCIA methodology that was selected for photochemical ozone formation (POF) (impact on human health) was EDIP 2003. Compared to ReCipe, TRACI and CML methodologies, CML was the only method that indicated Product Conversion to hold the largest contribution. Although all methodologies revealed the same impactful processes, which were the supply of natural gas utilisation of natural gas and coal for electricity; and the supply of glucose and sodium hydroxide, CML suggested that the supply of enzyme and recovery of solvent were also high contributors. Enzyme production and solvent recovery were associated with non-methane volatile organic (NMVOC) emissions, and although all methods consider NMVOC to contribute to POF, the CML method weighs these emissions to contribute more than other contributing substances.

Lastly, process contributions derived from using different LCIA methodologies for resource depletion followed a similar trend where the top two contributing blocks were Stirred Tank Fermentation and Product Harvest. However, only the ILCD method (adopted for this project) indicated that Product Harvest was the highest contributor. While the ILCD method considered all resources (fossils, minerals, and renewables), all other methods focussed only on fossil fuels or minerals. By taking account of renewables, ILCD showed that the use of butyl acetate was highly associated with resource depletion. The other methods favoured Stirred Tank Fermentation as the higher contributor.

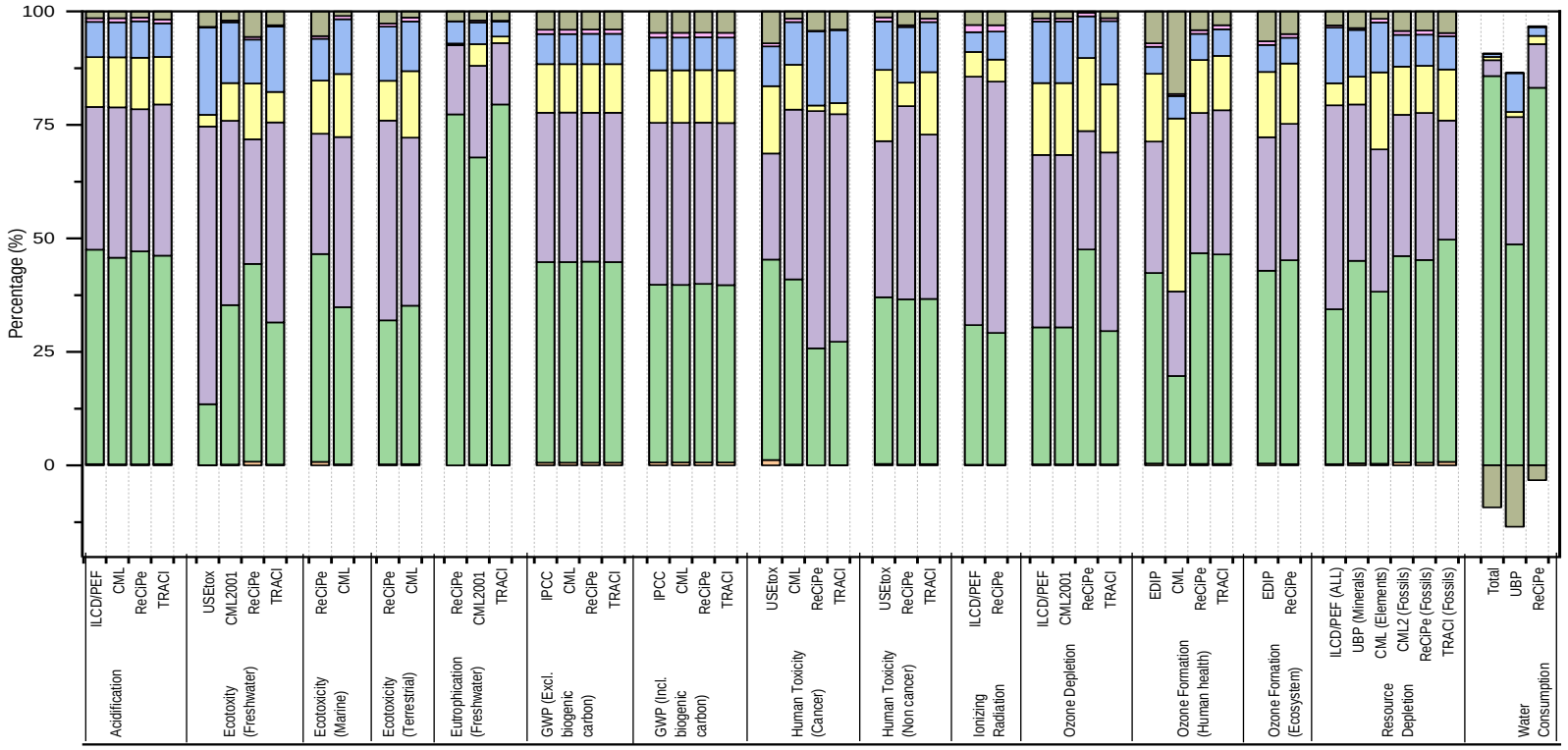

Figure 4.8: Hot-spot analysis comparison between LCIA methods. Process contribution results derived from LCIA methods selected (first method for each impact category) were compared with results derived from using CML, ReCipe and TRACI LCIA methodology packages where possible. The graph illustrates whether the diagnosis of high environmental impacts is independent of the LCIA methodology chosen. *Inoculation.* *Fermentation.* *Product Harvest.* *Product Conversion.* *Product Purification.* *Product Conditioning.*

4.4.5 Scenario Analyses Results

4.4.5.1 Analysis 1 - Product Titre

To understand the environmental effect of product titre, a two-step analysis was conducted by examining the changes in impacts per batch of product produced and then evaluating the change in impact if the demand for 6-APA remains at 2000 tonnes/yr. On a per batch basis, the scenario analysis results revealed that decreasing the titre to 35 g/L (by -38.4% - Scenario 1) generated decreases of impacts between 0.7 and 12.2%. When product titre was increased to 100 g/L (by 75.9% - Scenario 2), it increased impacts between 1.6 to 24.6%. See Appendix J – Table J.2 for impact changes to individual impact categories and correlation between product titre and batch per impact. The most sensitive impact categories were eutrophication – freshwater and marine, and freshwater consumption. These impact categories were particularly sensitive to the change in product titre was because of the level of contribution that the supply of glucose had towards each impact. Since hot-spot analysis showed that the supply of glucose was a high contributor to these overall impact values, changes to the mass input of this material due to changes in product titre would have a noticeable effect. Scenario 1 saw the glucose input decreased by 13.0%, whilst Scenario 2 increased glucose input by 27.9%, which were values similar to the maximum deviations generated by the two scenarios.

On the contrary, when impacts were scaled to meet the 2000 tonnes/year demand, decreasing product titre (Scenario 1) increased environmental impacts by 42.1% to 61.3% while increasing titre (Scenario 2) decreased impacts by 29.2% to 42.3% (See Table 4.15). The impact categories that emerged as most sensitive to the changes in the number of batches required per year were ozone depletion, human toxicity (cancer and non-cancer) and ecotoxicity - marine. Since the ozone depletion impact was derived mainly from the use of sodium hydroxide for cleaning, and that both human toxicity and ecotoxicity – marine were derived mainly from the use of natural gas to generate steam, the changes in product titre had an indirect effect on the number of cleaning cycles required between batches to support the yearly demand. Table 4.16 presents the changes to the input requirements because of the product titre changes.

The increase in product titre decreased the overall mass requirements for glucose and sodium hydroxide to produce 2000 tonnes of 6-APA, even though the amount of glucose increased per batch (Table 4.16). This was because glucose requirements do not correlate directly with product titre. Glucose is required for both microorganism growth and maintenance and penicillin synthesis during fermentation. It was assumed that the growth profile of cells remained the same, whilst glucose requirements for penicillin production changed due to the changes in product titre. This was justified by penicillin being a secondary metabolite, meaning that its production only occurs during the station phase of cell growth and should not interfere

with normal growth (James, 2017). Hence, the portion of glucose used specifically for cell growth was assumed to remain constant, and only the portion dedicated for product synthesis was changed proportionally to the assumed product titre/productivity.

Table 4.15: Environmental impact results for producing 6-APA 2000 tonnes/yr. in Scenarios 1 and 2 normalised using base-case impact results.

	Product Titre		
Impact Categories	**35g/L (Scenario 1)**	**57g/L (Base-case)**	**100g/L (Scenario 2)**
Acidification	1.54	1.00	0.60
Ecotoxicity - freshwater	1.55	1.00	0.61
Ecotoxicity - marine	1.58	1.00	0.60
Ecotoxicity - Terrestrial	1.54	1.00	0.60
Eutrophication - freshwater	1.45	1.00	0.69
Eutrophication - marine	1.42	1.00	0.71
Eutrophication - terrestrial	1.49	1.00	0.62
GWP100, excl biogenic carbon	1.53	1.00	0.60
GWP100, incl biogenic carbon	1.54	1.00	0.59
Human toxicity - cancer	1.61	1.00	0.58
Human toxicity - non-cancer	1.58	1.00	0.58
Ionizing radiation	1.43	1.00	0.60
Ozone depletion	1.61	1.00	0.58
Photochemical ozone formation - human health	1.52	1.00	0.61
Photochemical ozone formation - vegetation	1.52	1.00	0.61
Resource depletion - mineral, fossils and renewables	1.55	1.00	0.60
Total freshwater consumption	1.43	1.00	0.70
Water scarcity (impact on water resources)	1.53	1.00	0.62

Table 4.16: Glucose, sodium hydroxide and steam input requirements for each product titre scenario.

	Product Titre (g/L)		
Input Requirements	**57 (Base Case)**	**35 (Scenario 1)**	**100 (Scenario 2)**
Glucose (kg)			
Per batch	7.33E4	6.34E4	9.28E4
(Per $kg_{product}$)	(6.79)	(9.55)	(4.89)
Per year	1.43E7	2.01E7	1.03E7
Sodium Hydroxide (kg)			
Per batch	6.03E4	6.03E4	6.03E4
(Per $kg_{product}$)	(5.59)	(9.07)	(3.18)
Per year	1.18E7	1.91E7	6.70E6
Steam (MJ)			
Per batch	4.65E6	4.61E6	4.69E6
(Per $kg_{product}$)	(4.29E2)	(6.92E2)	(2.46E2)
Per year	9.07E8	1.46E9	5.21E8

By analysing scenario results further, it was found that changes to environmental impact values were relative to the change in product titre. Eq. 4.7 predicts the environmental impact value of an impact using the factor 0.17, which represents the average percentage change in impact per percentage change in titre (from the base-case scenario). It was also possible to generate factors for individual impact categories, which can substitute 0.17 to give a more accurate prediction (Eq. 4.8).

(Eq. 4.7) $$New\ Impact = \frac{1+\%Change\times 0.17}{1+\%Change} \times Original\ Impact$$

(Eq. 4.8) $$New\ Impact = \frac{1+\%Change\times F}{1+\%Change} \times Original\ Impact$$

Where F = the average percentage change in impact per parentage change in titre for a specific environmental impact category. Table 4.17 present the F value for each impact category. An observation made was that some F values calculated have a higher standard deviation than others. Those impact categories with higher deviations were ones where electricity usage was found to contribute greatly towards. Since product titre was found to have less effect on the emissions associated with electricity or fossil fuel usage on a per batch basis, impact categories where fossil fuel usage has a higher contribution, the correlation between the product titre and the overall impact would be lower.

Nonetheless, the impact associated with electricity generation were present in all impact categories. As demonstrated in Figure 4.9, the use of the equation can derive similar environmental impact values to those obtained through the scenario modelling using the GaBi software. The environmental impact per batch was calculated first before scaled to the number of batches required per year to generate the annual impacts. Acidification, ecotoxicity - freshwater and GWP were chosen to illustrate the potential differences between the calculated and GaBi values across the ranges of average environmental impact changes and their standard deviation (Table 4.17).

Since the fermentation input (media) density changed, the product titre indirectly altered the energy requirements for pumping and mixing. Results showed that pumping and mixing did not significantly affect the overall environmental impact scores on a per batch basis but were more pronounced on a per FU (kg and 2000tonnes/yr.) basis. The total pump electrical requirements per batch in the Stirred Tank Fermentation processing block was decreased by 0.47% and increased by 0.902% when the product titre was changed to 35 g/L and 100 g/L, respectively, from the base case scenario. This coincided with the sensitivity analysis that was carried out on pump parameters, where flow rates and pump efficiencies were varied by ±5% and generated <1% changes (Section 4.4.4). Since the change in product titre only altered pump power requirements up to ±1%, environmental impact changes were negligible. As a whole, as the overall amounts of the product produced per batch changed significantly, the impact allocable to 1 kg of 6-APA would noticeably change.

Table 4.17: The average changes to environmental impact values per batch due to changes in product titre. Eq. 4.8. New Impact = [1 + %Change x F]/[1 + %Change] x Original Impact. Where %Change = the percentage change to product titre from the base case scenario.

Environmental Impact Category	Average Environmental Impact %Change per Product Titre %Change (F)	Standard Deviation
Acidification	0.10	0.04
Ecotoxicity – Freshwater	0.11	0.01
Ecotoxicity – Marine	0.07	0.00
Ecotoxicity – Terrestrial	0.10	0.04
Eutrophication – Freshwater	0.28	0.00
Eutrophication – Marine	0.32	0.00
Eutrophication – Terrestrial	0.17	0.06
GWP (excluding biogenic carbon)	0.12	0.06
GWP (including biogenic carbon)	0.08	0.06
Human Toxicity (cancer)	0.02	0.00
Human Toxicity (non-cancer)	0.05	0.02
Ionising Radiation	0.19	0.16
Ozone Depletion	0.03	0.00
POF (human health)	0.13	0.05
POF (vegetation)	0.13	0.05
Resource Depletion	0.10	0.03
Water - Impact on Water Resources	0.14	0.01
Water Consumption – Total	0.32	0.00

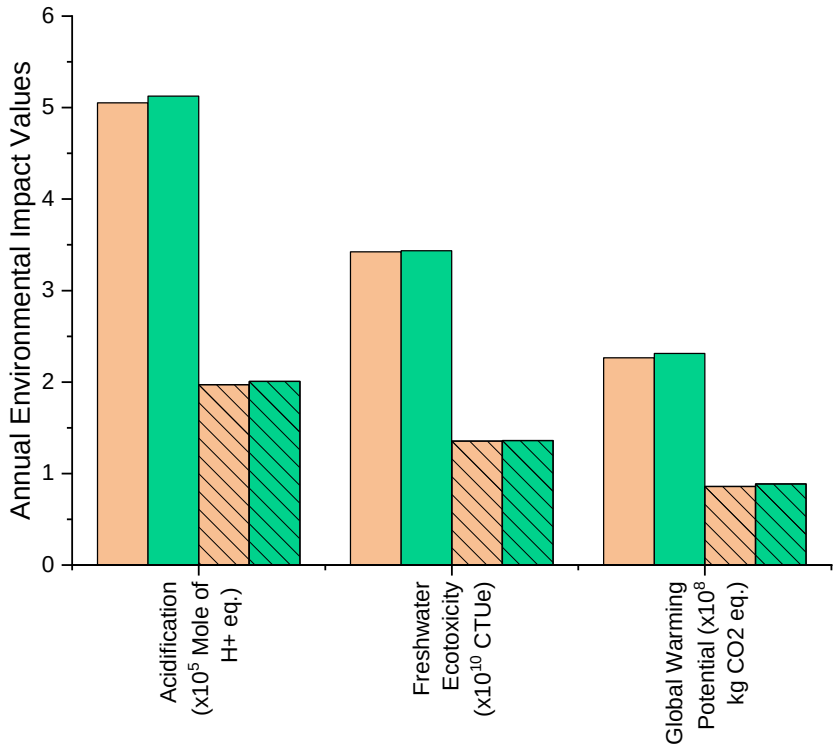

Figure 4.9: A comparison between the actual environmental impact values obtained through GaBi and the theoretical values calculated using Eq. 4.8 and factors in Table 4.17 for acidification, freshwater ecotoxicity and global warming potential 35g/L (GaBi value). 35g/L (calculated value). 100g/L (GaBi value). 100g/L (calculated value).

4.4.5.2 Analysis 2 - Production Scale

Figure 4.10 illustrates the environmental impacts generated for each production scale scenario (Scenarios 3 to 6) as compared to the base case scenario. The results suggested that the environmental impact scores decreased proportionally from the base case scenario (2000 tonnes production scale) at similar rates. From correlating the normalised environmental impact values (impact values as a percentage of the base-case value) with the production scales, an average linear equation was achieved (Eq. 4.9). The environmental impact values generated through producing 1000 tonnes/year (50% of base-case) was on average 53.1% (±2.0% STD) of the base-case impacts, using Eq. 4.9, 52.8% was generated. Similarly, for 500 tonnes/year, the value calculated was 28.9%, and the average value obtained through scenario analysis was 30.1% ±3.3% STD. For 100 tonnes/year the values were 9.8% and 9.6% ±2.9% STD respectively; and for 20 tonnes/year, the values were 6.0% and 5.1% ±2.5% STD respectively. See Appendix J – Table J.3 for correlations between the scale of production and environmental impacts.

(Eq. 4.9) $Y = 0.96X + 0.05$ (R^2=0.999)

Where Y = the environmental impact as a percentage of the base-case scenario, and

X = the production scale as a percentage of the base-case scenario

It was observed that environmental impacts did not decrease uniformly with the decrease in production scale. This suggested that some impacts were independent of scale. An example is the dry-inoculation on rice. The *Penicillium chrysogenum* spores were required to be inoculated onto rice before further cell expansion processes can occur. The step was assumed the same for all processes as this was a standard procedure to initiate cell growth. From this step, cells were expanded until there was a sufficient amount to produce the target quantity of product. Equipment fabrication and electrical usage were also reasons for a baseline impact (y-intercept) exhibited in the correlation model. Equipment sizing, and its subsequent power rating, were dependent on product scale but do not scale down proportionally like the mass flows of process materials. This was because off-the-shelf equipment that was assumed had operational ranges that could be used across multiple scenarios. In addition, power consumptions for some processes were dependant only on running time; throughputs were not particularly a factor. A good example of this would be the incubators assumed for flask cell culture, where it is possible to assume the same incubator at the lower production scale (i.e. at 100 and 20 tonnes/year).

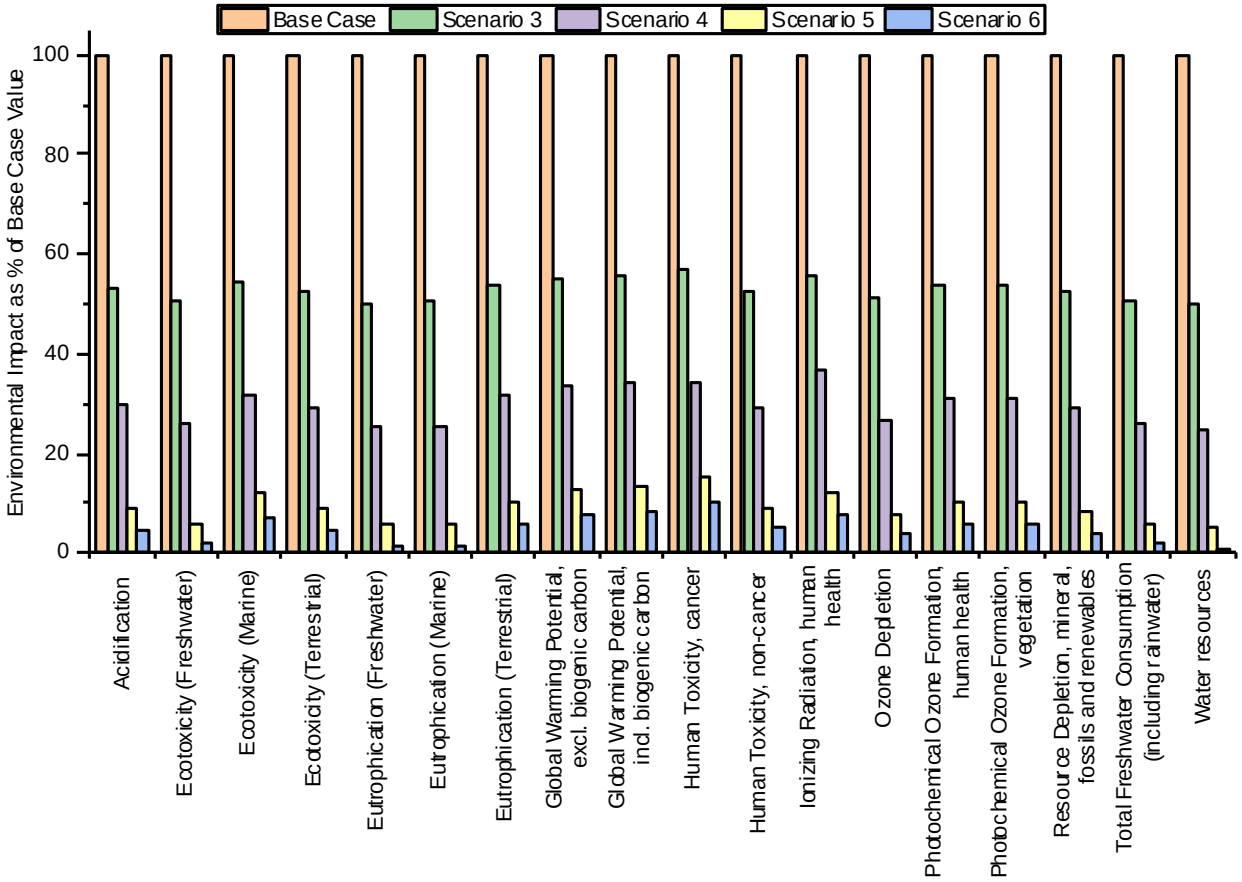

Figure 4.10: Environmental impact of production scale scenarios normalised by impact values of the base case scenario (300m³ scale). Similar trends are observed across all impact categories. 300m³ (base case). 150m³ (Scenario 3). 75m³ (Scenario 4). 15m³ (Scenario 5). 3m³ (Scenario 6).

4.5 DISCUSSION

The hot-spot analysis results have revealed the process steps that contributed the highest to each environmental impact category. It has, therefore, presented the potential areas that, when improved upon, from an environmental perspective, can reduce the overall impacts. However, to understand whether there is any urgency to optimise certain processes, the significance of the impact results generated by the 6-APA process must first be determined. Although normalising results to global per person emissions can reflect how much of our current emissions can be attributed to product production, it does not fully determine whether the environmental footprint is at a damaging level. Comparing against benchmarks can suggest if the process could be environmentally optimised, but the option to compare the results of this study against other biopharmaceuticals was not available. The unavailability was mainly due to the difference in LCA approaches (scope and methodology) applied amongst this study and the already limited LCAs studies on biopharmaceutical products in the literature. In addition, since the production of biopharmaceuticals can occur at different fermentation scales, with a different product titre, results would not be directly comparable.

Below presents three discussions topics that were explored. Firstly, on the significance of the overall impact values generated for the US 6-APA case study. Secondly, a discussion is presented on what process recommendations were drawn by understanding the LCA results fully; i.e. by relating the variety of results generated from LCI analysis, LCIA, hot-spot analysis, sensitivity analysis and scenario analysis. Lastly, whether scenario analysis results are comparable to and therefore capable of representing the environmental impacts of other biopharmaceutical products was discussed.

4.5.1 Significance of Results

Different methods and reference values were used to interpret the results to understand the significance of each environmental impact score generated in the base-case study on 6-APA production. Table 4.18 summarises whether the environmental impact results generated for each impact categories were deemed significant. The subsequent paragraphs detail further the reasoning and deductions behind the decisions made.

Ecotoxicity – terrestrial and freshwater generated results equivalent to the annual emission of 3.9 million and 2.6 million people respectively (approximate population of large cities such as San Francisco (United Nations, 2018)). The scale of these emissions from a single product intermediate can be classed as significant; as it is not the final product, the expected environmental impact of penicillin-derived antibiotics would result in a larger value

(cumulatively). When the overall impact was related to the amount of treatment that the manufacture of 6-APA can support, the significance of results was confirmed.

Table 4.18: Summary of environmental impact results and their significance.

Impact Category	Result Significance	Reason
Acidification	No	Accumulated exceedance value represents a low portion of annual exceedance in Europe.
Ecotoxicity (all sub-categories)	Yes	High people equivalency – except the marine subcategory.
Eutrophication (all sub-categories)	No	Accumulated exceedance value represents a low portion of annual exceedance in Europe.
Global Warming Potential	Moderate	73.9 kg CO_2eq / kg product is comparatively high compared to known products – hence there is room for improvement
Human Toxicity (all sub-categories)	No	When impact values were characterised to the human health damage category, their significance was found low.
Ionising Radiation	No	Low people equivalency.
Ozone Depletion	No	Low people equivalency.
Photochemical Ozone Formation (all sub-categories)	Moderate	This category is linked to climate change and therefore assumed significance to the effect of GWP.
Resource Depletion	Moderate	This category is linked to climate change and therefore assumed significance to the effect of GWP.
Water Consumption	Yes	Water consumption is comparable to the textile industry, which has been deemed to be water-intensive.

Based on molecule weights, 6-APA make-up 59% of amoxicillin, meaning that up to 3390 tonnes of beta-lactam antibiotics could be produced from 2000 tonnes of 6-APA. Using amoxicillin as the leading example, depending on the indication it is required to treat, one dose can range between 125mg to 3g, and a course of amoxicillin can range from two to four doses per day over a duration between 5 to 28 days (NICE, 2021). Taking the most mentioned course of treatment of 500mg - three times a day for seven days, 32.3 million treatments can be generated from the annual production of 6-APA. Here, the number of patients that can be treated is approximately eight times more than the people emission equivalence score (for ecotoxicity - terrestrial) for producing the product intermediate. This can be interpreted further as: “a course of treatment (over seven days) equates to approximately 1/8 (12.5%) of a person’s average yearly emission contribution to ecotoxicity – terrestrial”, which is arguably significant. For ecotoxicity – freshwater, the course of treatment is equivalent to 1/12 (8.3%) of a person’s average contribution. For ecotoxicity – marine, the equivalency was 0.37%. Although this sub-category of ecotoxicity did not generate a high score, the three compartments are interlinked such that substances with toxic properties can flow between them. For this reason, this thesis takes the point of view that ecotoxicity, in general, should be classified as significant. For other environmental impacts, as their normalised scores were an order of magnitude lower, the argument of whether or not the results were significant becomes more tenuous. Hence, other methods for comparing and understanding the significance of results were used.

Since the LCIA method used to calculate acidification and eutrophication – terrestrial measure the accumulated exceedance of acidifying (nitrogen (N) and sulphur (S)) and eutrophic (N) substances emitted to a location, results could be compared to the average accumulated

exceedances that occur at the specific location to understand its significance. Although there are N and S critical load values and quantifications for their emissions/deposition exceedances in the US presented in the literature (e.g. Bouwman et al. (2002) Clark et al. (2018) and Pardo et al. (2011)), average accumulated exceedance values for neither individual states nor the whole country were quantified. The average accumulated exceedance of critical loads per hectare per annum in Europe was taken from Slootweg et al. (2015); the values were 25 eq $ha^{-1}a^{-1}$ and 211 eq $ha^{-1}a^{-1}$ for the exceedance of acidity (N and S) and eutrophication (N) thresholds respectively. The acidification impact generated in this study was equivalent to the average exceedance of 13200 ha (132 km^2) of land in Europe, and for eutrophication, the impact was equivalent to 3290 ha (32.9 km^2). The land areas equated approximately to twice the size and half the size of San Marino (61 km^2) respectively and represent <0.000002% of the size of Europe. The emissions generated by the study were hence deemed low in significance.

Although the calculation for the impact of carbon emissions is a common practice in LCA, for studies to be comparable, factors such as annual throughput or product scale, functional unit, and the general scope of the study must be similar. While there are environmental assessments on penicillin, they did not include the conversion to 6-APA. Hence results cannot be compared. Nonetheless, the 6-APA production process emitted 73.9 $kg_{CO2eq}/kg_{product}$ (incl. biogenic carbon). It was in the same range as a chemical synthesised pharmaceutical API analysed under a similar scope in Wernet et al. (2010) – cradle-to-factory gate approach with the inclusion of plant-waste end of life treatment (67.6 $kg_{CO2eq}/kg_{product}$). Since 6-APA is a product intermediate, the carbon footprint associated with the final antibiotic product has the potential to be significantly higher than the reference pharmaceutical. With climate change currently being an imminent issue, it was deduced that the GWP value for 6-APA production could be improved upon despite its low people emissions equivalency (<20,000 per year; equivalent to 0.05% of a person's annual emission on a per treatment basis).

Because hot-spot analysis revealed that photochemical ozone formation (POF) and resource depletion impacts were derived greatly from the use of fossil fuel to generate electricity and steam, like GWP, it consolidated that processes can have a high impact on multiple impact categories. Processes that emit GHGs, such as the extraction and use of fossil fuels, often also release substances such as carbon monoxide (CO), sulphur dioxide (SO_2) and other carcinogens and contribute to POF. Since fossil fuel usage is also incorporated into resource depletion, there is an intrinsic link amongst the three impact categories. Optimising one impact category would inevitably decrease the impact score of the other two categories. Considering that their people emissions equivalency score was in the same order of magnitude, it was deduced that treating the three categories had the same levels of urgency.

Human health impact scores were calculated to their damage scores, measured in disability-adjusted life years (DALYs) by approximating the reduction in life years that would occur due to ill health. The results were 0.698 DALY/year (cancer effect) and 1.88 DALY/year (non-cancer effect). While the cancer effects sub-category has a higher impact value and people equivalency than the non-cancer effect sub-category, its damage score is lower. This suggested that people equivalency does not fully translate to the extent of harm towards the environment. However, the results suggested that reducing human toxicity - non-cancer may be necessary, as a near 2-year reduction in life years were associated with a year of 6-APA production. On the other hand, when the number of treatment courses 2000 tonnes of 6-APA can support, the reduction in life years per year translates to 6.19×10^{-8} DALY/course of treatment (~2sec), which is negligible.

Other impact categories that were considered negligible, mainly due to low normalised scores, were ionising radiation and ozone depletion. Their significance was sense checked by considering the processes that contributed to the overall scores. For ionising radiation, the impact was generated mainly from electricity generation from nuclear, which makes up 19.2% of the US electricity grid mix modelled for the LCA. Similarly, emissions that contribute to ozone depletion are typically chlorine, fluorine and bromine. As discussed previously, sodium hydroxide production is associated with chlorine gas release and hence, the main contributor of this impact category. Other materials supply generated impacts toward ozone depletion, for instance, butyl acetate. It was unclear whether the chemical processes involved in generating these materials require sodium hydroxide as a cleaning agent, but it was presumed likely as sodium hydroxide is commonly used for industrial cleaning (Chemical Toll, 2020; CSI, 2019). Another category where sodium hydroxide solely contributed over 60% of the impact was human toxicity – non-cancer. Since the human toxicity score was deemed insignificant, there is potential that emissions from the production of sodium hydroxide are limited as a whole, and therefore not environmentally significant. This transcends that the ozone depletion result generated was not significant either.

On an annual basis, the system consumed 8×10^{9} kg_{water}/yr. (3,800 $kg_{water}/kg_{product}$). The manufacturing plant itself withdrew high amounts of water for manufacturing (1.4×10^{9} kg/yr.), but a high proportion of the water was returned to the source via waste treatment. Hence, net consumption reduces to 5.8×10^{8} kg/yr. and 265 $L/kg_{product}$. Comparisons were made to the textile industry since the manufacture of clothing is known to consume high amounts of water. Their specific water consumption range has been reported to be between 10 $L/kg_{product}$ and 645 $L/kg_{product}$ with 138.9 $L/kg_{product}$ reported as a baseline (Emreol Gönlügür, 2019). Since the water consumption for 6-APA production was comparable to a high water impact industry, it suggested that water consumption due to 6-APA production was significant. The Swiss Eco-scarcity method

translated this into environmental impact points (UBP) by considering the scarcity of water at the source, which for the US, the scarcity was rated moderate and scored 232 UBP/m^3. However, there is no normalising factor for understanding the overall significance of the final impact score. With this, it was decided that the impact on water resources score should be used as a benchmark for comparison against processes situated in different countries (Chapter 5).

It was clear that comparing and normalising impact scores with global emissions cannot fully disclose the significance of the environmental impact results generated. Other references used to inform the significance of results included average exceedances per hectare per annum (however, US-specific values were not available), benchmarked emissions and resource consumption by other processes, and damage factors. In some cases, the function units were converted to an approximated number of treatments the product could provide annually, with impacts allocated according to total product mass for a course of treatment before being normalised to people emission equivalences. They all assisted in indicating the significance of impacts; however, the level of damage caused by these emissions remain unclear. Furthermore, 6-APA can be converted to other beta-lactam antibiotics and, as with other biopharmaceuticals, can be used at a range of dosages and course of treatments. Hence, the interpretation carried out using this method may not adequately represent how 6-APA is used in reality. This supports that the functional unit (FU) advised in Chapter 3 to be primarily mass-based such that the LCA results are then allocable to various treatment regime in a flexible manner. For instance, if the 6-APA produced in the assumed facility were wholly directed to manufacture a specific antibiotic, other than amoxicillin, that would be used for a specific indication with one standard course of treatment, the interpretation can be altered to suit its new end usage.

Due to inconsistencies in the LCA approaches used to analyse other biopharmaceutical processes, it was not possible to compare results from this study to existing ones. This suggested the necessity to carry out studies using a streamlined LCA methodology to begin benchmarking impacts across all biopharmaceutical product types to further review processes. In this thesis, an average 6-APA process was assumed as a benchmark for all 6-APA processes, and the LCA results in this chapter would act as benchmarks for comparison in Chapter 5, where locational factors were implemented into different production scenarios. Prior to this, the process improvements drawn from the results of this chapter must first be discussed such to set a baseline of recommendations that can be compared to once 6-APA production in different countries has been analysed.

4.5.2 Process Improvements

As highlighted in the previous section, the impact of ecotoxicity was shown to have the highest significance. Hot-spot analysis results indicated that reducing and/or finding environmentally

preferable alternatives to the use of butyl acetate, sodium hydroxide, and natural gas (for steam generation) could cause the greatest reduction in the overall impacts of 6-APA production. In addition to ecotoxicity, the three materials were found to contribute substantially to other impact categories deemed necessary to be reduced. This suggested further the benefit that their reductions can bring. A reduction in butyl acetate could improve ecotoxicity – freshwater, impact on water resources (water scarcity) and resource depletion scores. For sodium hydroxide, its reduction would benefit ecotoxicity – terrestrial and photochemical ozone formation impacts, and for the reduction of natural gas (or steam) use, ecotoxicity – marine, GWP, POF and resource depletion.

From the sensitivity analysis results, the LCA model was most sensitive to the cleaning-in-place (CIP) and steaming-in-place (CIP) of equipment, where a 20% change in caustic and acid mass inputs would deviate the impact categories of interest between 1% and 6% from the base-case values (Appendix I - Table I.2). As the parameters were varied individually, the change in two or more parameters, e.g. by changing both caustic and acid input requirements, the total impact change ought to be a summation of the model's sensitivity to each parameter. Table 4.19 shows the percentage change in overall impacts for when all cleaning-in-place (CIP) and steaming-in-place (SIP) requirements were reduced to the lower bound value (either 12% or 20% less than the assumed base-case input values). By deviating the input volumes of cleaning materials and energy requirements, it was found that ecotoxicity – terrestrial (the impact category with the highest significance) would decrease by 7.37%. However, when the new impact score was normalised to per-person emissions, and the theoretical number of treatment courses 6-APA can support (as Section 4.5.1), the score was equivalent to 11.2% (down from 12.5%) of a person's annual emission. Similarly, the impact reductions on ecotoxicity – freshwater and marine did not substantially change the base case per person emission scores. For GWP (incl. biogenic carbon) and water consumption, the impact values were reduced to 67.8 $kg_{co2eq.}/kg_{product}$ and 256L/$kg_{product,}$ respectively. While the GWP score was brought down to the impact value generated by the reference pharmaceutical in Wernet et al. (2010), the water consumption value remained above the baseline value for the textile industry. The small changes to overall impacts suggested that reducing cleaning materials alone would not be sufficient to reduce overall impacts.

Table 4.19: The change in environmental impacts, for key impact categories, if cleaning-in-place (CIP) and steaming in-place (SIP) inputs were lowered to the lower-bound amounts found in the literature (either 12% or 20%) and summed.

Impact Categories	Potential Impact Change - due to decreasing CIP and SIP input requirements
Ecotoxicity – freshwater	-1.87%
Ecotoxicity – marine	-9.80%
Ecotoxicity – terrestrial	-7.37%
Global warming potential (excl. biogenic carbon)	-8.70%
Global warming potential (incl. biogenic carbon)	-8.23%
Photochemical ozone formation (human health)	-6.84%
Photochemical ozone formation (vegetation)	-6.87%
Resource depletion	-3.75%
Water consumption	-3.34%
Water scarcity (impact of water resources)	-4.10%

Of the five materials that their use was shown to be most environmentally impactful (Table 4.12.), glucose is arguably the only input that cannot be replaced. Although glucose was associated greatly with high water consumption, the material directly influences the amount of product that can be produced via cell culture, as shown in Section 4.4.5 Scenario Analysis on product titre. This meant that to reduce water consumption associated with 6-APA, optimisation must occur at the glucose generation stage, i.e. starch hydrolysis process. However, this would be out of biopharmaceutical companies' control.

For butyl acetate, there are potentials for companies to reduce its mass requirements in the solvent extraction processes, and hence lower environmental impact contributions towards ecotoxicity – freshwater and terrestrial, resource depletion and water scarcity impacts. As shown in sensitivity analysis, the mass of butyl acetate used in the LCA model was determined by the mass ratio assumed between the solvent and aqueous phase, which was 7:1 in this case study. Sensitivity analysis tested the change in impact if the ratio was deviated from 7:1 to 5:1, the impact scores for ecotoxicity – freshwater and terrestrial would reduce by 15.4% and 7.72%, respectively. 10.9% and 4.49% reduction would also be observed for resource depletion and water scarcity, respectively. However, a decrease in the mass ratio would likely affect the flow rates assumed for solvent extraction to allow adequate mixing time between the solvent and aqueous phase; the lower mass input may mean a lower flow rate of both materials. Although changes in flow rates did not deviate substantially from the overall impact values for all impact categories (as shown by the sensitivity analysis), it can affect the overall batch running time and, therefore, the annual throughput of the production facility. Hence, further considerations must be made when changing operating parameters. Otherwise, lowering both cleaning agent requirements and butyl acetate can reduce ecotoxicity impacts by approximately 15%. Alternative materials were not fully considered, as both butyl acetate and sodium hydroxide are typical materials used for penicillin extraction and cleaning, respectively. Although there are

studies testing penicillin extraction using different solvents (such as (Lee, 2009; Rowley et al., 1946)), not all materials are available in the GaBi database for comparison. This suggested that the number of LCAs on pharmaceutical input materials is insufficient to make informative comparisons between input material choices. To allow process optimisation based on material selection, it would require life cycle impacts of all raw materials, which may be necessary for pharmaceutical production, to be known before evaluating the impacts of pharmaceuticals products.

Since the impacts associated with electricity and steam generation were attributed to fossil fuel use, an option to reduce the impact is to switch towards more renewable energy sources. On-site electricity generation can reduce reliance on the national grid, while an environmentally preferred process can be chosen. Dunn (2016) presented GlaxoSmithKline's initiative to install wind turbines and solar panels to provide 3.5% of a production facility's demand, which was stated to reduce 3000 tonnes of carbon emissions per year. Another potential is to utilise waste produced from the production process to generate both electricity and heat (combined heat and power - CHP) via incineration with energy recovery (Ryu and Shin, 2012) or via anaerobic digestion with subsequent use of biogas to generate energy (Dunn, 2016). However, it is understood that the installation of new infrastructure would require long-term planning, including further analyses to balance costs and environmental impacts, forecast governmental plans on future energy generation mixes, and the logistics for the supply of renewable resources. For instance, for wind and solar power, it would logistically depend on local weather norms as to determine whether they would be cost-effective; and if it is forecasted that the country would reach 100% renewable energy in the near future, the necessity for a facility to install their own renewable energy source may lessen. Focus may then switch to decreasing energy usage at the plant.

The prospect of reducing electricity and steam consumptions at the manufacturing facility would highly depend on the likelihood of which the power ratings of equipment or their running time can be reduced. In this case study, equipment other than reactors were assumed off-the-shelf units based on their capacity and equipment throughput specifications; this then determined the power ratings and equipment sizes. Recommendations would be to seek more energy-efficient equipment, which can replace those in the Stirred Tank Fermentation and Product Harvest stages as most energy usages were attributed to the two processing blocks (Section 4.4.3). Hence, optimising these blocks may offer the most reductions. Assuming that the overall process volume from production fermentation, and therefore the upstream operation, cannot be changed to ensure sufficient penicillin/6-APA are produced to meet demand, research should

be directed towards the Product Harvest stage to reduce its energy (electricity and steam) demand.

It was stated previously that the reduction of butyl acetate in the solvent extraction could reduce various impacts, but GWP and POF were not impact categories that were sensitive to the use of the material (Table I.2). This finding indicated that despite reducing the input of solvent, its cascading effect of lowering electricity use (a major contributor to both GWP and POF), due to lowering processing time, would not alter impacts drastically. The heat sterilisation of fermentation media required a large amount of steam and can be an area to improve upon. Although lowering the flow rate of steam would mean that less steam is required and, hence, lower various impact categories, the residence time for heat sterilisation would need to increase to provide sufficient heat transfer. This could affect the overall media processing time before fermentation, affecting the overall scheduling of production. Ideally, the viability of any chances must first be assessed technically, economically, socially and environmentally before implementation.

As shown in Section 4.4.5 Scenario Analysis, the increase in product titre positively affected the environmental impacts associated with producing 6-APA to meet the 2000 tonnes/year target. This has suggested that to reduce impacts, continual development on the host cell strain to achieve a higher titre can be highly effective. This can go hand in hand with continual studies to find more environmentally preferred solvents, cleaning agents and the operating procedures involving these materials. However, since not all materials necessary for pharmaceutical manufacture have been assessed to attain their environmental impact profiles from cradle-to-point of use, it is imperative to carry out LCAs on these materials to fully understand their impacts that can be attributed to pharmaceutical products.

4.5.3 The potential for estimating the environmental impact of other biopharmaceutical products

The scenario analysis carried out on the 6-APA process explored the impact changes associated with product titre and production scale changes. Since all of the materials (except butyl acetate) that were revealed as the largest contributors to all environmental impacts are commonly required for other biopharmaceutical processes, it was hypothesised that results for 6-APA production could be used to estimate impacts for the production of other products. Here, a discussion is presented to evaluate whether results generated for producing 6-APA at smaller scales are comparable to LCAs on other biopharmaceutical products.

From comparing the LCA results with other studies of biopharmaceutical products, it was found that Scenarios 5 and 6 produced a similar GWP as a study on monoclonal antibody (mAb)

(Ramasamy, 2015) (Table 4.20). Although product titres for penicillin production (57g/L used in this project) and mAb production (10g/L in Ramasamy (2015) were different, the scales of operation in terms of processing volume were similar. This suggested that the scale of operation determined a plant's annual environmental impacts on GWP. To test this hypothesis further, the correlations generated using scenario analysis results were used in an attempt to calculate the carbon emission of 6-APA production at the same production scale and product titre used in Ramasamy (2015) and additionally in Bunnak et al. (2016). Firstly, Eq. 4.10, interpreted from Table J.2, was used to estimate the GWP (incl. biogenic carbon) value of 6-APA production. In this case, the product titre of Scenarios 5 and 6 was reduced to 10 g/L and 5g/L, but the production scale and the number of batches remained the same. Then, using the correlation between the change in product scale and the change in GWP impact (Eq. 4.11 – interpreted from Table J.3), the GWP value for the new product titre was scaled to the production scales used in the referenced literature.

(Eq. 4.10) $GWP_{new\text{-}PT} = (0.06 \times \%\ \text{Change in Product Titre} + 1) \times GWP_{base\text{-}case}$

$GWP_{base\text{-}case}$ = the global warming potential value generated for the base-case scenario ($kg_{CO2eq.}$/yr).

$GWP_{new\text{-}PT}$ = an estimate of the global warming potential value for the new product titre scenario, provided that the production scale and the same number of batches produced are assumed the same as the base-case ($kg_{CO2eq.}$/yr).

% Change in Product Titre = [new product titre (g/L) divided by product titre at base-case – 1

(Eq. 4.11) $GWP_{new\text{-}PS} = (0.92 \times \text{Production Scale} / \text{Base-Case Scale} + 0.09) \times GWP_{base\text{-}case}$

$GWP_{new\text{-}PS}$ = an estimate of the global warming potential value for the new production scale scenario, provided that the production titre and the same number of batches produced are assumed the same as the referenced case ($kg_{CO2eq.}$/yr).

Table 4.20: Comparing GWP (incl. biogenic carbon) values obtained from this project for 6-APA production to values for mAbs from Ramasamy (2015) and Bunnak et al. (2016). Red = calculated value higher than the referenced value situated in the row below. Green = calculated value lower than the referenced value situated in the row below.

Study	Product Titre (g/L)	Fermentation Capacity (m^3)	Product Scale (tonnes/year)	Global Warming Potential (GWP) (kg_{co2eq}/yr.) Cradle-to-gate value
Scenario 5	57	15	100	$1.83x10^7$
Estimated from Scenario 5	10	15 10 – 40	-	$1.74 x10^7$ $2.82 x10^7$ (average)
mAb Production (Ramasamy, 2015)	10	10 – 40	-	**$2.3 x10^7$ (average)**
Scenario 6	57	3	20	$1.13x10^7$
Estimated from Scenario 6	10	3 1 – 2	-	$1.07 x10^7$ $5.88x10^6$ (average)
mAb Production for Clinical Trials (Ramasamy, 2015)	10	1 – 2	-	**$1.1x10^7$ (average)**
Estimated from Scenario 6	5	3 0.47 0.059	-	$1.07 x10^7$ $2.51 x10^6$ $1.17x10^6$
mAb Production (Bunnak et al., 2016)	5	*Fed-batch – **0.47 *Perfusion - **0.059	0.028	**Fed-batch – $1.7 x10^5$** **Perfusion – $2.0x10^5$**

*Fermentation mode – fed-batch fermentation and perfusion fermentation were compared by Bunnak et al. (2016)
**Working volumes were presented in the literature; 375L for fed-batch and 47L for perfusion fermentation was assumed to operate at 80% capacity.

While the results for Scenarios 5 and 6 held similarities to those generated for monoclonal antibodies, the calculations attempted generated GWP results that were not comparable. Estimating the emissions associated with 6-APA at low product titres at low production scales (< 3 m^3) resulted in GWP values nearly half of the value obtained by Ramasamy (2015) and an order of magnitude higher than Bunnak et al. (2016). Estimating the emissions for 10 g/L and at 10 m^3 to 40 m^3 scale generated a result ~22% higher than the literature obtained value as well. The inconsistent differences between the estimated values and those generated in the literature meant that estimations for one product could not represent another.

There may be several reasons for this. (1) The requirement for glucose and other manufacturing input materials varies largely among different products. Organisms are grown at different rates, and therefore, growth requirements assumed for *Penicillium chrysogenum* would not be the same as Chinese hamster ovaries cells (typically used to produce mAbs). The duration of cell culture and nutrients (including glucose) can also differ. Since the 6-APA case study results showed that most impacts were derived from the supply of materials, changes in overall materials requirements would affect the overall impact. (2) The extrapolation assumed that energy sources were equal. In this study, the US energy mix was used, but both Ramasamy (2015) and Bunnak et al. (2016) modelled mAb production using the UK mix. Since carbon emissions are mainly generated from fossil fuel combustion, differences in fossil fuel in the mix

would affect GWP calculations. In addition, the environmental impacts associated with the same material usually differ as a result of where it was sourced and/or generated. The effect of production location on the environmental impact of 6-APA production was explored and is presented in Chapter 5. (3) Since the correlation equations were derived using product titre and production scale ranges (35 g/L to 100g/L and 3 m^3 to 300 $m^{3,}$ respectively), estimating impacts for titres and scales outside these ranges would not generate accurate results. As the R-square values generated for the correlations shown in Tables J.2 and J.3 were over 0.9 for most impact categories, it was denoted that the correlations are suitable to predict impacts for 6-APA production. However, only if the production location, energy mix and other material sourcing assumptions are the same.

4.6 CONCLUSION

In this chapter, a life cycle assessment (LCA) on the production of 6-APA in a theoretical plant situated in the US was conducted under the goals and scope definitions set out in Chapter 3. Five types of analysis – life cycle inventory (LCI) analysis, life cycle impact assessment (LCIA), hot-spot analysis, sensitivity analysis and scenario analysis were carried out. Sensitivity analysis showed that the 6-APA production process modelled 85% robust and most sensitive to the potential deviations to the cleaning protocol, particularly the amount of sodium hydroxide required and the amount of butyl acetate necessary in the solvent extraction processes. LCI and hot-spot analyses showed that environmental impact scores for each processing block were highly correlated with the amounts of resources (materials, electricity, water and steam) required for production. Specific materials that were found to contribute highly to various impact categories were butyl acetate, glucose and sodium hydroxide. In addition, impacts associated with electricity and steam were attributed to the use of fossil fuels, and hence, put forward the suggestion of using a more environmentally preferable energy source.

Parameters that generated the highest deviations from the overall environmental impact scores (due to their uncertainty levels) were found to indicate the potential areas in the processes where process optimisation could occur. Although the use of sodium hydroxide and butyl acetate was one of the most impactful inputs, the possible reduction of both materials combined would not reduce the significance of the ecotoxicity – freshwater and terrestrial scores meaningfully. Suggestions were then made to investigate alternative materials. However, it was made known from building the LCA model for this case study that not all materials for biopharmaceutical products were available in the GaBi and EcoInvent databases. Hence, it would be imperative to conduct LCAs on biopharmaceutical input materials to assure that LCAs on biopharmaceutical products can be comprehensively conducted. Furthermore, it was made

clear that implementations to the existing production process must also consider technical and cost factors when optimising a process from an environmental perspective. This enforced the idea that cost and social analyses should be developed and used to complement LCAs.

Lastly, the scenario analyses illustrated how process decisions on product titre and production scale had a cascading effect on the overall environmental impact. However, it was possible to fit a linear correlation between the changes in each parameter to the changes in impact individually. Attempts were made to extrapolate the correlation models to estimate impacts generated for producing another biopharmaceutical, monoclonal antibodies, but it was found not possible. Reasons for this were presented, and the models were deemed only suitable for estimating impacts associated with 6-APA production.

Following the insight that the supply phase of the 6-APA production process was the most impactful, it was realised that materials supply, energy generation mix and water withdrawal methods are location dependant. To understand how process decisions on production location can also have cascading effects on the overall environmental impacts associated with 6-APA production, a comparative study between producing the product in nine countries was carried out. This is presented in the next chapter.

Chapter 5: COMPARATIVE STUDY: THE EFFECTS OF PRODUCTION LOCATION ON PRODUCT ENVIRONMENTAL IMPACTS

5.1 INTRODUCTION

The hot-spot analysis in Chapter 4 revealed that the supply phase, which denotes the supplies of materials and resources before the production of 6-APA, was the most significant contributor to all environmental impacts. Since the supplies of materials and resources are dependent on geographical variables, such as resource availability and national norms, the results suggested that the location of production was vital when defining the overall environmental impact of a 6-APA production process. A prime example is a countries' electricity mix, where renewable and non-renewable availability may differ. As discussed, lowering fossil fuel usage in energy generation would decrease particularly impact scores for global warming potential (GWP), photochemical ozone formation (POF) and resource depletion. Hence, if 6-APA were produced in countries with higher usage of renewable energy than the US (the base-case scenario, Chapter 4), the three impacts would be lowered. However, other impact scores would also change due to other location-dependent factors.

This chapter presents a comparative study on the production of 6-APA in nine countries, which was carried out to understand further how process decisions would affect a product's environmental impacts. The study took account of location-dependent factors (or country-specific variables): electricity grid mix, water scarcity and average inventories (resource use and emissions) for the supply of materials. With this, the chapter aimed to demonstrate how a comparative LCA study could be used to aid choices for siting a facility by exploring the effect manufacturing locations have on the environmental impacts of a given process. As part of the discussion, the significance of the impact results and how representative they are to 6-APA productions in the nine countries were presented. These were considered in the discussions that followed: the recommendations for siting biopharmaceutical facilities and the extrapolation of LCA results to estimate the global environmental impacts of 6-APA production. Relationships and correlations between the overall impacts and process design parameters (product titre, production scale and country-specific variables) were used to estimate the current global distribution of key impacts. This led to further considerations and recommendations drawn on how governments and the industry should reduce burdens on an international basis.

5.2 COMPARATIVE STUDY BACKGROUND

As mentioned in previous chapters, penicillin and its derivatives are produced widely across the globe. Figure 5.1 illustrates the various manufacturing locations for these products, which were assumed to indicate where the manufacturing and the reprocessing of 6-APA occur. The figure shows that in 2010, the product was produced in clusters mainly in the US, Europe, India and China. Although the production scales of the plants were not disclosed, it was assumed that environmental impacts associated with 6-APA production would concentrate in these locations. It was deemed necessary to carry out LCA on particular production locations to reflect the effects of this product. Nine countries were identified for this comparative study: Brazil, China, Germany, India, Singapore, South Africa, Spain, the UK and the US to represent the current demographics of producing 6-APA. Table 5.1 describes the reasoning behind the choice of each country location.

The chosen nine countries are currently at different stages of economic development. According to the International Monetary Fund, Brazil, China, India, and South Africa are emerging countries (i.e. developing); Singapore and Spain are advanced economies; Germany, the UK, and the US are major advanced economies (International Monetary Fund, 2019). The hypothesis was that countries classed as "developing" would have lower technical efficiency in the supply of materials and generate higher environmental impacts. Technical efficiency is the effectiveness of utilising inputs to produce an outcome (Palmer and Torgerson, 1999). For instance, a technically efficient process will entail the least resource materials, the minimal operators and from an environmental point of view, the lowest environmental emissions, associated with the higher efficacy in utilising input resources. Technical efficiency is associated with technology availability and skilled workers, which can be subjected to financial grounds to buy advanced technology and education access; hence the hypothesis. However, technology efficiency can also determined by the countries' terrain. The geology of the extraction site can also vary at different locations; depending on where resources are, the extraction methods required may be pre-determined. Thus, different emissions can occur during the extraction process. Hence, the comparison amongst the different 6-APA producing countries was indirectly aimed to understand whether there are environmental impact differences due to a country's development class.

Amongst differences in socio-economic class, the countries' water scarcity levels, main methods for energy supply and their proximity to material sources differed. As shown in Chapter 4, they were all factors that can affect the environmental impacts attributed to the production of 6-APA. Section 5.3 (particularly Table 5.2) highlights the different assumptions used to model each country scenario. While some countries may benefit environmentally due to their accessibility

to more efficient manufacturing supplies, others may have lower resource scarcity and more environmentally preferred energy mixes. The comparative study aimed to correlate the location-dependent factors with the impacts associated with 6-APA production to inform future choices between manufacturing locations, evaluate whether these factors should be considered when biopharmaceutical production siting, and suggest recommendations on ways of optimising current 6-APA production. As part of this exercise, in Section 5.5.3, the global impacts from 6-APA production were extrapolated using both scenario analysis results (Chapter 4) and this comparative study to derive further recommendations.

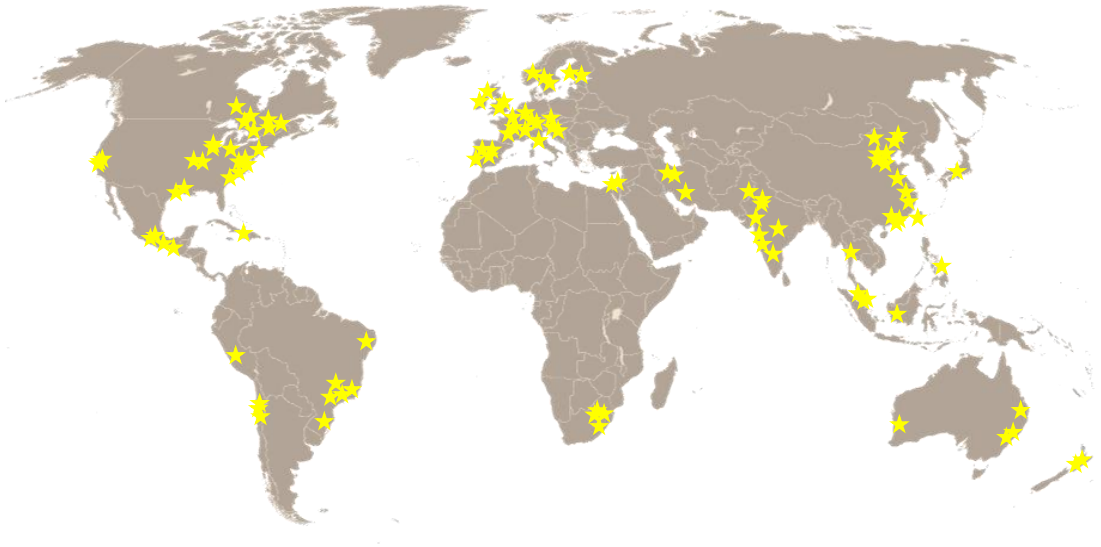

Figure 5.1: The manufacturing locations of penicillin and its derivatives; this was regarded to be indicative of the manufacturing and reprocessing locations of 6-APA. 6-APA required first the production of penicillin and was used to manufacture penicillin derivatives. Source: Antimicrobe (2010) *(not all plants are represented in the image).*

Table 5.1: Reasoning behind the choice to study the environmental impact of 6-APA production in the nine specified countries.

Studied Country	Reason of Choice
United States (Base-case Scenario)	As described in Chapter 4, the US was the first country that produced penicillin and its derivatives. It was assumed that production of 6-APA should be the most mature, and the scenario could be deemed the base-case scenario (Sampat et al., 2015).
Brazil	Brazil's national electricity mix comprised the least fossil fuel of the nine countries studied and had the lowest water scarcity level (Table 5.2). The country was chosen to provide an example of environmental impacts associated with the manufacturing of 6-APA when primarily renewable energy sources were used for electricity and a country that is currently developing its economic and technical status.
China	China was described as the biggest producer of penicillin salts, with a production capacity exceeding 100,000 tonnes per annum in PA International (2017). Since most penicillin are assumed converted to 6-APA (Chapter 4 – Section 4.2), China could be the biggest producer of 6-APA produced globally. For this reason, it was deemed that by understanding the environmental impact impacts associated with manufacturing 6-APA in this location, recommendations to reduce the global impact of producing this drug intermediate would be made possible.
Germany	Germany was shown to be populated with the most pharmaceutical manufacturing sites within Europe (Kurmann Partners, 2017). It was said to have the largest fermentation capacity in this continent (Germany Trade & Invest, 2018). Due to these reasons, it was deemed that this country would be a good representative of European production of biopharmaceuticals. Although it was noted that many antibiotic manufacturers were relocated from Germany to countries with lower operating costs (Roland Berger, 2018), it was believed that the capacity remains. By analysing the environmental impact of producing 6-APA in this country, insights into impacts and concerns are likely to be associated with other products as a function of manufacturing location.

India	India is a major supplier of 6-APA (Bhattacharyya and Sen, 2006; Ghosh and Bajaj, 1998). Hence, like China, it was deemed necessary to analyse this location to understand the overall environmental impact associated with 6-APA production globally.
Singapore	Singapore was chosen to represent an emerging country for biopharmaceutical production. From conducting interviews with industry experts (Appendix D), it was clear that Singapore was deemed a prime location for future biopharmaceutical development. This information concurred with the 2016 Scientific American Worldview Scores for biotechnology innovation, where Singapore was ranked second (the US in the first place) (Scientific American, 2016). The ranking of countries' strength in biotechnology considered many factors; this included strength of IP protection, efforts and capability for biotech innovation (production intensity/number of occurrences); governmental support for enterprise, etc. The high rankings in these categories suggested why Singapore was an attractive location for biotechnology business development (Benner, 2016).
South Africa	South Africa was one of the named emerging economies in 2010 (Ezziane, 2014) and represents Africa's largest life science market (Deloitte, 2017). This country was chosen to represent manufacturing in Africa and give insights into potential environmental impacts present in the future when further development occurs. It was reported that trade deals between China and South Africa had allowed the expansion of China's economic activities within South Africa (Wasserman, 2018). Through consultation with industry experts (Appendix D), pharmaceutical production in South Africa was foreseen to support Chinese demands for providing lower-cost products.
Spain	Spain was another European country chosen for the study due to suppliers of beta-lactam antibiotics, Reig Jofre (Laboratorio Reif Jofre, 2019) and Centrient (Centrient Pharmaceuticals, 2018), have manufacturing locations within this country. Another reason for choosing Spain was its energy mix. The mix was found to be predominantly renewable resources and nuclear energy (25.9% and 20.8%, respectively) (Table 5.2). Compared to other locations analysed, Spain utilised the highest percentage of both renewable and nuclear energy. Including a country with such an energy mix helped understand the changes to environmental impacts when fossil fuels are not in primary use.
United Kingdom	Both penicillin and 6-APA were discovered in the United Kingdom (UK) (Carrington, 1971; Gaynes, 2017). Although the US was the first to mass-produce penicillin, UK was among the first to commercialise it (Gaynes, 2017). The production of 6-APA in the UK is mature, GSK's production site in Worthing does not produce 6-APA anymore, but Irvine remains the company's UK production site (Dunn, 2016). The study of 6-APA production in the UK was assumed directly comparable to the US base-case study. Both producing locations were first to produce this product and hold the most mature techniques for production.

5.3 METHODOLOGY AND ASSUMPTIONS

5.3.1 Goal and Scope Definitions

The ultimate goal of this comparative study was to understand the differences in the environmental impacts associated with the production of 6-APA when the manufacturing location was to change. The life cycle approaches used for this study were kept the same as Chapter 4, such that LCA for each country scenario would be comparable to the US base case study. Hence, the functional units (per kg and 2000 tonnes/yr.) and system boundaries (cradle-to-gate) remained the same. In addition, the fundamental assumptions were that the process schematic and therefore the scale of production, i.e. fermentation sizing, also remained the same. As suggested in Chapter 4, as the production of this substance is well established, the assumption that overall material utilisations and yields do not vary drastically was deemed appropriate.

5.3.2 Life Cycle Inventory (LCI)

While the 6-APA production process was assumed the same (as presented in Chapter 4), the differences amongst the scenarios lied with location-dependent factors. Table 5.2 shows the electricity mix and water scarcity for each country scenario that was modelled. In addition, the table presents the geographic codes of which processes and materials life cycle inventories (LCIs) were selected for the LCA models. As stated in Chapter 4, the LCIs, obtained from databases

and employed for the US base-case study, were location-specific (country-specific LCIs); otherwise, regional or global LCIs were used. Similarly, for all country scenarios, country-specific LCIs were primarily chosen where possible before selecting regional-specific or global LCIs (see Table 5.2 for the preferred geographic LCIs for each scenario). For the Singapore scenario, due to proximity to Malaysia, where Singapore-specific LCIs were not available, preference was given to Malaysia-specific LCIs.

It must be noted that not all LCIs used in the LCA study fitted the locational criteria set out in Table 5.2. Because only US or North America specific life cycle inventories were available for glucose, corn steep liquor and sulphuric acid, all scenario was modelled using the same LCIs for these materials. Since the impact associated with manufacturing materials should differ due to location-dependent factors, the gaps in the LCI databases on biopharmaceutical materials meant that a fully comprehensive comparison between different countries producing the same product was not possible. Hence, this has posed a limitation of this comparative study.

Another locational factor that was incorporated into modelling was transport distances. For each scenario, a manufacturing site belonging to a company that produces beta-lactam antibiotics was assumed the 6-APA production location for each scenario from the interactive map developed by Kurmann Partners (2017), where they indicated all manufacturing sites approved by EMA and FDA. The locations considered for the study were also sites where pharmaceuticals concentrate. Hence, the study was also configured so that the analysis would help gauge the environmental impact at locations undertaking high-level manufacturing activities.

Process materials were assumed to be sourced locally, the average distances between the assumed biopharmaceutical plant location and local vendors were calculated. The process for this calculation was by first researching suppliers for materials, which were greatly used for 6-APA production, then by analysing the proximity of these suppliers to the assumed manufacturing location. For equipment supply, it was assumed that raw materials extraction and equipment fabrication occurred in China for all scenarios except for India. It was believed that equipment was locally sourced in India as the country is known to be a large exporter of steel like China (OECD, 2019). The modes of transport were assumed according to the distance and medium between the supplying location and the plant. See Appendix F for the assumed locations, transport distances and modes of transport.

Table 5.2: Location-dependent factors studied in the comparative study.

	Countries Modelled								
	Brazil	**China**	**Germany**	**India**	**Singapore**	**South Africa**	**Spain**	**UK**	**US**
Energy Mix*	63.2% Hydroelectricity, 13.7% Natural Gas, 9.9% Renewables, 6% Oil, 4.5% Coal, 2.6% Nuclear, 0.1% Other	72.6% Coal, 18.6% Hydroelectricity, 4.2% Renewables, 2.3% Nuclear, 2% Natural Gas, 0.2% Oil, 0.1% Other	45.8% Coal, 23% Renewables, 15.6% Nuclear, 10% Natural Gas, 3.1% Hydroelectricity, 0.9% Oil, 1.6% Other	75.1% Coal, 10.2% Hydroelectricity, 5.2% Renewables, 4.9% Natural Gas, 2.8% Nuclear, 1.8% Oil	95.3% Natural Gas, 1.7% Renewables, 1.1% Coal, 0.7% Oil, 1.2% Other	93% Coal, 5.5% Nuclear, 1% Renewables, 0.4% Hydroelectricity, 0.1% Oil	25.9% Renewables, 20.8% Nuclear, 17.2% Natural Gas, 16.5% Coal, 14.2% Hydroelectricity, 5.1% Oil, 0.3% Other	30.4% Coal, 30% Natural Gas, 19% Nuclear, 17.7% Renewables, 1.8% Hydroelectricity, 0.5% Oil, 0.6% Other	39.7% Coal, 26.9% Natural Gas, 19.2% Nuclear, 6.9% Renewables, 6.1% Hydroelectricity, 0.9% Oil, 0.3% Other
Water Scarcity (UBP/m³)**	0.48	360	421	1300	5	600	811	74.7	232
LCI Selection by Geographic Codes (in GaBi)†	[BR], [RoW], [GLO]	[CN], [RoW], [GLO]	[DE], [EU-28]/ [RER], [GLO]	[IN], [RoW], [GLO]	[SG], [MY], [RoW], [GLO]	[ZA], [RoW], [GLO]	[ES], [EU-28]/ [RER], [GLO]	[GB], [EU-28]/ [RER], [GLO]	[US], [RNA], [GLO]

*Energy mix of 2014 was used to model the LCA of each country scenario. Renewables include solar, wind, geothermal and biomass. Others include various waste to energy methods. The data was obtained from the "Statistical Review of World Energy" provide on the BP website *(BP plc., 2019)*.

**UBP/m^3 is the environmental impact point allocated per volume of water consumed depending on the country's scarcity level. Higher the UBP, the higher the water scarcity level. *(FOEN, 2013)*

†In the order of preference. [BR] = Brazil, [CN] = China, [DE] = Germany, [IN] = India, [SG] =Singapore, [MY] = Malaysian, [ZA] = South Africa, [ES] = Spain, [GB] = Great Britain, [US] = United States, [EU-28] = European Union, [RER] = Europe, [RNA] = North America, [RoW] = Rest of the World, [GLO] = Global.

5.3.3 Life Cycle Impact Assessment and Analysis of Results

Life cycle impact assessment (LCIA) was carried out as stated in Chapter 4 – Section 4.3.3, where calculations for the foreground process (6-APA production) were transferred onto the LCA modelling software, GaBi (Sphera, 2020b), from Microsoft Excel (Microsoft Corporation, 2021). Relevant LCIs were obtained from databases within the software for the LCA models. The LCIA methods proposed in Chapter 3 were used for all scenarios such that the results generated were comparable.

Impact scores were normalised using two methods: (1) by global per capita emissions normalisation factors presented in Table 4.5 to understand the significance of each impact score; and (2) by the US base-case scenario results. Comparing overall environmental impact results for each scenario with the US scenario conveyed the overall changes to impact scores due to changes to the various location-dependent factors.

5.3.4 Analysis of Results

5.3.4.1 Hot-spot Analysis and Comparisons

Hot-spot analysis was carried out as stated in Chapter 4 – Section 4.3.4.1, where the areas of high environmental impacts were diagnosed by allocating impacts scores to the different processing blocks first and then investigating which process inputs contributed highest to each block. Given that each process inputs were modelled using location-specific LCIs, the differences between the magnitude and contribution generated toward each environmental impact by the nine scenarios showed the effect of production location on overall impacts. Relationships and correlations between the locational factors were analysed to understand further the cascading environmental effect of the choice of production location. The analysis involved comparing the highest contributing factors with the overall impacts across the different scenarios. For instance, where fossil fuel usage was found as a large contributor and the magnitude of overall impacts varied among the scenarios, steps were taken to understand the difference (demonstrated as part of 5.4 Results – Section 5.4.2).

5.4 RESULTS

5.4.1 Life Cycle Impact Assessment Results

Presented in Tables 5.3 and 5.4 are the normalised results for each country scenario. Direct comparison of all environmental impact results revealed that South Africa, China and India consistently generated the highest impacts whilst Germany, the US and Spain consistently scored lowest for most categories (Table 5.3). The most significant impact scores were observed

in ecotoxicity – terrestrial, ecotoxicity – freshwater and acidification. The China scenario's ecotoxicity scores were up to 16 times higher than the US base-case value. Impact categories that generated the least deviations were ecotoxicity – marine, eutrophication – marine and total freshwater consumption. Normalisation by global per capita emissions showed that ecotoxicity as a whole remained the highest for all scenarios (Table 5.4). Since the ecotoxicity scores for the US scenario were classified as significant, all other country scenarios that have a higher score would also be classified as significant. Otherwise, all other people equivalence scores were mainly similar to the US values; see Section 5.5.1 for the discussion on their significance. The total impact results per FU are presented in Appendix H – Tables H.21-22.

Table 5.3: Environmental impact results normalised by the US base-case scenario. Dark red = highest result; Light red = results >50% higher than the base-case value; Light green = results >50% lower than the base-case value; Dark green = lowest result.

	Impacts Normalised with US Base-Case Scores								
	Brazil	**China**	**Germany**	**India**	**Singapore**	**South Africa**	**Spain**	**UK**	**US**
Acidification	1.56	2.12	0.87	3.61	1.20	5.50	0.99	1.21	1.00
Ecotoxicity – freshwater	3.65	5.00	2.73	4.92	3.92	3.89	3.29	3.43	1.00
Ecotoxicity – marine	0.90	2.03	0.63	1.24	0.89	0.95	0.76	0.86	1.00
Ecotoxicity – terrestrial	4.94	16.24	2.29	7.28	3.01	4.31	2.63	2.60	1.00
Eutrophication – freshwater	1.70	2.19	1.46	2.09	1.88	1.92	1.64	1.71	1.00
Eutrophication – marine	1.14	1.03	1.12	0.99	1.02	1.07	0.98	1.01	1.00
Eutrophication – terrestrial	0.95	1.98	0.82	3.12	1.53	3.86	0.90	1.16	1.00
Global warming potential, excl. biogenic carbon	0.73	1.15	0.90	1.18	1.03	1.50	0.76	0.90	1.00
Global warming potential, incl. biogenic carbon	0.70	1.16	0.90	1.20	1.04	1.52	0.74	0.79	1.00
Human toxicity – cancer	0.09	0.75	0.07	0.12	0.10	0.11	0.08	0.09	1.00
Human toxicity – non-cancer	1.30	2.48	1.28	2.26	1.27	1.76	1.24	1.42	1.00
Ionising radiation	0.26	0.44	0.93	0.43	0.24	0.51	1.06	1.20	1.00
Ozone depletion	1.28	2.10	1.56	2.06	1.95	1.96	1.73	1.75	1.00
Photochemical ozone formation – human health	0.87	1.47	0.80	1.97	1.18	2.71	0.87	0.85	1.00
Photochemical ozone formation – vegetation	0.88	1.51	0.80	2.07	1.20	2.82	0.87	0.98	1.00
Resource depletion, mineral, fossils and renewables	1.28	2.02	1.29	2.51	1.80	1.89	1.35	1.60	1.00
Total freshwater consumption	1.25	1.17	1.02	1.13	1.05	1.11	1.05	0.98	1.00
Impact on water resources (scarcity)	0.73	1.32	1.14	2.17	0.65	1.58	2.15	0.69	1.00

Table 5.4: Environmental impact results normalised to global people emission equivalence (People Eq./yr.) and expressed as a percentage of the total global population (% Glo. Pop.). Red = results ≥ 0.001% of the global population.

	Brazil		China		Germany		India		Singapore		South Africa		Spain		UK		US	
	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.	People Eq./yr.	% Glo. Pop.
Acidification	9.3E+03	<0.001	1.3E+04	<0.001	5.2E+03	<0.001	2.2E+04	<0.001	7.2E+03	<0.001	3.0E+04	<0.001	5.9E+03	<0.001	7.2E+03	<0.001	6.0E+03	<0.001
Ecotoxicity – freshwater	9.6E+06	0.125	1.3E+07	0.172	7.2E+06	0.094	1.3E+07	0.169	1.0E+07	0.135	9.4E+06	0.123	8.6E+06	0.113	9.0E+06	0.118	2.6E+06	0.034
Ecotoxicity – marine	1.1E+05	0.001	2.5E+05	0.003	7.6E+04	0.001	1.5E+05	0.002	1.1E+05	0.001	1.1E+05	0.001	9.2E+04	0.001	1.0E+05	0.001	1.2E+05	0.002
Ecotoxicity – terrestrial	1.9E+07	0.253	6.3E+07	0.832	9.0E+06	0.118	2.8E+07	0.373	1.2E+07	0.154	1.6E+07	0.204	1.0E+07	0.134	1.0E+07	0.133	3.9E+06	0.051
Eutrophication – freshwater	1.2E+04	<0.001	1.5E+04	<0.001	1.0E+04	<0.001	1.5E+04	<0.001	1.3E+04	<0.001	1.3E+04	<0.001	1.2E+04	<0.001	1.2E+04	<0.001	7.1E+03	<0.001
Eutrophication – marine	4.9E+02	<0.001	4.5E+02	<0.001	4.8E+02	<0.001	4.3E+02	<0.001	4.4E+02	<0.001	4.3E+02	<0.001	4.2E+02	<0.001	4.4E+02	<0.001	4.3E+02	<0.001
Eutrophication – terrestrial	3.7E+03	<0.001	7.8E+03	<0.001	3.2E+03	<0.001	1.2E+04	<0.001	6.0E+03	<0.001	1.4E+04	<0.001	3.5E+03	<0.001	4.5E+03	<0.001	3.9E+03	<0.001
Global warming potential, incl. biogenic carbon	1.2E+04	<0.001	2.1E+04	<0.001	1.6E+04	<0.001	2.1E+04	<0.001	1.8E+04	<0.001	2.5E+04	<0.001	1.3E+04	<0.001	1.4E+04	<0.001	1.8E+04	<0.001
Human toxicity – cancer	3.2E+04	<0.001	2.7E+05	0.004	2.5E+04	<0.001	4.5E+04	0.001	3.6E+04	<0.001	3.5E+04	<0.001	2.9E+04	<0.001	3.2E+04	<0.001	3.6E+05	0.005
Human toxicity – non-cancer	2.2E+04	<0.001	4.1E+04	0.001	2.1E+04	<0.001	3.8E+04	<0.001	2.1E+04	<0.001	2.7E+04	<0.001	2.1E+04	<0.001	2.4E+04	<0.001	1.7E+04	<0.001
Ionising radiation	3.8E+02	<0.001	6.3E+02	<0.001	1.3E+03	<0.001	6.2E+02	<0.001	3.4E+02	<0.001	6.8E+02	<0.001	1.5E+03	<0.001	1.7E+03	<0.001	1.4E+03	<0.001
Ozone depletion	1.0E+02	<0.001	1.7E+02	<0.001	1.2E+02	<0.001	1.6E+02	<0.001	1.5E+02	<0.001	1.4E+02	<0.001	1.4E+02	<0.001	1.4E+02	<0.001	7.9E+01	<0.001
Photochemical ozone formation – human health	1.1E+04	<0.001	1.9E+04	<0.001	1.0E+04	<0.001	2.6E+04	<0.001	1.5E+04	<0.001	3.3E+04	<0.001	1.1E+04	<0.001	1.1E+04	<0.001	1.3E+04	<0.001
Photochemical ozone formation – vegetation	7.5E+03	<0.001	1.3E+04	<0.001	6.8E+03	<0.001	1.7E+04	<0.001	1.0E+04	<0.001	2.2E+04	<0.001	7.4E+03	<0.001	8.2E+03	<0.001	8.5E+03	<0.001
Resource depletion, mineral, fossils and renewables	2.0E+04	<0.001	3.2E+04	<0.001	2.0E+04	<0.001	4.0E+04	0.001	2.9E+04	<0.001	2.8E+04	<0.001	2.1E+04	<0.001	2.5E+04	<0.001	1.6E+04	<0.001

5.4.2 Hot-spot Analysis Results and Impact Correlations

The percentage impact contributions that each life-cycle phase and processing block have toward the different environmental impact categories were calculated and are presented for each country scenario in Appendix H – Tables H.23-38. For all scenarios, the supply phase generated the highest impact, consistent with the US base case scenario results (Chapter 4). The same two processing blocks (Stirred Tank Fermentation and Product Harvest) ranked highest across all scenarios. However, in the Brazil, Germany, India, Spain and the UK scenarios, Stirred Tank Fermentation dominated as the top contributor in most impact categories by generating a wider percentage gap to the contribution generated by the Product Harvest block as compared to the US scenario. Further hot-spot analyses were carried out on the Stirred Tank Fermentation processing blocks to understand the different impacts of the scenarios better.

As a whole, results concurred with Chapter 4, where the five key inputs, butyl acetate, glucose, electricity, sodium hydroxide and steam, were also shown to have the greatest impacts across all scenarios. However, an additional input, ammonium sulphate, was found to contribute highly towards many impact categories, depending on the scenario in question. These impact categories were: acidification, ecotoxicity (all subcategories), eutrophication – freshwater, human toxicity – non-cancer, ionising radiation, ozone depletion and resource depletion.

Ammonium sulphate is an inorganic chemical that is frequently used as a nitrogen source for organisms. In the 6-APA production process, the material is required heavily in the Stirred Tank Fermentation block; this may explain this block's higher process contribution in most other scenarios compared to the US scenario. The substance can be extracted or produced via many pathways (James and Speight, 2017). For the US scenario, the ammonium sulphate LCI used modelled the chemical production (in the US) through ammoxidation of propene (reaction with ammonium and air), which produces acrylonitrile and hydrocyanic acid as well. For other scenarios, country-specific LCIs were unavailable, regional LCIs [RER] (Europe) and [RoW] (“Rest of the World” – countries not in Europe or North America) were used according to the location of each country. Both [RER] and [RoW] LCIs for the substance referenced its production from ammonia and sulphuric acid, which is said to be highly exothermic (Symeonidis, 2020a, 2020b). Analysing and comparing the LCIs individually, [RoW] generated the highest impacts; it was followed by [RER] LCI and then the US-specific LCI. Hence, according to the databases, obtaining the material through the co-production process (assumed for the US scenario) was environmentally favourable to the dedicated production process (assumed for other scenarios).

For the impact categories that ammonium sulphate contributed significantly to, the material played a role in determining the rank order among the scenarios. However, as shown in Chapter

4, each impact category often had multiple hot spots, i.e. more than one high contributor (Table 4.12). The numerous contributing factors made the relationship between manufacturing location and the changes in impacts a complex paradigm. For example, acidification results showed that the highest contributors were fossil fuels percentage in the electricity mix and the supplies of glucose and ammonium sulphate. Since the supply of glucose was modelled the same for all scenarios, it was known not to have determined the rank order. On the other hand, since the supply of ammonium sulphate and electricity were modelled using different and more location-specific LCIs for each scenario, they were the factors that affected the overall ranking.

Figure 5.2 presents a graph comparing the overall **acidification** scores generated by each scenario and the electricity grid mix assumed. It shows that all countries scenarios that were modelled using the [RoW] LCI for ammonium sulphate, except Singapore, generated the highest scores. The analysis showed that using coal for electricity generation, which releases sulphur dioxide emissions during coal combustion (Häsänen et al., 1986), was the highest contributor for South Africa, India and China scenarios. The percentage used of coal energy in the mix also correlated directly with acidification results for these countries. However, Germany used the 4th highest level of coal in their mix but ranked lowest for acidification potential. In addition, both the UK and Spain scenarios were modelled using the same LCI for ammonium sulphate, [RER], as Germany and used a lower coal percentage in their electricity mixes, but resulted in a higher acidification score. These results suggested that the technical efficiencies, in the extraction or the processing of raw materials, for generating electricity (in different countries) have affected the acidification scores.

Similarly, the Brazil and Singapore scenarios assumed the same LCI for ammonium sulphate. Although Singapore used a higher fossil fuel percentage in the energy mix, it generated a lower score than Brazil. The determining contributor for Brazil's ranking was the use of heavy fuel oil. The results suggested that both the type of feedstock and technical efficiency affected the impact scores.

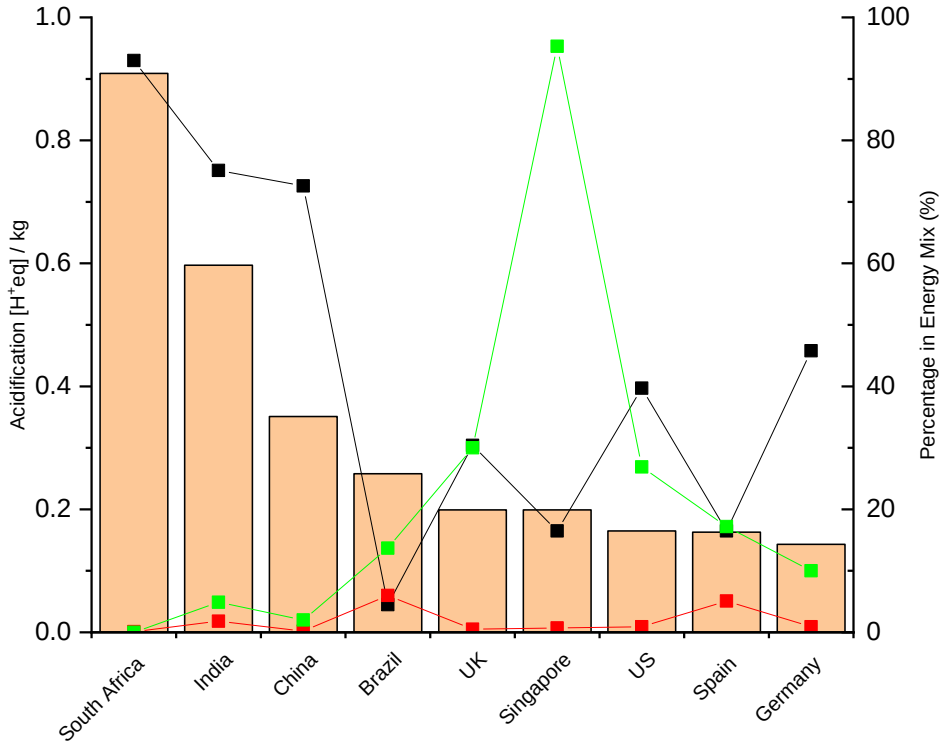

Figure 5.2: (Left axis) Acidification potential per kilogram of 6-APA produced. (Right axis) Percentage of fuel in the national energy mix. ■ Coal in energy mix. ■ Heavy fuel oil in energy mix. ■ Natural gas in the energy mix. The graph highlights the acidification potential of each country scenario for the manufacture of 6-APA.

Although modelling each country scenario with the same LCI for the supply of glucose has highlighted more clearly how other inputs have affected the overall impacts of 6-APA production, it was clear that using non-specific LCIs for glucose was a source of inaccuracy. Environmental impacts from material supply should change with the material source. Hence, the impact profile of glucose should vary for each country scenario. **Eutrophication – marine and total freshwater consumption** were the two impact categories where glucose contributed over 70% towards the total scores for all scenarios. These two impact categories were also shown to deviate the least when comparing 6-APA production in different countries – Table 5.3 showed a difference of up to 15% for eutrophication - marine (between Brazil and Spain) and up to 22% for freshwater consumption (between Brazil and the UK). The variation in impacts was derived from energy generation; for eutrophication – marine, it was due to biomass and natural gas energy generation, and for freshwater consumption, it was due to hydroelectricity. However, since the impact of glucose had such a high dominance in these categories, it had masked the effect of changing production location. Country-specific data on the supply of glucose is needed to check how variable are the environmental impacts associated with this material. It would also affirm whether the differences in the impacts scores generated by the scenarios were representative.

Like ammonium sulphate, butyl acetate and sodium hydroxide were also modelled using regional-specific or global LCIs. The regional codes used for sodium hydroxide supply were: [US] for the US, [EU-28] for European countries, but [GLO] (global) for all other scenarios since [RoW] values are not available. For butyl acetate, since [GLO] and [RNA] (North American) LCIs for the production of the material was unavailable, the US scenario was modelled using the [RER] LCI as for European countries due to known similarities in their electricity mixes and levels of socio-economic development. An [RoW] LCI for butyl acetate was available for all other country scenarios. When the LCIs for individual materials were compared, it showed that the [GLO] LCI for sodium hydroxide, produced via the average global mix of chlorine-alkali-electrolysis, generated the highest impact. This LCI was followed by the [EU-28] LCI, where the production mix employed mainly the membrane chlorine-alkali-electrolysis method, and then the [US] LCI, where the primary method used was diaphragm chlorine-alkali-electrolysis. For butyl acetate, both [RoW] and [RER] coded LCIs represented its production via the same process (the esterification of 1-butonal with acetic acid), but the [RoW] LCI generated the higher impacts; similar to the case for ammonium sulphate.

Since the two materials contributed highly towards various impact categories, the LCIs used to model their supply also helped determine the scenarios' impact rankings. It was found that the ranking for each impact category largely followed the trend of RoW countries > European countries > the US. For instance, ammonium sulphate, butyl acetate and sodium hydroxide, all contributed toward **ecotoxicity – freshwater and terrestrial**; and the overall rankings from the highest to the lowest score were: China > India > Singapore > South Africa > Brazil > UK > Spain > Germany > US; and China > India > Brazil > South Africa > Singapore > Spain > UK > Germany > US, respectively. Both rank orders can be simplified to “Rest of the World” countries > EU countries > the US. Country-specific factors then determined the ranking amongst countries in the same regional class. These factors included the countries’ electricity mix, the country-specific LCIs used for energy production (electricity and steam) and other processes, namely the supply of nitric acid, the assumed acid cleaning substance, and water purification.

Similarly, all three input materials also contributed toward **resource depletion**; the ranking was India > China > South Africa > Singapore > UK > Spain > Germany > Brazil > US. Here, this ranking largely followed RoW countries > EU countries > US schematic, but Brazil was ranked eighth. The ranking is an example of how inputs modelled using country-specific LCIs have affected the overall ranking. The main country-specific LCIs that affected the overall impact values were nitric acid, purified water, and electricity supply, like for ecotoxicity. The Brazil LCI for producing purified water and nitric acid was the least impactful to resource depletion as compared to other

country scenarios and were sufficient to compensate for the higher impact associated with using [RoW]/[GLO] over [RER] and [US] LCIs for ammonium sulphate and sodium hydroxide.

It must be noted that the rankings amongst country-specific LCIs for individual processes and/or materials did not follow the ranking trends exhibited by regional LCIs, i.e. countries outside of Europe and America do not necessarily generate higher impacts. It was also found that the order of impact scores generated by country-specific LCIs do not follow any trends in general. For instance, the ranking of the environmental impacts associated with one process/material by location was not the same for another.

A clear example of this would be **ecotoxicity – marine**; the ranking was: China > India > US > South Africa > Brazil > Singapore > UK > Spain > Germany. The order was determined mainly by the use of coal to generate electricity and the supply of natural gas to generate process steam. Table 5.5 highlights the ecotoxicity – marine potentials for generating 1 kWh of electricity from coal and 1kWh of steam from natural gas, and the percentage of coal in the electricity grid mixes are also presented for reference. The table highlights the ranges of emissions from carrying out similar processes and that the rankings of countries per kWh were not consistent between coal energy generation and steam generation. Although South Africa employed the highest amount of coal in its electricity mix, the impact per kWh produced was the lowest compared to all other countries, i.e. the least impactful per kWh of energy produced. China and India employed a relatively high percentage of coal in their grid mixes and generated high impact per kWh. For producing process steam from natural gas, the US was found the highest, followed by China and Germany. As China ranked high in both processes, the China scenario ranked highest for this impact category.

In addition, Table 5.5 demonstrates that the ability to generate lower impacts does not necessarily depend on a country's stage of economic development. This finding suggested that the range of emissions may have resulted from where coal and natural gas were extracted or the specific techniques that were in place. The land composition combined with the default methods for extraction would result in different emission profiles. As a whole, this has suggested that technical efficiencies cannot be generalised; i.e. a country does not exhibit the same level of technical efficiency for all processes it conducts.

Table 5.5: Ecotoxicity – marine potentials for generating 1kwh of steam from natural gas and electricity from hard coal at different locations. Life cycle impact assessment was carried out on life cycle inventories provided by GaBi databases (Sphera, 2020a) *to understand the impact per kilowatt-hour (kWh) of electricity produced. Red = highest ecotoxicity score in the column. Green = lowest ecotoxicity score in the column.*

Country	% Coal in Electricity Mix	Ecotoxicity – Marine (kg 1,4-DB eq. / kWh)	
		Electricity from Coal	Process Steam from Natural Gas
South Africa	93.0	3.67E-05	6.37E-06
India	75.1	1.66E-04	9.63E-06
China	72.6	5.67E-04	1.97E-05
Germany	45.8	7.51E-05	1.67E-05
US	39.7	1.19E-04	2.57E-05
UK	30.4	9.11E-05	8.53E-06
Spain	16.5	1.36E-04	5.26E-06
Brazil	4.5	5.13E-04	1.24E-05
Singapore	1.1	1.70E-04	7.49E-06

Other impact categories where impacts associated with electricity and/or steam production determined the rank order of the scenarios were: eutrophication – terrestrial, global warming potential, human toxicity – cancer, ionising radiation, and impact on water resources. For the hot-spot analysis of the US scenario, **human toxicity (cancer)** was shown to be dominated by the supply of natural gas for steam production at the plant. Although this was also the case for the China scenario, for all other scenarios, the supply of natural gas contributed below 0.5%, and the highest contributing factor was the supply of glucose and then butyl acetate. Hence the rank order, US > China > India > South Africa > Singapore > Brazil > UK > Spain > Germany, was first determined by the emissions associated with steam-generation, then by the regional LCIs used for butyl acetate, which followed the [RoW] > [RER] order of impacts. Since the results showed that the US and China's processes involved in supplying natural gas were significantly more impactful than in all other countries studied, it presented a potential area for both countries to lower toxic emissions associated with natural gas supply.

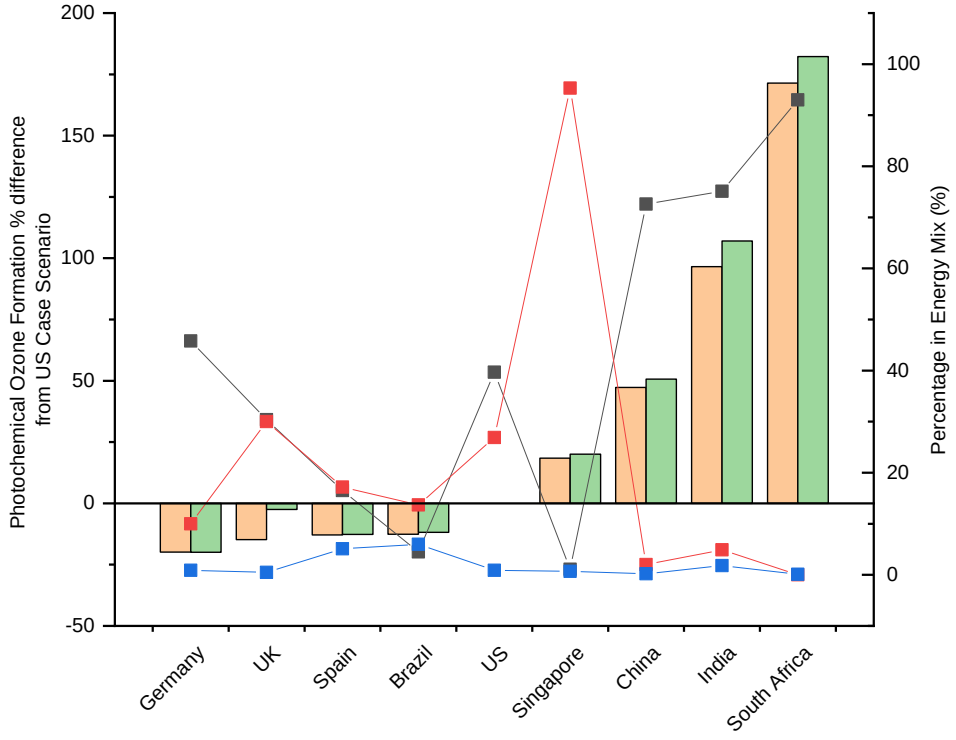

Figure 5.3: The effect of different fossil fuel percentages in the energy mix on the photochemical ozone formation (POF) potentials of each country scenarios normalised to the US base case scenario. POF potentials (impact on human health and impact on vegetation) were normalised to the results generated from the US scenario. ■ Coal % in the energy mix. ■ Natural gas % in the energy mix. ■ Oil % in the energy mix. Countries with higher fossil fuel percentage in the energy mix than the US are typically shown to have a greater impact on POF.

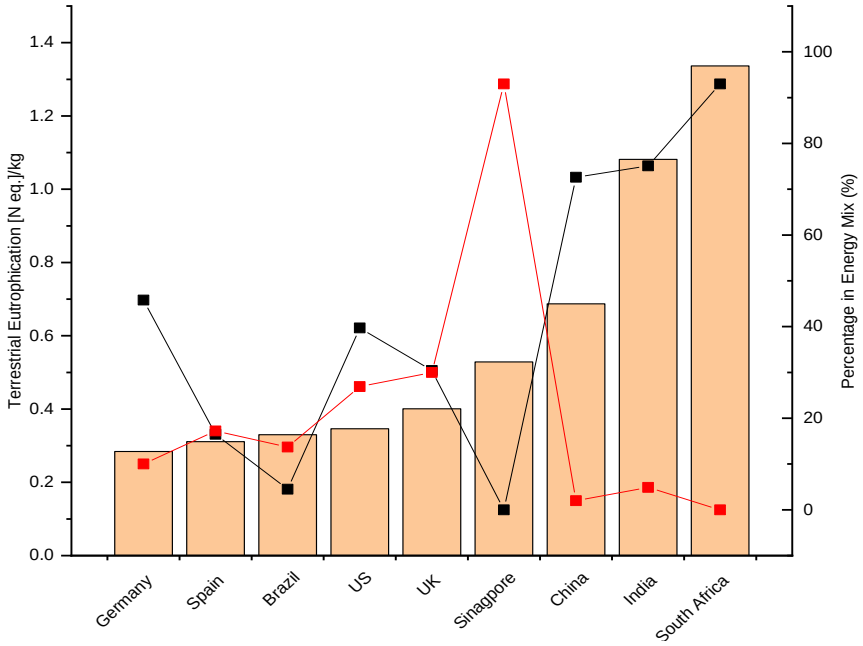

Figure 5.4: (Left axis) Terrestrial eutrophication potential per kilogram of 6-APA for each country scenario. (Right axis) Percentage of fuel in the national energy mix. ■ coal in the energy mix. ■ Natural gas in the energy mix. The graph highlights the terrestrial eutrophication potential of each country scenario for the manufacture of 6-APA.

The ranking for **photochemical ozone formation (POF) and eutrophication – terrestrial** followed a similar trend to acidification. In Figures 5.3 and 5.4, all "Rest of the World" classed countries except Brazil were ranked highest; this was attributed to the LCI used to model sodium hydroxide and the level of coal assumed in the electricity mix. As for the acidification impact category, Germany ranked lowest despite using >50% coal in its mix. From analysing the individual LCIs, the German LCI for coal energy production generated the 2nd lowest POF score (2.88 m^2*UES*ppm*hours/kWh, ranged – 2.26 (US) to 10.7 (South Africa) m^2*UES*ppm*hours/kWh). The supply of natural gas determined Brazil's ranking, while the country used the least coal energy, its impact deriving from steam generation was the highest (ranged 0.95 (UK) to 3.75 (Brazil) m^2*UES*ppm*hours/kWh). The different rank orders reiterated that a country with a lower emission for one form of energy production (compared to other countries) does not mean it would generate a lower emission for another process.

As shown in Chapter 4, the main factors contributing to the overall **global warming potential (GWP)** scores were the level of fossil fuels in the electricity mix and the use of natural gas for steam generation. Unlike the previously discussed impact categories, the level of fossil fuels (coal and natural gas) used in the mix did correlate with the overall impacts generated by the country scenarios. Figure 5.5 presents the percentage differences between the GWP values generated by each country scenario and that of the US scenario. South Africa utilised the most coal energy in its energy mix and generated the highest GWP values. Singapore employed the least coal energy but the most energy natural gas in its mix, which led it to generate the 4th highest scores. Lastly, Brazil had the least fossil fuel in its mix and resulted in the lowest GWP values.

From analysing individual LCIs for electricity generation and studies in the literature, they showed that, on average, the combustion of coal for electricity generation emits 2.5 times more GHG than using natural gas to generate the same electrical output (Fout et al., 2015). With this information, it was possible to express natural gas in terms of coal according to their relative GHG emission rates during combustion. Hence, a fossil fuel index was developed to represent the total percentage of fossil fuel in an electricity mix as the total share of coal energy in the mix (Eq 5.1) - coal percentage in mix equivalence. Table 5.6 presents the fossil fuel index calculated for each scenario, which was overlaid onto Figure 5.5 and demonstrated the correlation visually. Figure 5.6 illustrates that the correlation between the calculated index and GWP per kilogram of 6-APA produced was 91% accurate using the R-squared method.

(Eq. 5.1) $$Fossil\ Fuel\ Index =$$

$$[Coal\ \%\ in\ energy\ mix + (Natural\ gas\ \%\ in\ energy\ mix\ \times 0.4)]\ \times 100$$

The sources for the variances obtained for the correlations was assumed likely to be from the technical differences for carrying out similar processes (as discussed impact categories). Other factors, such as the use of natural gas for steam generation and transport, which contribute to the overall GWP scores, were attributed to the baseline values (the y-intercept) in the correlation models. When the fossil fuel index equates to 0, the GWP values associated with 6-APA, 37.1 kg and 45.0 $kg_{CO2}/kg_{product}$ for excluding and including biogenic carbon, respectively, indicating the average impacts associated with other fossil fuel usages.

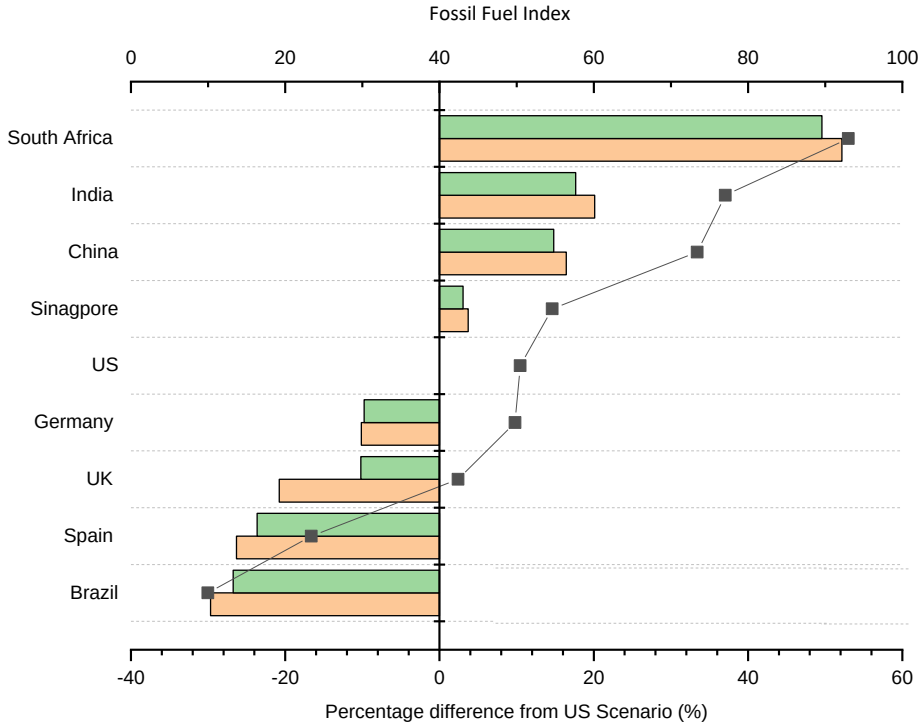

Figure 5.5: Comparing global warming potential relative to US base case scenario. Global warming potential (GWP) results of each country scenario were normalised with the US GWP results to obtain the percentage difference to the US scenario. The graph illustrates that GWP is a function of the fossil fuel index, calculated using Eq. 5.1. The index expresses the percentage of fossil fuel in the energy mix to the percentage of coal equivalence in the mix regarding their GHG emission per kWh of electricity produced. ■ *Fossil fuel index* *GWP excl. biogenic carbon.* *GWP incl. biogenic carbon.*

Table 5.6: Fossil Fuel Index for each country scenario using (Eq.5.1). Coal % in energy mix

Country	Coal % in Energy Mix	Natural Gas % in Energy Mix	Fossil Fuel Index
Brazil	4.5	13.7	9.98
China	72.6	2	73.4
Germany	45.8	10	49.8
India	75.1	4.9	77.1
Singapore	1.1	95.3	39.2
South Africa	93	0	93.0
Spain	16.5	17.2	23.4
UK	30.4	30	42.4
US	39.7	26.9	50.5

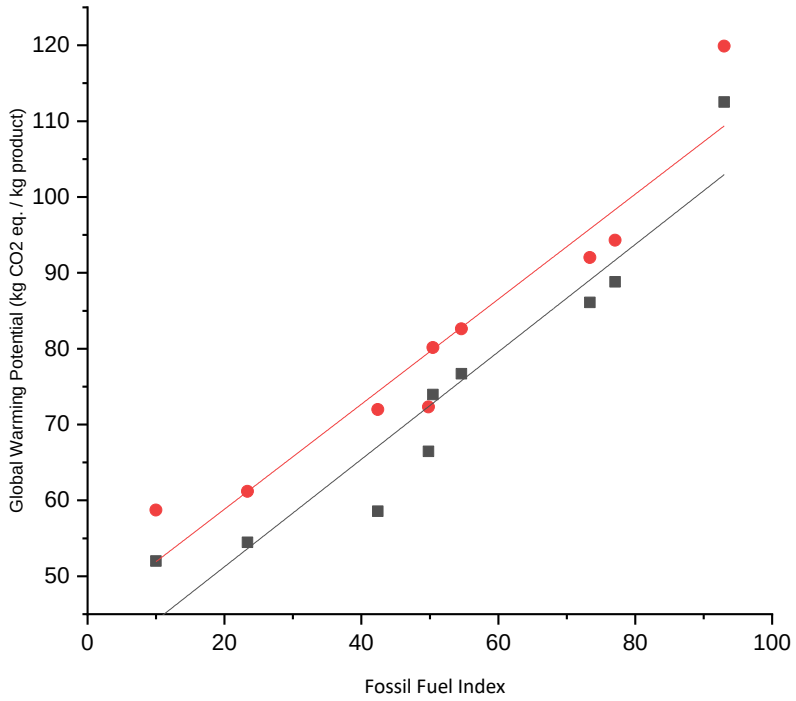

Figure 5.6: Correlation between Fossil Fuel index and global warming potentials (GWP) per kilogram of 6-APA produced for each country scenario. Fossil fuel index calculated using Eq. 5.1 expresses each country's fossil fuel percentage in the energy mix as coal percentage by considering average GHG emission per kWh of electricity produced by the particular fuel. Data points: ■ GWP excl. biogenic carbon. ● GWP incl. biogenic carbon. Trend lines: ━ y = 0.71x + 37.1 (R-square = 0.91) ━ y=0.62x + 45.0 (R-square = 0.91). y = GWP and x = Fossil Fuel index.

In the case of **ionising radiation**, the percentage of nuclear energy in the energy mix influenced the overall impact scores for each scenario. Although Singapore had no nuclear power in its energy mix and generated the lowest ionising radiation score, it was very similar to the results obtained for the Brazil scenario, which assumed 2.6% nuclear power in its mix. Both the US and the UK scenarios assumed 19% nuclear power in their mixes and generated the highest scores. Figure 5.7 presents the correlation between nuclear power in mix percentage and overall ionising radiation scores per kilogram of 6-APA produced. The graph shows that the linear correlation model fitted had a 92.8% fit (R-square). Like the GWP correlation models, other processes associated with ionising radiation, such as the supplies of glucose, butyl acetate and ammonium sulphate, can explain the baseline value for when no nuclear power was employed. The overall contributions from butyl acetate and ammonium sulphate to this impact category were low but have helped determine the rank order amongst countries with similar percentages of nuclear power in their energy mixes. The variance in the linear model can again be explained by emission differences between carrying out similar processes in different countries.

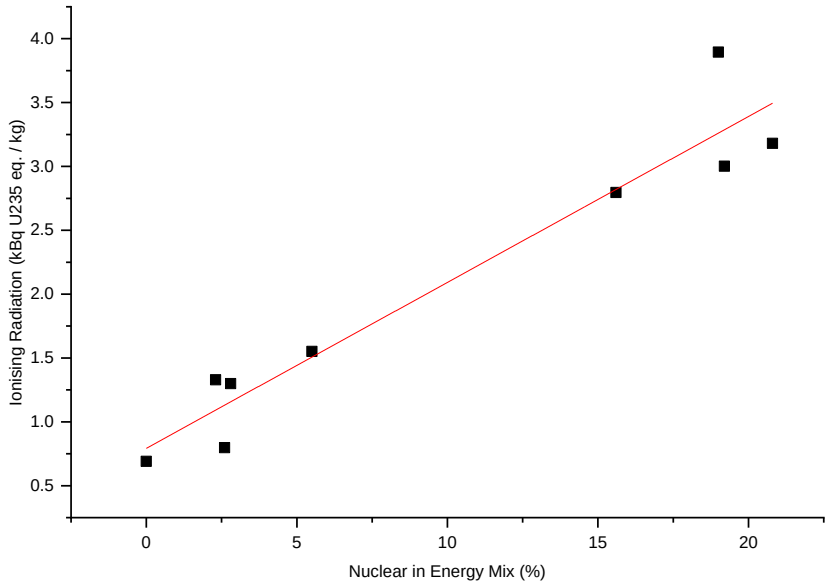

Figure 5.7: Correlations between nuclear energy in each scenarios' electricity mix and their overall ionising radiation potential per kilogram of 6-APA produced. — *y = 0.130x + 0.793 (R-square = 0.928)*

Lastly, the **impact on water resources (water scarcity)** varied greatly amongst the country scenarios despite consuming similar amounts of freshwater (Figure 5.8). The impact on water resources considered the local water scarcity levels and the amount of freshwater consumed. As discussed in Chapter 4, the main reason for water consumption was the supply of glucose and other water-consuming processes, including the generation of purified water for plant use, generating electricity with hydropower, and using cooling water during fermentation processes. Brazil was the highest consumer of freshwater because of the high hydropower percentage in its national energy mix. However, the scenario generated the third-lowest impact score due to freshwater being not scarce (water scarcity level - 0.48 UBP/m^3). South Africa was assumed to have only 0.4% of hydroelectricity in its mix but consumed a relatively high amount of freshwater; it was diagnosed that coal combustion, which made up 93% of the country's energy mix, was associated with high water usage (Mielke et al., 2010). Since South Africa has a relatively high water scarcity level (600 UBP/m^3), it, in turn, generated the third-highest impact score.

Hot-spot analysis revealed that the total freshwater consumption score was determined mainly by the level of hydroelectricity, then coal and then natural gas in the energy mix. A comparison of water consumption rate due to different electricity generation methods was carried out by reviewing the GaBi database (Sphera, 2020a) (Table 5.7). The review showed that the average water consumption associated with hydroelectricity was approximately 30 times higher than the average for coal electricity generation. The difference in consumption was similar to the findings presented by Mielke et al. (2010). European countries were observed to incur lower water

consumptions during electricity generation for all three methods. This observation suggested that technical efficiency in preventing water loss was better in Europe than in other countries.

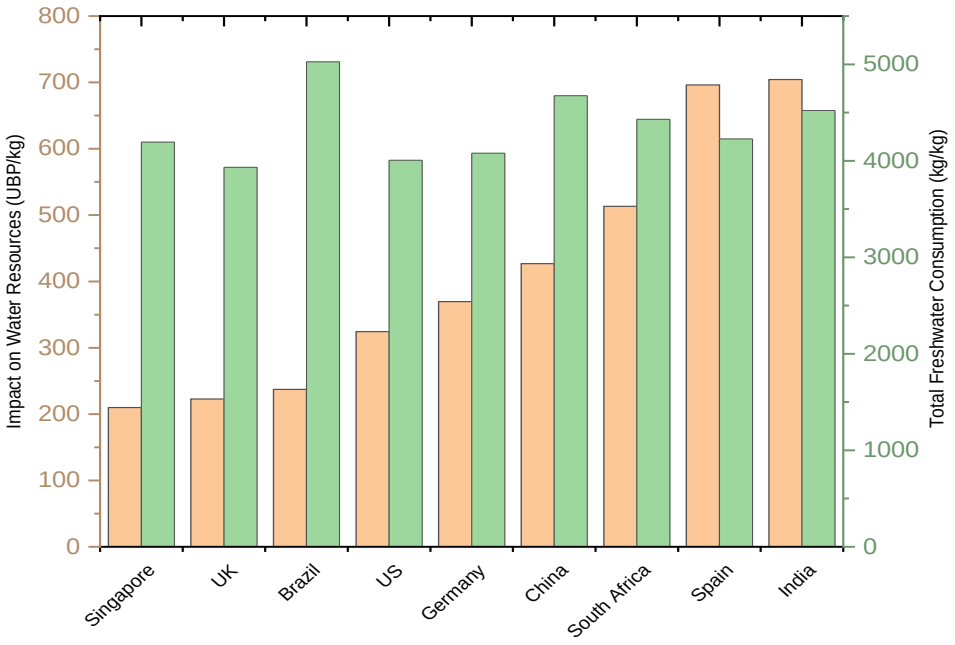

Figure 5.8: Comparing environmental impact potentials on water resources (due to water consumption) and total freshwater consumption between location scenarios. Total freshwater consumption per kilogram of 6-APA produced. Impact on water resources per kilogram of 6-APA produced.

Table 5.7: A review of water consumption rates by different electricity production methods at different locations. Data was sourced from the GaBi database and represents water consumption from the cradle-to-gate of electricity production, including sourcing resources (Sphera, 2020a).

	Water Consumption per kWh net energy produced ($kg_{water}/kWh_{electricity}$)		
	Hydroelectricity	**Electricity from Coal**	**Electricity from Natural Gas**
Brazil	19.27	0.31	0.21
China	16.26	0.54	0.31
Germany	3.19	0.43	0.18
India	8.32	0.55	0.32
Singapore	-	0.42	0.26
South Africa	18.97	0.58	-
Spain	23.29	0.47	0.25
UK	12.34	0.47	0.31
US	16.48	0.45	0.29
Average	**14.76**	**0.47**	**0.27**

A water index was first developed (Eq. 5.2) to begin evaluating the relationship between freshwater consumption due to energy generation, the country's water scarcity level and the overall impact on water resources for 6-APA production. The water index was designed to express water consumptions due to electricity generation in terms of hydroelectricity percentage in energy mix equivalences. As derived from Table 5.7, it was assumed that every 30% of coal energy in the electricity mix would consume the same amount of freshwater as 1%

of hydroelectricity in the mix. For simplicity, calculations to convert natural gas percentage was not included in the equation. The index for each country scenario is presented in Table 5.8.

(Eq. 5.2) $$Water\ Index = [Hydroelectricity\ \%\ in\ energy\ mix + (Coal\ \%\ in\ energy\ mix\ \div\ 30)]\ \times 100$$

By plotting this index against water scarcity and overall impact on water resources, a correlation was found with an R-square of 0.91 (Figure 5.9). Similar to the correlation model created for GWP, there was a baseline impact, as indicated by the z-intercept of the correlation model. The baseline impact accounted for other factors that were not considered in generating the correlation model, such as purified water production at the manufacturing facility and water consumption associated with the supply of process materials. The variance was explained by the technical efficiency differences in minimising water consumptions when conducting similar processes in different countries.

Table 5.8: Comparing the ranking of each scenario's impact on water resources to their water scarcity level, hydroelectricity and coal power in the energy mix. The water index represents the potential for water consumption due to the combination of resources used for electricity generation. The water index expresses the energy mix in terms of hydroelectricity percentage equivalence (in the mix) and is derived using Eq. 5.2.

		Factors			
Rank (Highest to Lowest Impact)	**Country**	**National Water Scarcity (UBP/m3)**	**Hydroelectricity in Energy Mix (%)**	**Coal Power in Energy Mix**	**Water Index (by Energy)**
1	India	1300	10.2	75.1	12.70
2	Spain	811	14.2	25.9	15.06
3	South Africa	600	0.4	93.0	3.50
4	China	360	18.6	72.6	21.02
5	Germany	421	3.1	45.8	4.63
6	US	232	6.1	39.7	7.42
7	Brazil	0.48	63.2	4.5	63.35
8	UK	74.7	1.8	30.4	2.81
9	Singapore	5	0.0	1.1	0.04

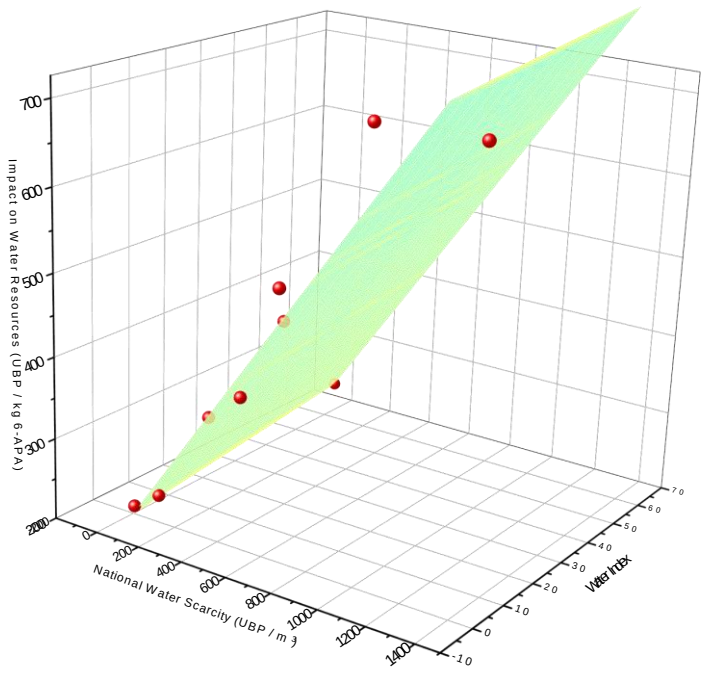

Figure 5.9: Correlation showing the effect of a country's water scarcity level and energy mix on overall environmental impact on water resources. ▭ *Plane Fit z = 221 + 0.431x + 0.593y (R-Square = 0.91), z = impact on water resources (UBP/kg$_{produced}$); x = national water scarcity level (UBP / m^3); y = Water Index as calculated using Eq. 5.2 to represent each country scenario.*

5.5 DISCUSSION

As Chapter 4, before recommendations can be drawn on the siting of 6-APA production, the significance of the impacts generated must first be discussed. However, in addition to understanding the magnitude of impacts, the limitations of the results must be explored to understand its legitimacy. For instance, while the significance of impacts provided insights on the order of environmental urgency for each impact category, the additional critiques provided boundaries for which recommendations can be drawn from the LCA comparative study. Hence, the significance and limitations are provided first before presenting the considerations for siting biopharmaceutical facilities.

Next, with considerations for the significance and limitations of the results, the thesis explores by estimating the distribution of environmental impacts due to global 6-APA manufacture. This aimed to understand whether the relationships and correlations drawn from Chapter 4 (between product titre and production scale, and impacts) and this chapter (between country-specific variables and impacts) can further provide insight into the environmental sustainability of the biopharmaceutical industry. The practical steps and policy recommendations derived below enforces the need for industry and government to work cohesively to achieve global sustainable development.

5.5.1 Significance and Limitations of the Impacts Generated

As the environmental impact results for the US scenario were thoroughly analysed in Chapter 4, the significance of the results generated by all other scenarios was determined mainly by comparison to the base-case study. First, impact categories that were previously determined as negligible for the US scenario: acidification, eutrophication – all subcategories, human toxicity – all subcategories, ionising radiation and ozone depletion were reviewed to understand whether it was equally the case for all other country scenarios. For human toxicity – cancer and ionising radiation, as results generated by all scenarios were either lower or similar to those generated by the US scenario, both impact categories were automatically deemed to have remained insignificant. For the other impact categories, the highest impact scores were assessed on their significance using the same methods used in Chapter 4. This exercised confirmed that all acidification, eutrophication, human toxicity – non-cancer and ozone depletion scores were also negligible.

Those impact categories previously determined as significant, ecotoxicity – all subcategories, global warming potential (GWP) – all subcategories, photochemical ozone formation (POF) – all subcategories, resource depletion, and total freshwater consumption, impact values that were higher than the value generated for the US scenario were assumed significant. These were ecotoxicity, resource depletion and total freshwater consumption scores for all scenario. Although freshwater consumption remained relatively the same for all scenarios due to process needs, the maximum change from the US value was +25% due to higher hydroelectricity use in the Brazil scenario. The impact on water resources varied substantially (~200 to 700 UBP/kg). While there were no benchmarks to suggest at what level of impact would a process be determined as significantly impactful, the results did indicate that Singapore, the UK and Brazil had the lowest impacts (< 250 UBP/kg). For GWP and POF, the maximum changes from the US impact values were -30% (Brazil) and +182% (South Africa) and were found related to the percentage of fossil fuels in the countries' electricity mix. The GWP (incl. biogenic carbon) result generated for the Brazil scenario was 51.7 $kg_{CO2eq.}/kg_{product}$ and therefore lower than the literature referenced value for a traditional pharmaceutical (67.6 $kg_{CO2eq}/kg_{product}$ (Wernet et al., 2010)). Since 6-APA is a product intermediate and its transformation into the final API would generate further impacts, it was unclear whether the GWP value required improvement. It would be more informative to compare GWPs when 6-APA is converted to an antibiotic, i.e. when both products are at the same life cycle gate, it would help gauge whether the impacts associated with the production of 6-APA are significant as a whole. The need for reference values are applicable for POF and impact categories as well; otherwise, it was unclear whether environmental impacts generated by a process require immediate attention. From comparing

the nine scenarios only, it was clear that countries with lower use of fossil fuels, such as Brazil and Spain, were environmentally preferred in terms of GWP.

The most significant difference from the US impact scores was observed in ecotoxicity. Table 5.9 presents the percentage contributions a course of treatment has towards an average person's annual impacts toward ecotoxicity – freshwater and terrestrial. It was found that all scenarios generated impacts equivalent to substantial portions of a single person's emissions in one year. Hence, ecotoxicity can be classified as significant for all scenarios. The ecotoxicity – terrestrial score generated by the India and China scenarios, in particular, represented nearly 90% and more than one person's annual impact. These results suggested that the reduction of ecotoxicity – terrestrial in the India and China was essential. However, the legitimacy of these results must first be considered.

Table 5.9: The emissions contributions of a course of treatment, which can be supported by the production of 6-APA, towards a person's average annual emissions. Normalised impacts (i.e. people equivalence scores) were divided by the number of treatment courses (32.3 million) by assuming that all 6-APA would be converted to amoxicillin and that the average course of treatment requires 500mg, three times a day for seven days. The calculated value was then represented as the percentage of a person's yearly emission. Red = highest value; Green = lowest value.

	% annual per capita emission per course of treatment	
	Ecotoxicity – Freshwater	Ecotoxicity – Terrestrial
Brazil	29.6	59.8
China	40.6	186.5
Germany	22.2	22.8
India	39.9	88.1
Singapore	31.8	36.4
South Africa	29.1	48.3
Spain	26.7	31.8
UK	27.8	31.4
US (base case)	8.1	12.3

From hot-spot analysis, it was found that ecotoxicity – freshwater and terrestrial scores were derived mainly from the supply of ammonium sulphate, butyl acetate and sodium hydroxide amongst energy generation from fossil fuels. While the LCIs for both butyl acetate and sodium hydroxide modelled the average production mixes for each region, i.e. the average impacts generated by the range of processes used in the region, the LCIs used for ammonium sulphate assumed specific processes. As shown above, the production of ammonium sulphate via a co-production process, assumed for the US scenario, was significantly more environmentally preferable to the dedicated process that was assumed for both European and "Rest of the World" countries. Since ammonium sulphate is often produced as a by-product/co-product (IHS Markit, 2019; Symeonidis, 2020a, 2020b), all regions are likely to have the capabilities to manufacture the material by the more environmentally preferred option. This could mean that the models employing [RER] and [RoW] LCIs for ammonium sulphate may not be representative of the supply chain employed by a company. The comparison between the scenarios can only

suggest that when companies choose suppliers, they should consider how materials are sourced and manufactured.

Nonetheless, for materials that were assumed produced via similar processes for the different regions, for instance, butyl acetate and sodium hydroxide, it was clear that [RoW] and [GLO] geographically coded LCIs generated consistently higher impacts than [EU-28], [RER], [RNA] and [US] LCIs. This trend suggested that if all countries scenarios were modelled using regional-specific LCIs for the "same" process, the overall ranking for ecotoxicity would remain in the order of "Rest of the World" countries > European countries > the US. The reason why [RoW] and [GLO] LCIs for process materials generated higher impacts than other LCIs was unclear, as readily available documentations do not disclose extensively the processes that were considered into developing the inventories. This uncertainty presented a limitation for using readily available LCIs from databases, as processes become black boxes in the LCA study.

From reviewing the available documents for EcoInvent LCIs (for example, Symeonidis (2020a)), the common datasets used to model cradle-to-gate inventories for processes included input materials, energy uses, infrastructure and emissions. Out of the four sets of data, the factor determining the overall impact differences between geographically different LCIs could be the methods assumed for energy generation. The combustion of fossils fuels for energy is known to generate higher environmental impacts than other energy sources. Since different countries employ different energy mixes, it is plausible that the results generated through producing materials in different countries and regions would also differ. Since many [RoW] countries, including China, India and South Africa, employ a higher percentage of fossil fuels in their electricity mixes than European countries and the US, the datasets assumed for energy use in [RoW] LCIs could be the reason for their higher impacts.

While fossil fuel usage was shown to be a high contributor to ecotoxicity, the South Africa scenario, which employed the highest percentage of coal in its energy mix, generated impacts lower than most other "Rest of the World" classified country scenarios (Table 5.9). The impacts due to electricity generation for the China and India scenarios, where >70% of the electricity mixes were from coal combustion, were ranked highest. The Brazil scenario employed little fossil fuel in its mix but generated the third-highest ecotoxicity – terrestrial score. It showed that the percentage of coal in the electricity mix and a country's efficacy in preventing toxic emissions when extracting coal and generating electricity (technical efficiency) determined a production's contribution towards various impacts. As a whole, the result suggested that coal energy production in South Africa, despite used in higher quantities, the process may be more technically efficient than China and India's coal energy production process. Hence, there is room

for China and India to reduce their impacts from coal energy production by optimising their processes and reducing their coal percentage in their electricity mixes.

Furthermore, examples set by South Africa and Brazil may disprove the presumption that higher impacts generated by [RoW] LCIs for process materials were due to the likely high fossil fuel usage for energy. Instead, other factors, including technical efficiencies, are in effect. Findings in the hot-spot analysis support this, as the level of impacts deriving from generating electricity via the same energy source was not consistent with each country's socio-economic or regional class. Since there is such variety in impacts arising from simply energy production, using regional-specific LCIs may be an over generalisation, particularly if the comparison was aimed to understand the impact differences deriving from potential supply chains. Other factors that might affect the environmental impacts generated by a process include resource availability, transport distances for supplying raw materials, and production efficacy. Since they are primarily location dependant, it enforces the preference for using country-specific LCIs and the necessity to develop country-specific LCIs for biopharmaceutical process materials to enable better comparisons. Otherwise, further understanding into each regional-specific LCIs is needed to best judge the factors behind impact differences and evaluate whether they would adequately represent a particular production scenario.

Although there are limitations associated with employing regional-specific LCIs as compared to the country-specific LCIs, the environmental impacts generated were in the same order of magnitude. Since it was found that a country’s technically efficiency in avoiding impact for one process does not equal the efficiency for another process, the use of multiple regional average inventories for different materials may give a balance of all locational factors. LCA practitioners should understand that if the manufacturer's location operates vastly different from other countries in the same regional class, regional LCIs would not be representable. Next, by considering both the significance and limitations of the LCIA results, environmental considerations for siting biopharmaceutical facilities are provided.

5.5.2 Considerations when Siting Biopharmaceutical Facilities

The discussion above on the generated impact results presented readily environmental considerations for producing 6-APA; in this section, considerations are expanded to the broader biopharmaceutical industry. This extension of recommendations was possible because key inputs that were diagnosed as environmentally impactful are required to produce all other fermentation-derived biopharmaceuticals. For instance, biopharmaceuticals are both water-intensive due to the nature of fermentation processes (Budzinski et al., 2019). Ramasamy (2015) suggested that monoclonal antibodies production was equally energy-intensive as this project

has presented. Process materials that have contributed highly to multiple impacts include sodium hydroxide, glucose and ammonium sulphate, commonly used for equipment cleaning and fermentation in other biopharmaceutical manufacture. Below discusses the practical (or logistical) and policy considerations for the siting of biopharmaceutical facilities regarding the environmental sustainability of the supply of energy, water, and process materials.

There were three key recommendations directly drawn from the generated results for siting biopharmaceuticals facilities. Firstly, companies should consider the percentage of fossil fuels employed within the electricity grid mix and/or install site own renewable energy generation capabilities to reduce reliance on the grid. Secondly, companies should ensure that the water scarcity level at the intended production location is low. Lastly, the source and supply chain of process materials should be understood, and where possible, companies should opt for suppliers using environmentally preferential processes for generating materials, particularly glucose, sodium hydroxide and ammonium sulphate, required for biopharmaceutical manufacture. When all three recommendations are satisfied, the environmental impact categories that presented the most significant results in this thesis, ecotoxicity, GWP, POF, resource depletion, and impact on water resources, would be best reduced.

Although the LCA has pinpointed the areas that have generated high impacts for which the production of 6-APA can improve, other considerations influence a company's choice of location. Out of the nine scenarios, due to low fossil fuel usage in its energy mix and low water scarcity rating, only the Brazil scenario generated low impacts in both the GWP and the impact on water resources categories. However, the scenario ranked the third-highest for ecotoxicity – all subcategories. As noted in the previous sections, although the production processes assumed for ammonium sulphate and sodium hydroxide were different for each scenario depending on their regional class, suppliers are likely capable of supplying these materials in a more environmentally friendly way. It would mean that ecotoxicity has the potential to be reduced and resemble closer to the US scenario, which generated the lowest score for this impact category. Hence, choosing Brazil as the biopharmaceutical producing location would likely cause the least environmental impact. However, this choice does not consider any political and financial factors that must be accounted for when developing a process. The discussion with Arlington (personal communications, 2017) (Appendix D) revealed that biopharmaceutical development in South America is generally low due to security reasons. Brazil suffers from regulatory issues and corruption, which means a secure supply chain may not be possible (Arlington, personal communications, 2017). Hence, producing in Brazil may not be economical nor socially viable. To understand whether development in Brazil is plausible, a multi-objective

decision tool that combines the assessment of environmental, cost, and social impacts of development would be beneficial (Chapter 6 - Conclusions and Future Works).

Reviewing current environmental policies could direct companies on the technologies and support required for their processes to minimise impacts. The next country that had the most potential to become the best location for biopharmaceutical manufacture was Spain. The Spain scenario employed a low percentage of fossil fuel in its mix but had a high water scarcity rating and therefore generated a high impact on water resources score. As a country part of the EU, it does not have security issues associated with its supply chain. Its potential for biopharmaceutical development would depend largely on corporate policies, tax incentives and costs associated with development and operation, which are higher than developing in Asian countries (Arlington, personal communications, 2017). The issue with water scarcity is being rectified under the governmental agendas, which means the impact on water resources may reduce in the future.

Under the objectives of Directive 2000/60/EC of 23 October 2000, which advice on community action in the field of water policy, and the 2030 Agenda for Sustainable Development, in particular, SDG 6 – Clean Water and Sanitation, the Spain government published a White Paper on Water in 2000, heavily promoting the use of desalination and reuse technologies (Navarro, 2018). By 2007, the Royal Decree 1620/2007 (RDR) provided stringent regulations on the reuse of reclaimed water. While reclaimed water for human consumption is prohibited, it can be used for water processing and cleaning. In a biopharmaceutical process, reclaimed water can be used for cooling reactors, generating process steam for media sterilisation and general cleaning, which can reduce the level of freshwater that would be consumed. Hence, Spain is an example of how a country's environmental policy and cooperation by industry can reduce the environmental footprint of a production process. This example also suggests that companies can consider current environmental policies on water and energy when siting a biopharmaceutical facility to gauge the future environmental impacts of the production facility. As indicated in Chapter 4, since many countries aim to reduce fossil fuel usage, companies may not need to install their own capabilities for renewable energy generation.

In the context of governmental policy, there are potentials for government to publish guidance on the type of materials that are suitable for biopharmaceutical manufacture, based on their overall environmental impacts. This would mean that companies may require to modify their production processes to suit the choice of location. For instance, there are reference documents - best available techniques (BATs) for various industries, that provide guidance on the technologies to use for specific productions. However, there is not one for the biopharmaceutical industry. Acknowledging that input materials are part of the technology to

manufacture a particular product, governments may want to give guidance on the best available techniques (and materials) with the lowest environmental footprint over their life cycles. For example, a co-production process producing ammonium sulphate can be classified as a BAT; or, if there is an environmentally preferable alternative to ammonium sulphate that can act as a nitrogen-source in the fermentation process, then it can also be classed as a BAT. However, thorough LCA comparisons would be required to make this judgement.

5.5.3 Estimating the Environmental Impacts of Global 6-APA Production and Global Recommendations

In Chapter 4, the correlations generated between process design parameters, product titre and production scale, and environmental impacts associated with 6-APA production were stated as viable for estimating impacts for producing 6-APA at other product titres and scales. This was based on the R-square values for all correlation models obtained. In this chapter, correlations were also drawn for GWP, ionising radiation and impact on water resources. Since it was deemed not possible to use models to estimate the impact associated with producing other biopharmaceutical products, due to different fermentation requirements, it can be inferred that correlations generated in this chapter would not estimate the impact for other products correctly either. However, it would be plausible to estimate those three impacts associated with producing 6-APA in countries beyond the nine analysed.

Since 6-APA is the most produced biopharmaceutical product globally, it was hypothesised that estimating the global environmental impact for producing this product can help gauge the distribution of impacts provided by the biopharmaceutical industry. As both GWP and impact on water resources were found as significant environmental impacts, it would be beneficial to understand the extent of impact globally to provide recommendations to companies and governments on an international level. In addition, while there were no correlations drawn for ecotoxicity, it was noted that due to its high significance in most scenarios, attempts should be made to extrapolate this impact category as well. Hence, to further understand the environmental impacts of 6-APA, calculations were carried out using the LCA results obtained in Chapters 4 and 5 to estimate its global impacts for key impact categories. In this section, the assumptions and approaches taken for the calculation are first described. The environmental impacts derived are then discussed before drawing further recommendations.

5.5.3.1 Calculation Approach for Estimating the Global Impact of 6-APA

The approach taken for the calculation was by first reviewing the countries that manufacture penicillin and its derivatives and then assuming the production throughput and the number of manufacturing plants within each country (Table 5.10). The calculation was carried out by assuming that countries that export penicillin and its derivatives also produce 6-APA at the same

proportions. Hence, the export quantities of "Penicillin and Derivatives with a Penicillanic Acid Structure; Salts Thereof" within the HS96 dataset (OEC; 2018) was used to assume each country's contribution towards the global manufacture of penicillin products (Table 5.10). The countries' contributions were then multiplied by 103,000 tonnes, as calculated in Appendix C as the global tonnage 6-APA production, to generate the tonnage produced in each country. The number of 6-APA production plants and their annual throughput were assumed from the literature, market research and companies' websites for countries that were assumed to produce >10,000 tonnes/yr. (China, Spain and India) (See Appendix K for the assumptions used for the global distribution of production). For all other countries, this thesis took the simplifying approach of dividing the annual throughput by 2000 tonnes (the assumed average plant size) and then rounding it up to generate the number of plants needed.

Table 5.10: Countries that exported the most "Penicillin and Derivatives with a Penicillanic Acid Structure; Salts Thereof" in 2017 according to HS96 dataset (OEC, 2018)*. In this project, it is assumed that the percentage of the global export of penicillin and derivatives is equal to the percentage of global production of this type of production. These percentages were used to calculate the amount of 6-APA produced in each country. *Countries that were studied as part of this project.*

Country	Share of global export of "Penicillin and Derivatives with a Penicillanic Acid Structure; salts Thereof" (%)	Estimated Production (Tonnes)	Assumed Number of Production Facilities
*China	43	44300	7
*Spain	13	13400	5
*India	10	10300	6
*Singapore	4.7	4840	3
*UK	3.4	3500	2
*US	1	1030	1
*Germany	0.54	556	1
*Brazil	0.05	51.5	1
Austria	5.5	5670	3
Italy	4.3	4430	3
Mexico	2.7	2780	2
Japan	2.5	2580	2
Netherlands	1.6	1650	1
Belgium	1.5	1550	1
Luxembourg	1.5	1550	1
Korea	1	1030	1
France	0.95	979	1
Bulgaria	0.34	350	1
Hungry	0.27	278	1
Oman	0.26	268	1
Slovakia	0.21	216	1
Ireland	0.13	134	1
Other	0.02	2000	7
TOTAL	n/a	103000	49

It was assumed that each production plant employed the "average" 6-APA process described in Chapter 4. The environmental impact categories estimated were GWP, impact on water resources, and ecotoxicity – freshwater and terrestrial, as they were deemed significant categories. The specific impact calculations procedures were as follows before they were summed and normalised.

For countries that were analysed as part of this thesis, environmental impacts associated with the 2000 tonnes manufacturing facility at each location were scaled to the assumed average plant throughput impacts using the relationship presented in Appendix J – Table J.3. It was then multiplied by the number of plants sited in the country to generate the total impacts of 6-APA production. For all other countries, the trends observed in Chapter 5 were used to estimate the impacts at the 2000 tonnes production scale before using the productivity correlations as stated previously. For GWP, countries' Fossil Fuel Index (Eq. 5.1) were first generated before using Eq. 5.3, obtained from Figure 5.7, to make the estimates. Similarly, impacts on water resources were estimated by generating a country's Water Consumption Index (Eq. 5.2), which was then used in the equation Eq. 5.4, obtained from Figure 5.9.

(Eq. 5.3) Annual $GWP_{2000t\ throughput}$ ($kg_{co2eq.}$ /yr.) = (0.708 x Fossil Fuel Index + 37.1) x 2010000

(Eq. 5.4) Annual Impact on $Water\ Resources_{2000t\ throughput}$ (UBP/yr.) = [(0.428 x Water Scarcity (UBP/m^3) + 0.588 x Water Consumption (by Energy) Index + 219] x 2010000

Estimations for ecotoxicity – freshwater and terrestrial were based on a country's "region". For these impact categories, it was found that the materials supply of ammonium sulphate and butyl acetate were the highest contributing factors to their overall environmental impact values. Since the values were dependant on which regional LCIs were chosen to model the LCA of 6-APA manufacture (either [RoW], [RER] or [RNA]), the impact values result from the Brazil, China, India, Singapore and South Africa scenarios were averaged to form the estimates for developing countries. Whilst Germany, Spain and UK were averaged for European countries, and the US was used to estimate Canada. Like other GWP and impact on water resources, the averaged impacts were then scaled to the correct plant throughputs for each country using the relationship provided in Table J.3.

5.5.3.2 Global 6-APA Environmental Impact Results and Discussion

Presented in Table 5.11 are the environmental impact calculations for global 6-APA production. The normalised results showed that the total GWP associated with 6-APA was 7.7 $MtCO_{2eq.}$, 0.012% of total global annual emissions. Since 6-APA is the largest manufactured product in the biopharmaceutical industry, the results suggested that the industry might not contribute highly to this impact category in general. This presumption took into consideration that, although 6-APA is not the final product, studies in the literature showed that the API production stage is more impactful than subsequent biopharmaceutical production stages (de Jonge, 2003; de Soete et al., 2014a, 2013; McAlister et al., 2016). For instance, if the later stages of semi-synthetic beta-lactam antibiotic production are as impactful as the 6-APA production phase and that this class of antibiotics contribute to only 25% (arbitrary) of the total outputs of

biopharmaceuticals, then global GWP of the industry would amount to 0.096% of the global annual impact. Nonetheless, to confirm whether the API and product intermediate production phase is the most impactful in a biopharmaceutical's life cycle, LCA should be carried out on the later stages.

For impact on water resources, as there were no reference values to normalise the results, the results were only compared with the impact scorings set out in FOEN (2013). In general, the water scarcity of a location can be classified as low, moderate, medium, high, very high and extreme, and the respective scorings for consuming one cubic metre of freshwater from each location are 24, 220, 880, 2400, 6200, 22000 UBP (FOEN, 2013). The impacts generated by the global production of 6-APA was 26.6 UBP/course of treatment (that 6-APA can theoretically support as described in Chapter 4) and 430 UBP/$kg_{product}$. They resembled the impacts of consuming 1 and 17 m^3 of water from a low scarcity area, respectively, and consuming 0.0012 and 0.020 m3 of water from an extreme scarcity area, respectively. Arguably, consuming 1 L of water per 1 kg of 6-APA may not be considered high even in extremely scarce locations, but since 6-APA is produced in tonnes, the consumption would accumulate. Critical thresholds/allowance for freshwater consumption may be needed to guide plant sizing. The amount of freshwater consumed by the 6-APA process was calculated to be 593 L/$kg_{product}$ (excluding water consumed at the supply and end-of-life phases). The impact and litre per kilogram values combined meant that the product was produced at an average 725 UBP/m^3, reflecting moderately scarce locations. Again, determining a critical allowance for freshwater consumption at moderately scarce locations would help judge whether 593L/$kg_{product}$ is acceptable.

Results for ecotoxicity freshwater and terrestrial were taken under caution and to represent the worst-case scenario as the production process for multiple materials (i.e. LCIs) assumed might not be representative of how it would be produced in all countries (discussed above). As shown in Table 5.11, both ecotoxicity subcategories generated high impacts. The overall ecotoxicity – terrestrial impact results, in particular, was equivalent to 24.2% of the global annual emissions; the average impact per course of treatment was equivalent to 110% of one person's yearly impact towards this impact category as well. These results can be attributed mainly to the high portion of 6-APA produced in China. The impact value generated by this country was equivalent to 17.8% of global emissions (74% of the global ecotoxicity – terrestrial score generated). For this country, it was found that 78% of the impact generated was derived from the production of electricity from coal; and the next highest contributors were ammonium sulphate and nitric acid, which had a combined contribution of 15%. The ecotoxicity – terrestrial impacts associated with ammonium sulphate and nitric acid for all scenarios were isolated to evaluate the potential

changes to the overall impact if all countries assumed the US LCIs for both materials, instead of [RoW] and [RER] LCIs that were assumed. The maximum decrease in overall impact was estimated to 15%, but the resulting impact would still represent 20.5% of the global annual ecotoxicity – terrestrial impact. This value indicated that although the impact from supplying process materials was an overestimate, the aspect of which the overall 6-APA production process can improve upon was energy generation.

Since energy generation, unlike raw material supplies, occurs mainly in the country they are used, results showed that ecotoxicity impacts associated with global 6-APA productions would concentrate in China (and India). Since the country produces many other products, it can be presumed that the ecotoxicity associated with productions within China represents an even more significant portion of the global total. The effect of this level of concentration is not yet researched, but results suggest that both China and India should reduce its coal energy in their grid mix. The switch from coal energy to other energy resources would also decrease GHG emissions. Although GHG emissions associated with 6-APA production presented a small percentage of the global total, the urgency to reduce carbon emissions are well documented by in the Paris Agreement (UFCCC, 2015) and literature studies highlighting trends on global temperature increase (Betts and McNeall, 2018; Le Quéré et al., 2018).

Governmental policies are constantly evolving based on issues that must be rectified; if there is an accumulation of impacts, for instance, ecotoxicity, due to a specific industry, then restriction could be laid. An example of this is that due to the high water demands and pollutions that stems from the textile industry in India, there are higher levels of governance on this water issue (Araral and Ratra, 2016; Restiani and Khandelwal, 2016). As there are currently no guidance or thresholds available on how much freshwater and terrestrial ecotoxic emissions can be released before it would cause damaging effects, governments, particularly the Chinese government, may want to investigate environmental impact thresholds for their countries. With known thresholds, countries can then better relate the ecotoxicity potentials of the production processes they host with their potential damages. To prevent further contributions to this impact category, the government may need to set targets for companies to strive for and prevent new developments that may contribute highly towards this impact. For example, governments could prevent new coal energy generation plants and ammonium sulphate production via energy-intensive processes from being developed. Furthermore, it may be plausible for governments to require companies to present LCAs on their production process with particular regards to ecotoxicity results before new biopharmaceutical processes can be established. If the impacts toward this impact category are too high, companies must not

develop their process unless changes are made to ensure ecotoxicity potentials are acceptable. Otherwise, it may give rise to other industries with lower impact to grow instead.

Noting that ecotoxicity impacts deriving from coal combustion in China were higher per kWh than other countries, the results from this chapter suggested further that China should consider increasing their technical efficiency from the extraction and processing of coal. Technical efficiency is the effectiveness of generating outputs from set inputs. It is dependent on many factors such as the resources available and the level of skilled workers but can also depend on how strict local policies are (Duman and Kasman, 2018; Forstner and Isaksson, 2002; Madau et al., 2017). Literature suggested that more governmental guidelines are necessary to minimise emissions to air, water and land in China (Jin, Andersson, and Zhang 2016b; Shin 2013; Wang 2010; Zhang, Wen, and Peng 2007; Y. Zhao et al. 2009; Zhen-guang et al. 2013; Zhen 2015). It was also reported that governmental inputs are essential to increase the efficiency of power plants, set water quality criteria and begin accounting for pollutants (Huang et al., 2015; Tian et al., 2015; Zhao et al., 2015, 2009). As limited systems were placed to prevent emissions toward each environment, companies had no targets to meet. The lack of emission restrictions could be the reason that high impacts were generated as a whole. This insight enforces the need for the Chinese environmental policy to progress to minimise production impacts. In addition, it was noted that the EU had "become an increasingly central player in the international politics" (Vogler, 2005) due to its co-ordinating role in committing EU countries to the Kyoto Protocol and other climate regimes. Since the EU has placed great efforts in globalising their environmental policies (Conca, 1995; Duit et al., 2016; Kelemen, 2010), it is conceivable that with EU policies providing guidance, China can adopt effective environmental policies in a short time.

Besides lowering coal energy in electricity mixes and introducing more stringent policies, emissions can be lowered by diverting 6-APA production to other countries. Ideal locations for 6-APA manufacture should be low in water scarcity, low in fossil fuel usage for energy generation, and the supply of materials is mostly more environmentally efficient (as shown in Section 5.5.1). Example countries include Austria, Canada, and Switzerland, where water scarcities are low (< 25UBP/m^3) and the percentage of both coal and natural gas in the energy mix are all below 10%. Austria is the 4th highest producer of penicillin and its derivatives, and hence the experience of manufacturing the product is present in the country. However, to divert production from China, Austria's production capacity must be increased, which is dependent on land availability and the cost of development. In addition, the government may also be required to provide incentives for developing beta-lactam antibiotics and/or biopharmaceutical productions.

*Table 5.11: Environmental impact estimations of global 6-APA production. Estimations were made based on producing 2000 tonnes per year then scaled to the respective throughputs using correlations presented in Appendix J. "Other" aggregates the estimates for Portugal, Hong Kong, Turkey, Canada, Israel, Slovenia and Switzerland. (1) GWP and Impact on Water Resources were calculated using correlations generated from Chapter 5. (2) Average impacts of [RoW] and [RER] countries were taken and used as the estimate for respective countries. *impact values from Chapter 5 were scaled using correlations from Appendix J. ** Global population assumed: 7.63 billion – 2018 value provided by Worldometer(2020). † 6-APA was assumed to support (converted into) 32.3 million courses of treatment per 2000 tonnes produced – see Chapter 4 - Section 4.5.1 for further explanation.*

				Annual Impact				People Emission Eq. / Global Population**		
Country	Estimated Production (Tonnes)	Assumed Plant Number	Average Throughput (Tonnes)	GWP (incl. biogenic carbon)[1] ($x10^8$ $kg_{co2eq.}$)	Water[1] ($x10^9$ UBP)	Ecotoxicity - Freshwater[4] ($x10^{11}$ CTU_e)	Ecotoxicity - Terrestrial[4] ($x10^9$ kg $_{1,4\text{-DB eq.}}$)	GWP (incl. biogenic carbon)	Ecotoxicity - Freshwater	Ecotoxicity - Terrestrial
*China	44300	7	6300	36.1	18.6	25.0	8.07	0.000	0.038	0.178
*Spain	13400	5	2700	7.28	9.38	5.08	0.41	0.000	0.008	0.009
*India	10300	6	1700	9.32	7.23	5.75	0.87	0.000	0.009	0.019
*Singapore	4840	3	1600	3.81	1.02	2.16	0.17	0.000	0.003	0.004
*UK	3500	2	1800	2.16	0.81	1.42	0.11	0.000	0.002	0.002
*US	1030	1	1000	0.82	0.33	0.40	0.01	0.000	0.001	0.000
*Germany	556	1	560	0.46	0.22	0.18	0.02	0.000	0.000	0.000
*Brazil	51.5	1	52	0.12	0.03	0.03	0.01	0.000	0.000	0.000
Austria	5670	3	1900	2.63	0.28	2.06	0.17	0.000	0.003	0.004
Italy	4430	3	1500	2.75	1.11	1.63	0.13	0.000	0.002	0.003
Mexico	2780	2	1400	1.80	0.38	1.41	0.26	0.000	0.002	0.006
Japan	2575	2	1300	2.00	0.49	1.31	0.24	0.000	0.002	0.005
Netherlands	1650	1	2600	1.90	0.15	0.94	0.07	0.000	0.001	0.002
Belgium	1550	1	1600	0.70	0.76	0.58	0.05	0.000	0.001	0.001
Luxembourg	1550	1	1600	0.70	0.76	0.58	0.05	0.000	0.001	0.001
Korea	1030	1	1000	0.82	0.56	0.51	0.09	0.000	0.001	0.002
France	979	1	980	0.44	0.10	0.36	0.03	0.000	0.000	0.001
Bulgaria	350	1	350	0.36	0.13	0.13	0.01	0.000	0.000	0.000
Hungry	278	1	280	0.25	0.00	0.11	0.01	0.000	0.000	0.000
Oman	268	1	270	0.28	1.11	0.14	0.03	0.000	0.000	0.001
Slovakia	216	1	220	0.18	0.00	0.09	0.01	0.000	0.000	0.000
Ireland	134	1	130	0.21	0.00	0.05	0.01	0.000	0.000	0.000
Other	2000	7	290	1.96	0.80	0.90	0.11	0.000	0.001	0.002
TOTAL	103000	49	n/a	77.07	44.27	50.86	10.93	0.000	0.076	0.242
				($kg_{co2eq.}$)	(UBP)	(CTU_e)	(kg $_{1,4\text{-DB eq.}}$)	People Emission Eq. / Course of treatment†		
Impact /$kg_{product}$	-	-	n/a	74.8	430	49400	106	-	-	-
Impact /Course†	-	-	n/a	4.64	26.6	3060	6.57	0.001	0.349	1.083

As mentioned by Arlington (personal communications, 2017) (Appendix D), the policy in place and the cost of production usually determines the location of manufacture. At current, Singapore and South Africa are popular locations for biopharmaceutical development (Arlington, personal communications, 2017). Singapore has low water scarcity and low levels of coal usage but a high natural gas percentage in the energy mix. Producing in Singapore instead of China will greatly reduce 6-APA carbon emissions and the burden on water. The Singapore scenario also returned a lower ecotoxicity value than the China scenario. Hence, the overall environmental impact would decrease if China's current 6-APA production is diverted there. On the other hand, from comparing the South Africa and China scenarios, a trade-off would be observed if 6-APA production were to move to South Africa. While ecotoxicity impacts would decrease, both GWP and impact on water resources would increase. Whether developing 6-APA plants in South Africa is plausible, it would be necessary to understand the country's critical threshold for various emissions and impacts.

5.6 CONCLUSION

In this chapter, a life cycle assessment comparative study was carried out on producing 6-APA in nine different countries. Hot-spot analysis was conducted on all scenarios. The results mostly concurred with the results generated in Chapter 4, where energy generation via fossil fuels and the supplies of sodium hydroxide, butyl acetate and glucose remained as high contributors to many impact categories. Ammonium sulphate was found as an additional input that can generate high impact. As a whole, the chapter highlighted the importance for companies to understand the supply route of their materials as a way to minimise impacts, i.e. choose suppliers that use the most environmentally preferable process to generate process materials, particularly ammonium sulphate.

This chapter showed the limitations associated with using non-country-specific LCIs when comparing production at different locations. The isolated comparison between LCIs for the same process and different geographical codes, carried out as part of the hot-spot analysis, showed that the socio-economic development and regional class of a country do not determine whether they would generate high environmental impact for a given process. This finding highlighted that the use of regional LCIs, i.e. [RoW], [RER], [EU-28] and [GLO] LCIs, was an over generalisation, where [GLO] and [RoW] LCIs were consistently generating higher impacts than other geographically coded LCIs. For a comprehensive LCA comparison between manufacturing locations, country-specific LCIs for biopharmaceutical process materials need development.

As a whole, the relationship between the environmental impacts associated with producing 6-APA and location-dependent factors (or country-specific variables), particularly countries'

electricity mix and water scarcity, were discussed. It was found that countries' electricity mix directly correlated the global warming potential (GWP) and ionising radiation impacts generated and that there is a strong correlation between countries' electricity mix, water scarcity and the impact on water resources. The correlations showed that low coal energy in the electricity mix and water scarcity rating would be preferred when siting a biopharmaceutical facility. Using the relationships and correlations gathered in both Chapter 4 and this chapter, an estimation for the environmental impacts of global 6-APA production was possible. The results showed that impacts associated with 6-APA production concentrate in China and that due to producing electricity heavily from coal, large ecotoxicity impacts were generated. This finding has prompted suggestions on how governmental policies should be developed to reduce impacts. Otherwise, productions of 6-APA should be moved from China to prevent over-concentration of impacts and irreversible damage to one local environment.

Chapter 6: CONCLUSIONS, FURTHER RECOMMENDATIONS AND FUTURE WORKS

6.1 INTRODUCTION

This chapter aims to provide the conclusions and future works drawn from this project. Firstly, the chapter concludes how the work presented in this thesis has fulfilled the aims and objectives of the project by highlighting the project outcomes. The chapter further summarises the lessons learnt from applying the life cycle assessment (LCA) methodology to a relevant biopharmaceutical product, 6-APA. The learnings from using the LCA methodology have given rise to recommendations for conducting LCA on biopharmaceutical processes. The findings on the significant environmental impacts of biopharmaceutical manufacturing highlighted the process stages that the relevant companies should consider during process development. Lastly, the chapter summarises the four major challenges and limitations in this work. (1) Unavailability of data in the literature (and databases) for the life cycle inventory (LCI) and potential errors from using. (2) Assuming the same average 6-APA process for the comparative analysis may be an over generalisation as different producing countries may have different technical efficiencies for producing biopharmaceuticals. (3) Overall process recommendations required support from economic and social analyses. (4) Subsequent life cycle environmental impacts associated with the further processing of 6-APA remained unknown. The associated future works necessary to address each limitation for future LCAs on biopharmaceutical processes are presented concurrently.

6.2 CONCLUSIONS AND FINAL RECOMMENDATIONS

The main aim of this thesis was to address the limited knowledge in the study of the environmental impacts associated with the biopharmaceutical industry. To this end, this thesis operationalised the application of LCA to a case study related to the biopharmaceutical industry showing the advantages and limitations of the LCA approach. Following a literature review presented in Chapter 2, Chapter 3 presents a set of guidelines to apply the LCA methodology to biopharmaceutical manufacture from a company's perspective (Section 3.3). The guidelines suggested were then applied fully in Chapters 4 and 5, where the LCA methodology was operationalised to 6-APA manufacture. Advantages and limitations were also presented in these chapters providing some further guidance to the application of LCA.

Chapters 4 and 5 presented hot-spot analysis, sensitivity analysis, scenario analyses and comparative study. The results showed the causes for high environmental impacts in the 6-APA

production, enabling recommendations on practical steps to reduce the environmental impacts of related biopharmaceutical products (see Section 6.2.2). In particular, the results provided an in-depth understanding of how product titre, production scale, and manufacturing location affect the overall environmental impacts (Sections 4.4.5 and 5.4.2).

6.2.1 Conducting LCA on Biopharmaceutical Products

By operationalising the LCA methodology to evaluate 6-APA, lessons were learnt on how best to conduct LCA on biopharmaceutical processes. Particularly under the limitations associated with the data available to model the production process and the lack of benchmarks to interpret LCI and life cycle impact assessment (LCIA) results comprehensively.

On inventory building, the thesis recommends conducting inventory analysis on the use phase first, as this would be a company's data, before carrying out analyses on the supply and end-of-life phase. The data should ideally be empirical (primary data) from the industry, as theoretical values may not accurately reflect the manufacturing process. Where values must be calculated or assumed, this must be made known (documented). If possible, a range should be provided to reflect the potential error of the calculations so that sensitivity analysis can be conducted. While industry-based process data were unavailable for use in this thesis, the possible data available (as discussed with industry experts – Appendix D) was restricted to site-wide electricity, water, and steam usage. The data would have then required assumptions for allocating site utilities across the processes. To collect data from production processes, companies may put in place steps to measure utility usage for the different unit operations. The calculation of PMI and E-factors was found beneficial to check against known values from the pharmaceutical industry to assure the completeness of the inventory generated.

Since there were limited LCA studies on biopharmaceutical products, there were no benchmark values available to compare against the generated LCIA results; normalisation was used as a reference to establish the pressure on the environment for certain impact categories. The normalisation factors used were global per capita emissions, which resulted in people emissions equivalences for each impact category. The issue arising from normalising impacts with these factors was that it remained unclear whether the impacts generated were environmentally damaging or not. To understand better the level of environmental damage a process may have, companies may choose to normalise their LCIA results using global carrying capacities. Since carrying capacity based normalisation factors are developed only for impacts generated using the EF methodology package (Sala et al., 2020), companies will first need to carry LCIA using EF methodologies.

Concerning the LCA approaches used as a whole, the use of the processing blocks, as described in Chapter 3, for allocating the inventory was shown to be beneficial. The approach offered a system to mask production processes by grouping them into blocks when reporting results. This approach would potentially be advantageous for public reporting on the environmental sustainability of a company. Although the underlying processes were masked, results were represented adequately and still enabled the diagnosis of impactful areas within the overall process, as shown in Chapters 4 and 5. As a whole, recommendations derived demonstrated the capabilities of LCA to assist with biopharmaceutical process optimisation and development.

6.2.2 Sources of High Environmental Impacts within the Biopharmaceutical Industry

The LCA analyses in Chapters 4 and 5 showed that the supply phase of the 6-APA manufacturing life cycle generated the highest environmental impacts. The most impactful process materials were ammonium sulphate, glucose, sodium hydroxide and butyl acetate. Except for butyl acetate, all materials are commonly used to produce most biopharmaceutical products in general. Ammonium sulphate and glucose are essential for cell growth and product generation; hence, replacing these materials may not be possible. Instead, companies should consider how their input materials are sourced and manufactured. This suggestion was supported by the considerable variation in the impacts generated between the regional-specific LCIs used to model ammonium sulphate production in Chapter 5. The analysis showed that the material produced by a co-production process was more environmentally friendly than a process that was dedicated to manufacturing it. A potential policy consideration by governmental bodies could be to classify the environmentally preferred manufacturing processes as the best available techniques (BATs), which means there would only be one possible method to produce certain materials. For instance, in the case of ammonium sulphate, this could be the co-production method. Unfortunately, the supply of glucose was modelled using the same inventory data for all scenarios due to limited country-specific LCI available in the databases. Therefore the impact differences arising from different production methods were not analysed (see Section 6.4 – Limitations and Future Works).

Due to pharmaceuticals' health and safety regulations, production processes must be sterile to avoid contamination before their use in humans. Since sodium hydroxide is the most common cleaning agent used within the pharmaceutical industries, it is unlikely that it would be replaced. Like ammonium sulphate, the production methods used to produce sodium hydroxide in different regions generated varying impact results. Results showed that the US production mix for this cleaning agent, which mainly used the diaphragm chlorine-alkali-electrolysis method, was more environmentally preferable to the EU production mix for the material, which primarily used the membrane chlorine-alkali-electrolysis method. In addition, the sensitivity and scenario

analyses in Chapter 4 showed that the cleaning protocol, which affected the amounts of cleaning reagents (sodium hydroxide and nitric acid), water (water for injection) and pure steam required, had a large effect on the results for all environmental impact categories. Since the level of freshwater consumption (due to 6-APA production) and the use of natural gas to produce steam were both found environmentally burdensome (Chapter 4), the results suggested that steps to optimise cleaning regimes so that input requirements are minimised should be conducted.

While butyl acetate is not a common material used to produce biopharmaceuticals, results highlighted the consequences of employing a solvent. According to the principles of green chemistry, the use of solvents should be minimised. As it is not possible to use alternative process materials once pharmaceuticals have gained regulatory approval, the LCA results enforce the necessity to seek environmentally preferred process materials and operations at process development stages. As shown in the scenarios analyses and the comparative study, decisions at process development stages, such as product titre, production scales and production location, could subject a manufacturing process to avoidable environmental impacts.

The thesis showed that the electricity mix and water scarcity level of a given country, and therefore the manufacturing facility's location, have greatly affected the overall environmental impacts allocable to 6-APA. In addition, the estimated global distribution of 6-APA production and the subsequent environmental impact results showed that the percentage of coal energy in China's electricity mix meant that ecotoxicity impacts concentrate heavily in this location. These findings have provided three suggestions to reduce impacts. Firstly, companies should consider the electricity mix and water scarcity in the local area when placing biopharmaceutical facilities. For instance, develop production processes in areas where there are low fossil fuel usage in the electricity grid mixes and low water scarcity levels. Secondly, companies may install "green" energy production capabilities at the manufacturing site to reduce energy demands from the grid, particularly if the fossil fuel in the grid mix is high. Green-energy production on-site would also apply to steam generation. Since the LCA on 6-APA production assumed that steam was generated via natural gas, switching to a renewable feedstock would lower impacts associated with the use of steam. Lastly, governments may take steps into developing and enforcing emissions and/or impact limits on local and national levels to prevent impacts over concentrating in an area causing irreversible damage.

6.3 LIMITATIONS AND FUTURE WORKS

6.3.1 Limited life cycle inventory data on biopharmaceutical-specific inputs

A major limitation to carrying out the LCA on 6-APA production was the inventory data available for various input materials. For modelling the environmental impacts associated with the supply of materials, cradle-to-gate LCIs for process inputs were obtained from the Gabi (Sphera, 2020a) and Ecoinvent (Ecoinvent, 2019) databases. Where possible, these LCIs were material-specific and country-specific. When materials were not found in the databases, generic LCIs for organic and inorganic chemicals were used. However, this assumed that materials in the same category would have similar emissions, which may not be the case. Inventory gaps that were found through this project included substances: phenylacetic acid, pluronic (a surfactant) and various solvents that may substitute butyl acetate in solvent extraction processes. A way to ensure future biopharmaceutical LCAs reflect the actual production processes, work should be carried out to diagnose gaps in inventory databases and develop LCIs for the biopharmaceuticals process materials that are lacking.

When country-specific LCIs were unavailable, preference was given to regional-specific LCIs before choosing global average entries. However, there were incidents where only the US or North America-specific LCIs were available. For example, glucose; this led to the modelling of glucose produced under US conditions for all country scenarios. The issue arising from not using country-specific inventories was that the LCA could not fully represent a product's manufacture in a given location. The lack of representativeness was particularly problematic when comparing differently-located productions as the full extent of the environmental impact differences could not be evaluated. In addition, Chapter 5 highlighted that inventories representing "Rest of the World" [RoW], i.e. countries that were not within Europe and North America, typically generated higher impacts than other geographically coded LCIs. However, as shown by comparing country-specific LCIs for energy production, countries classified to the "Rest of the Word" group do not necessarily generate higher impacts than European countries and the US when carrying out the same process. Since biopharmaceuticals are manufactured worldwide, country-specific LCIs should be developed for all process materials so that future LCA on comparing process can assist companies in choosing environmentally optimal supply chains.

6.3.2 Technical efficiencies associated with biopharmaceutical production

From comparing the impacts generated from energy production, it was found that while different countries may use the same feedstock for a given process, a range of impacts was generated. This suggested that the technical efficiency for preventing environmental impacts is different depending on its location. As discussed, the location-dependent factors that may

influence technical efficiency could be the skill levels of workers, the efficacy of machinery available, land composition, accessibility of where materials are extracted or grown, and local climate, if applicable. For national energy production, Duman and Kasman (2018) stated that availability of resources, energy loss rates in transmission, conversion, and distribution, significantly affects overall energy efficiency and, therefore, the overall environmental technical efficiency of a country. This suggested that the overall technologies employed could be the underlining factor to technical efficiency. Duman and Kasman (2018) also observed that developed countries have a higher environmental technical efficiency than others. This suggested that these countries may be more capable of employing technically more efficient technologies. However, the difference in efficiency could also lie with that developed countries are likely to host service industries over manufacturing industries that require more energy than developing countries (Duman and Kasman, 2018). Nonetheless, Barasa et al. (2015) showed that the adoption of foreign technology by developing countries had a minor positive effect on manufacturing technical efficiency. It suggested that since foreign technologies are often imported from more advanced companies, they are more efficient and that more technical support may be available for their use.

For the LCA on 6-APA manufacture, by modelling the "average" production process requiring and producing the same inputs and outputs, the technical efficiency of each manufacturing facility was assumed the same for all country scenarios. It was assumed viable for the technology and the skill levels of workers employed to be the same due to the type of equipment needed and that operational training should be standard practice for biopharmaceuticals. However, many other factors can contribute to a manufacturing plant's technical efficiency: social factors, such as work culture or wages, and natural factors, such as extreme weather occurrences, can affect plant productivity. Hence, assuming 6-APA production to have equal performance in the various scenarios may have been an over-generalisation. Analyses are required to understand whether there would be productivity and environmental differences in producing biopharmaceuticals in different countries; beyond those already observed in the supply of materials and energy generation.

6.3.3 Subsequent impacts associated with the use of 6-APA not modelled

The assessments on 6-APA suggested that specific environmental impacts were significant. This confirmed the need to conduct LCA on the product intermediate and active pharmaceutical ingredient (API) productions to find areas of high impacts for process optimisation. The next step would ideally be to evaluate the impacts of further processing of biopharmaceuticals, i.e. further transformation, formulation, packaging, and distribution. Assessing the later stages of biopharmaceutical production will allow a further understanding of the environmental impacts

of the biopharmaceutical industry and know whether the API/product intermediate production stages are truly the most impactful. Hence, a set of future works is to operationalise the LCA methodology to all other life cycle stages of biopharmaceutical products.

As shown in Chapter 2, there are limited LCAs on the formulation of biopharmaceuticals. Only Renteria Gamiz et al. (2019) have conducted an LCA study assessing the freeze-drying of liquid formulations. However, the study only accounted for the exergy of the system. A few LCA studies in the literature evaluated tabletting synthetical pharmaceuticals(de Jonge, 2003; de Soete et al., 2014a, 2013; McAlister et al., 2016), which can be used to infer environmental impacts associated with tabletting biopharmaceuticals. However, since some bio-therapeutics must be stabilised at low temperatures and that their formulation, storage and distribution would incur additional requirements, they would generate different impacts to traditional pharmaceuticals. Hence, there is a need to apply the LCA methodology to the later stages of the biopharmaceutical supply chain to eliminate the gaps in the literature.

The life cycle of a biopharmaceutical product has three distinct stages: API manufacture, drug formulation, and packaging and distribution. Depending on the company, each stage can occur at different locations and operate under different managements. In the case of 6-APA, its transformation to beta-lactam antibiotic APIs can either happen within the same facility or another site. If transformation occurs on-site, the LCA conducted as part of this project can be expanded to include the added transformation process. The function of the facility would be to produce the specific antibiotic API and, the functional units would be altered to reflect this; i.e. "1 kg of antibiotic" and "producing x kg of antibiotic/year" (where "x" is the production mass per year). However, if transformation occurs offsite, to ensure that LCA can be conducted on a per facility basis, an additional LCA study for the transformation would be advised. Hence, for semi-synthetic pharmaceuticals, there are up to four distinct life-cycle stages before they reach consumers.

For developing a cradle-to-grave LCA on a specific biopharmaceutical, the thesis notes that LCA studies on processes after the API or product intermediate stage take the gate-to-gate approach. For instance, carrying out gate-to-gate LCA studies on the conversion of 6-APA to a beta-lactam antibiotic and the subsequent formulation into a drug product would allow impacts generated to be summed with the initial cradle-to-gate study (6-APA production). This summation would achieve a comprehensive cradle-to-gate assessment of the final drug product. However, it must be noted further that LCA approaches, particularly the LCIA methodologies employed, should be consistent across all studies to allow the summation.

6.3.4 Economic and Social Considerations

While the project focused only on the environmental aspects, the conventional LCA, this thesis recognised that process decisions could not be made considering only the environment. Given that the biopharmaceutical industry relies heavily on financial support from shareholders and trust from the wider community, companies need to consider their processes' economic and social aspects. It was clear from analysing the productions of 6-APA that economic and social factors should be considered besides environmental prospects when assessing the sustainability of a product's manufacture. Economic and social data can be collected as part of inventory building, but the specific data necessary will depend on the study's goal. Economic metrics, including electricity cost, water cost, raw material cost, fuel cost, workers' pay and sale of end products, can be found in the literature (Martins et al., 2010; Mata et al., 2012).

On the other hand, the choice of social metrics is more complex. Social topics, including health and safety, well-being, employment, and basic rights (World Business Council for Sustainable Development, 2016), can be hard to quantify as they are subjective (Zamagni et al., 2015). Quantitative metrics are commonly used to extrapolate information on social concerns (Martins et al., 2010; Zamagni et al., 2015). For instance, the number of workers employed and its gender and ethnicity breakdown can be used to address equality concerns, while injury rates can give insights into the health and safety of the process (Martins et al., 2010). There are also qualitative methods, such as the five-point reference scales (Goedkoop et al., 2018), which are becoming more popular for understanding the social impact of a system. Qualitative methods often require a performance rating for certain topics, such as average job satisfaction or even the level of compliance to a specific law. The gathering of performance rating commonly requires surveys and audits.

The employment of life cycle costing (LCC) and social life cycle assessment (S-LCA), in addition to LCA, would be beneficial for companies. LCC and S-LCA are sophisticated sustainability assessment tools that allow the complete analysis of a system's economic and social sustainability (Gundes, 2016). The integration of LCC, S-LCA and LCA into a multi-objective decisional tool would assist companies in designing their production processes in a truly sustainable manner and abide by the goals of sustainable development further than analysing the environmental impact of operations alone. Due to the complexity of each assessment, this thesis suggests separate projects for operationalising the application of LCC and S-LCA to biopharmaceuticals. Establishing the two evaluations individually would convey better their benefits and provide better guidance on their use. Once the use of the methodologies has matured, a multi-objective decisional tool could be developed more efficiently.

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APPENDIX A: MODES OF LIFE CYCLE ASSESSMENT CONSIDERED

Table A.1: The different modes of life cycle assessment as summarised by Guinée et al. (2018). (All information presented are as Guinée et al. (2018)).

Type	Description	Questions addressed (example)	Key method	Objective of analysis	Scope			Other methods/models used	Allocation method
					Temporal	Processes	Data		
Attributional (A)	To provide information on what portion of global burdens can be associated with a specific product life cycle.	What are the environmental impacts of a product system as it currently functions?	LCA	Commercially existing product system; as it is or was	Present, past	All	SDb	n.a.	Variable
Backcasting (B)	Exploring ways—in a life-cycle perspective—to meet normatively defined sustainability levels (planetary boundaries) through adapted affluence (as consumption levels), population growth, and/or technologies (Heijungs et al., 2014).	What is a region's maximum attainable affluence to meet its planetary boundaries at time t with constant technologies and population?	IOA	Regional/global consumption; as it should be	Future, past	All sectors		Linear programming (LP_ simplex algorithm	Variable
Consequential (C)	To provide information on the environmental burdens that occur, directly or indirectly, as a consequence of a decision (usually represented by changes in demand for a product).	What are the consequences of increased demands of a certain product system?	LCA	Commercially existing product system as it changes due to decision	Future	Marginal, market	SDb	CGEM; PGEM; IAM; LOM	Substitution
Decisional (D)	Based on CLCA but using the actual or anticipated financial and contractual relations between economic actors (business-to-business relations) as the main basis of information (Frischknecht and Stucki, 2010)	What are the consequences of increased demands of a certain product system?	LCA	Commercially existing product system as it changes due to decision	Future	Marginal, B2B	SDb	n.a.	Substitution
Integrated (I)	LCA integrated with other modelling approaches such as input-output analysis, energy-scenario modelling, and, for example, material flow analysis (Hertwich et al., 2014); method for assessing the environmental and resource implications of scenarios for large-scale adoption of climate change mitigation measures (Gibon et al., 2015)	What are the global life-cycle impacts of a specific energy transition?	LCA	Global energy consumption	Future	All	SDb and TI, B, IOA	IOA, IEA, Blue Map scenario	Variable
Anticipated (N)	A forward-looking, non-predictive tool that increases model uncertainty through the inclusion of prospective modelling tools, decision theory, and multiple social perspectives (Wender et al., 2014)	What are the expected environmental impacts of an emerging product system?	LCA	Emerging product system	Future	All	SDB and TI, F, B (optional)	Learning curves; technology and chemical models	Variable
Prospective (P)	Estimating future life-cycle environmental impacts using scenarios (Spielmann et al., 2005; Walser et al., 2011)	What are the expected environmental impacts of an emerging product system?	LCA	Emerging product system	Future	All	SDB and TI, F, B (optional)	Learning curves; technology and chemical models	Variable
Scenario-based (Sb)	LCA based on scenarios separating three modelling processes, life-cycle modelling, scenario modelling, and valuation modelling (Fukushima and Hirao, 2002).	What are the expected environmental impacts of a certain future scenario of a product system?	LCA	Emerging product system	Dynamic from past to future	All	Calculated	Life-cycle modelling language	Variable

"n.a. not applicable; *product system* (or technology system) a set of unit processes interlinked by material, energy, product, waste, or service flows and performing one or more defined functions (Guinee et al., 2002); *SDb* = standard LCA data(bases), such as ecoinvent, GaBi, ILCD, and USDA; *TI* = assumptions on technical improvements in key energy and material production technologies (Hertwich et al., 2014); *F* = foreground processes; *B* = background processes; *CGEM* = computable and partial general equilibrium model; *PGEM* = partial general equilibrium model; *IAM* = integrated assessment model; *LOM* = linear optimization model; *All* = all processes included for supplying the functional unit; *All sectors* all industry sectors included for supplying the region's/global consumption; *Marginal* = processes actually affected by the decision; *Market* = affected processes are determined by using market information and price elasticities; *B2B* = affected processes are determined by factual or anticipated economic business-to-business relationship" Guinée et al. (2018)

APPENDIX B: THE DEMOGRAPHICS OF THE BIOPHARMACEUTICAL INDUSTRY

B.1 PRODUCTS AND ANNUAL PRODUCTION

Table B.1: Traditional Biotechnological Product [Source: (Walsh, 2003)*]*

Source	Product type	Example(s)
Animal / Human	Proteins	Blood derived clotting factors, polyclonal antibodies
	Peptides	Peptide hormones
	Toxins	Anti-hypotensive
Microbial	Antibiotics (including peptide antibiotics)	Penicillin, cephalosporin, carbapenems, tetracyclines and their derivatives
	Other metabolites, toxins, alkaloids, vitamins)	Botoxin, riboflavin, vitamin B12
Plant	Alkaloids	Morphine, codeine
	Steroids (may require further biotransformation)	Digoxin, progesterone, hydrocortisone
	Salicylate	Aspirin

Table B.2: Market value of individual pharmaceutical product category, produced via biotechnology. Note that the estimates assume that selling prices do not fluctuate drastically. In bold is the top sold antibiotic based on mass. Note that market values are obtained from various sources and can reflective in different years.

Pharmaceutical Type	Annual Market Value ($)	Annual API production (tonnes)	Source
Antibiotics	45 billion	-	(CDDEP, 2015; CenterWatch News Online, 2014)
e.g. Beta-lactams	25 - 29 billion		(Elander, 2003; Meštrović and Chow, 2015)
†Cephalosporin & derivatives	11.9 billion	98,900*	(Pandey and Sumant, 2019).
†Penicillin & derivatives	10.8 billion**	269,000**	
Steroids	10 billion	1 million	(Margaret Smith, 2013)
e.g. Hydrocortisone	145 million	7	(Chib, 2014)
Alkaloids	34.9 billion	-	(Persistent Market Research, 2016)
e.g. Narcotic drugs		1100	(International Narcotics Control Board, 2016)
Morphine		408	
Codeine		334	
Thebaine		93.5	
Cannabis		100	
Vaccines	32.5 billion	-	(The Vaccine Reaction, 2016)
e.g. †Influenza	3.8 billion (est.)	3.75 -15 kg	(Vajo et al., 2019)
Toxins	-	-	
e.g. † Botulinum toxin	3.6 billion	1.15 mg***	(Hopkins, 2019)
†Insulin	24 billion	35***	(Somya, 2015)
†Proteins		26.4	(Walsh, 2014)
e.g. Antibodies	100 billion	25.7***	(van Huijsduijnen et al., 2020)

†Produced via fermentation/cell culture technology, as defined to be a biopharmaceutical product for this project.
* Annual production was estimated by dividing market value with the average selling prices of each product, which were calculated from prices indicated on the British National Formulary website (https://bnf.nice.org.uk/) and uses currency conversion from GBP to USD provided by XE (https://www.xe.com/) in January 2016.
**Penicillin annual production value assumed that production rate is reflective of consumption rate. (Refer to Appendix F)
Antibiotic consumption breakdown: Penicillin and derivatives = 44.3% and Cephalosporin and derivatives = 16.3% (ECDC, 2018)
Penicillin and derivatives mass = 98900 x 44.3 / 16.3 = 269,000
Value check: Average pricing of Penicillin and derivatives = $\$40/kg_{product}$; estimated global penicillin market = 40 x 269,000 = $10.8 billion
*** The mass produced were extrapolated from Table B.4

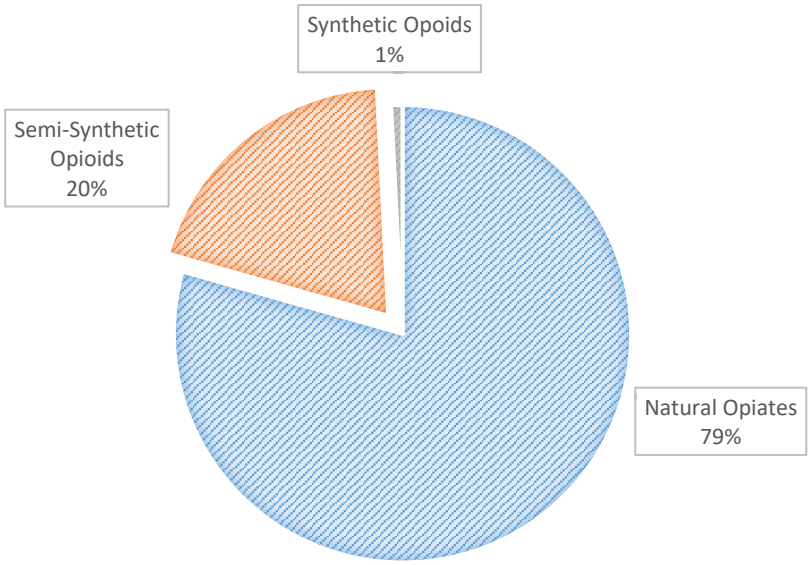


Figure B.1 Global production of narcotic drugs in 2015 (International Narcotics Control Board, 2016)

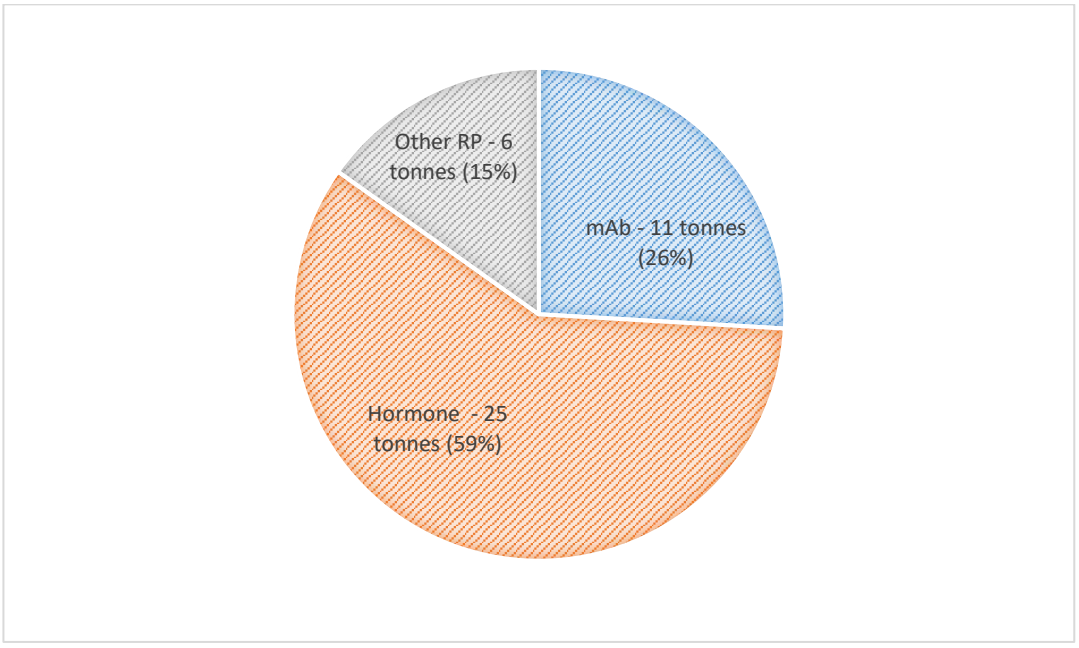


Figure B.2 Production split of the top 20 Biologics in 2014, visual representation of Table B.3

*Table B.3 Highest Revenue Biological Drugs in 2014. mAb = Monoclonal Antibody, FP = Fusion Protein, H = Hormone, SF = Stimulating Factor, V = Vaccine, GF = Growth Hormone, C = Cytokine, T = Toxin, CHO = Chinese Hamster Ovaries, MM = Murine Myeloma, SP = Streptococcus Pneumoniae, SC = Saccharomyces Cerevisiae, CB = Clostridium Botulinum *Estimation for the amount of product sold involved utilizing net prices for the given drug per mg from https://www.evidence.nhs.uk/; the prices were converted from GBP to USD using the exchange rate on 16/01/16. Various sources were used to compile this table, this includes reports from pharmaceutical consultancy evaluation reports (EvaluatePharma, 2015; Langer, 2015), journal papers* (Levine and Ph, 2014; Walsh, 2014), *documents from European Medicine Agency* (EMA, 2014b, 2014a, 2009, 2006, 2005; EMEA, 2012a, 2012d, 2011a, 2011b, 2010b, 2010a, 2010c, 2009b, 2009a, 2009c, 2009d, 2008, 2007c, 2007d, 2007b, 2007a, 2006, 2005d, 2005b, 2005f, 2005e, 2005c, 2005a, 2004b, 2004d, 2004a, 2004e, 2004c, 2004f, 2003, 2015a, 2015b, 2012e, 2012b, 2012c; MHRA, 2012). *Note: Botox, is traditional biopharmaceutical, the toxin is extracted from natural occurring source as mentioned previously. All other products are modern biotechnology product which will be reviewed in the following paragraphs.*

Rank	Brand	Chemical Name	Molecule Type	Expression System	Company	2014 Sales ($m)	Estimated Kg Sold (3s.f.)*	Manufacture Locations
1	Humira	Adlimumab	mAb	CHO	Abbie / Eisai	12890	1030	Massachusetts, USA / Barceloneta, Puerto Rico / Pontevedra, Spain / Singapore, Singapore
2	Enbrel	Etanercept	FB	CHO	Amgen / Pfizer / Takeda	8915	1750	Biberach, Germany / Dublin, Ireland
3	Remicade	Infliximab	mAb	MM	JNJ / Merck&Co / Mitsubishi Tanabe	8807	1470	Leiden, Netherlands / Pennsylvania, USA
4	Lantus	Insulin Glargine Recombinant	H	*E. Coli*	Sanofi	8428	7460	Frankfurt, Germany
5	Rituxan	Rituximab	mAb	CHO	Pfizer / Daewoong	7547	3580	California, USA
6	Avastin	Bevacizumab	mAb	CHO	Roche	7018	2080	California, USA / Basel, Switzerland / Singapore, Singapore
7	Herceptin	Trastuzumab	mAb	CHO	Roche	6863	2020	Penzberg, Germany / California, USA / Singapore, Singapore
8	Neulasta	Pegfilgrastim	SF	*E Coli*	Amgen / Kuowa Hakko	4599	28.2	California, USA / Juncos, Puerto Rico
9	Lucentis	Ranibizumab	mAb	*E Coli*	Novartis / Roche	4301	9.35	California, USA / Stein, Switzerland / Singapore, Singapore
10	Prevenar 13	Pneumococcal Vaccine	V	SP	Pfizer / Daewoong	4297	7.67	Massachusetts, USA / North Carolina, USA / Dublin, Ireland
11	Epogen / Procrit	Epoetin Alfa	GF	CHO	Amgen / JNJ / Kyowa Hakko Kim	3292	3500	California, USA
12	NovoRapid	Insulin Aspart Recombinant	H	SC	Novo Nordisk	3109	4640	Kalunborg, Denmark / Bagsvaerd, Denmark
13	Avonex	Interferon beta-1a	C	CHO	Biogen	3013	0.388	Massachusetts, USA / North Carolina, USA
14	Eylea	Aflibercept	FP	CHO	Regeneron / Bayer / Santen	2972	10.2	New York, USA
15	Humalog	Insulin Lispro Recombinant	H	E Coli	Eli Lilly	2785	3870	Indiana, USA / Carolina, Puerto Rico
16	Levemir	Insulin Determir Recombinant	H	SC	Novo Nordisk	2533	9050	Kalunborg, Denmark / Bagsvaerd, Denmark
17	Botox	OnobotulinumtoxinA	T	CB	Allergan / GSK	2496	0.0000147	Mayo, Ireland
18	Aranesp	Darbeopoetin	GF	CHO	Amgen / Kuowa Hakko	2454	1.17	Juncos, Puerto Rico
19	Rebif	Interferon beta-1a	C	CHO	Merck KGaA	244	0.0918	Corsier-Sur-Vevey, Switzerland / Aubonne, Switzerland
20	Xgeva / Prolia	Denosumab	mAb	CHO	Amgen / Daiichi Sankyo	2411	655	Colorado, USA / California, USA / Juncos, Puerto Rico / Biberach, Germany
					Total (3s.f)	**99000**	**42300**	

B.2 PRODUCTION METHOD

Table 1.6: Expression Systems/Host Cells for U.S./EU-Marketed Cultured Biopharmaceuticals

	System Type	Number of Biopharmaceuticals
Microbial		
	Bacteria (*E. coli*)	75
	Yeast (*Saccharomyces cerevisiae*)	26
	Yeast (*Pichia pastoris*)	3
Insect cells		
	Trichopulsia ni (High Five)	1
	Spodoptera frugiperda Sf21	3
Mammalian, non-human		
	Chinese hamster ovary (CHO)	57
	Murine myeloma/hybridoma	17
	Baby hamster kidney (BHK)	3
	Madin-Darby canine kidney (MDCK)	2
	Chicken embryo culture	6
	Chicken eggs (influenza vaccines)	27
Mammalian, human		
	Human fibroblasts	4
	Human kidney cells (HEK)	1
	Human foreskin	4
	Human autologous cells	6
	Human cells, gene activation	2
	Human cells, EBV-transformed	1
Goats, transgenic		1
Rabbits, transgenic		1

Source: *BIOPHARMA: Biopharmaceutical Product in the U.S. and European Markets*, (at www.bioplanassociates.com/biopharma), early 2014

Figure B.3 Expression systems/host cells employed for US and EU markets (Langer, 2015)

APPENDIX C: GLOBAL 6-APA PRODUCTION ESTIMATE

Table C.1: Information obtained on the global antibiotics used to calculate annual global production of 6-APA.

	Literature obtained values	Source
Global antibiotics market	$45 billion	(CDDEP, 2015; CenterWatch News Online, 2014)
Beta-lactam antibiotic share of the antibiotics market	57 to 65%	(Elander, 2003; Meštrović and Chow, 2015)
Global Cephalosporin market	$11. 9 billion (2017)	(Pandey and Sumant, 2019).
Average cephalosporin and its derivatives pricing	$120 / kg	(PharmaCompass, 2019)
Antibiotic consumption breakdown	Penicillin and derivatives – 44.3% Cephalosporin – 16.3% (2017)	(ECDC, 2018)
Average penicillin and its derivatives pricing	~ $40 / kg	(Cuthbertson et al., 2019; PharmaCompass, 2019)
6-APA to amoxicillin mass ratio	216.25g/mol : 365.4g/mol 0.59	(NCBI, 2019)
Percentage of beta-lactam antibiotics are derivatives	Up to 85% (Penicillin G/V conversion to 6-APA = 59-65%; conversion to 7-ADCA/other= 20-23%)	(Bhattacharyya and Sen, 2006)

Table C.2: List of assumptions and calculation used to obtain annual global 6-APA production mass

Assumptions	Calculations
Market value is reflective of annual production. Market value divided by average pricing equates to massed produce.	Global cephalosporin and derivatives mass = $11. 9 billion / $120/$kg_{product}$ = 98,900,000 kg or 98,900 tonnes
Annual consumption is reflective of annual production. The mass ratio of antibiotics consumes = the mass ratio in which they are produced.	Global penicillin and derivatives mass = 98,900 tonnes / 16.3 x 44.3 = 269 000 tonnes
All penicillin derivatives share a similar molecular weight. The mass ratio between 6-APA and amoxicillin was used to convert total penicillin derived antibiotics to 6-APA	Global 6-APA mass = 269 000 tonnes x 0.65 x 0.59 = 103,000 tonnes

Value Check: The beta-lactam antibiotics market is made up of penicillin and its derivatives, cephalosporin and its derivatives and synthetic beta-lactam antibiotics. The global penicillin market value should be lower than the global beta-lactam antibiotics market value minus the cephalosporin market value.

Global Penicillin market = Total penicillin x average pricing = $10.8 billion

Beta-lactam antibiotic market = Global antibiotics market x beta-lactam antibiotics share

= $25.7 to $29 billion

Global beta-lactam antibiotic market – global cephalosporin market = £13.8 to $17.6 billion

APPENDIX D: PERSONAL COMMUNICATIONS

D.1. PERSONAL COMMUNICATION – PEOPLE AND TIMELINE

*Table D.1: Correspondences with **Amanda Weiss**, [redacted] at Fujifilm.*

Correspondence	Topic	Response / Action Summary
Meeting Oct 2015	Fujifilm expansion	Acquired Kalon Biotherapeutics. Added latest high throughput equipment for R&D and constructed 62,000ft^2 facility. They expanded capacity with new single-use technology but keeping existing stainless steel tech.
Meeting Oct 2015	Drivers and trends of biopharmaceutical business	Mammalian cells tend towards disposables – 2000L, 1000L and 200L scales. Microbial cell cultures remain high. Upstream is still in convention stainless steel equipment, but downstream tend towards single-use (columns and filters). The capacity of mammalian cell culture increasing – for MAbs and FAbs. Most are in clinical trials - >90%, 2/180 are in commercial. Mentions Mark Douglas – Strategic Business Development Manage at Fujifilm.

*Table D.2: Correspondences with **Steve Carleysmith**, [redacted] at Reo Process Improvement Ltd.*

Correspondence	Topic	Response / Action Summary
Email March 2016	History with penicillin production	Steve worked on penicillin fermentation at GSK, Worthing site, many years ago. Moved off fermentation in 1995, moved away from the Worthing factory into R&D in 1996 and left GSK in 2008. Know that penicillin G (PenG) is only fermented at Irvine for many years and production seems to be expanding.
Phone March 2016	Penicillin and 6-APA production	We discussed penicillin and 6-APA production. Steven noted that penicillin must be separate from other drugs because of allergy. Steve gave particular details on the upstream processing of penicillin: fermentation configurations, the scales of production, pooling of fermentations working in parallel. Typically 5 to 7 days of fermentation and chilled with cooling water. However, glycol or plate heat exchange is used to chill product downstream of fermentation. Steve recommended the unit operations to research; this included upstream and downstream process equipment. Discussed waste treatment – lots of diluted waste, too expensive to heat treat, so flocculate solid waste; pH treatment (alkaline to neutral) – previously filtrate were released to sea containing biological waste – Worthing plant has a full waste treatment capabilities (primary and secondary waste treatment). (Steven – in 1990 six-sigma internal consultant, in 1996 became Engineering Manager)
Phone June 2016	Contacts	Steve provided email introductions with Peter Hillier.
Phone July 2017	Validating assumptions for 6-APA production.	Quality checked various assumptions on a 6-APA production facility, including: - Fermentation - reactor sizes – 200m^3 and 100m$^{3;}$ around 40g/L, 300 batches/year, eight days fermentation; fermentation did not have HVAC / work under GMP, flame proof for extraction – explosion risk. - Process specifics – oxygen transfer, heat removal, inline cooling, HVAC system needed further research - Downstream processing duration sounds viable; confirmed 54% 6-APA yield from penicillin sounds correct - Production rates of purified water and WFI - It was reiterated that waste requires acidification before leaving the plant. Incineration/landfill processes offsite.

*Table D.3: Correspondences with **Laura Diaz Anadon**, [redacted] at UCL Science Technology Innovation and Public Policy.*

Correspondence	Question / Topic	Response / Action Summary
Meeting March 2016	Research to policy	Law/Act address social, health, economic needs/problems Public policymakers need persuasion/engagement on the strategic use of knowledge. Technology to improve performance needs short payback time (<2-3 years), need regulatory policies if the payback is > 3 years. Environmental policies can be co-designed Best Available Technologies (BATs) Tech Standards and Voluntary Agreements Governmental bodies of interest: - Medical Manufacturing Industrial Production (MMIP) - Department for Environment, Food and Rural Affairs (DEFRA) - Business Innovation and Skills (BIS)

*Table D.4: Correspondences with **Peter Hillier**, [redacted]; **Frank Wayman**, [redacted] & **Fiona Reid**, [redacted] at GlaxoSmithKline.*

Correspondence	Question / Topic	Response / Action Summary
Phone June 2016	Introductions with Peter Hillier	We talked about PenG / 6APA manufacture at the GSK Irvine facility. Peter offered a tour of the site to help understand the process further. He also said he could arrange a meeting with the utility team to discuss some data regarding energy usage and water usage.
Site Visit August 2016	See D.2 for: - A document sent ahead of the site visit - A presentation was given at the site visit - Site visit Report	
Email October - December 2016	Data sharing	Due to process sensitivity, information on energy and water usage cannot be disclosed per batch production, even when processes are masked. Only yearly figures can be given and would apply to the whole manufacturing site, including clavulanic acid production. A publically available presentation was received from GSK - Dunn, 2016

*Table D.5: Correspondences with **Stephen Arlington**, [redacted] at Pistoia Alliance Inc.*

Correspondence	Topic	Response / Action Summary
Phone December 2017	Locations of where biopharmaceuticals are being developed.	Location for developing pharmaceuticals highly dependent on cost and policies, environmental aspects, such as water impact, is not high on the list for consideration. Security of where the plant is located is also important; they need a secure supply chain. For instance, development in South America is low due to political and financial factors. There is ambition from Brazil, but they suffer from regulatory issues, waste issues and corruption. Mentioned risk of natural disasters such as earthquake is a factor, e.g. Mexico. Before the 2000s, UK/US our perform developing countries. Countries are catching up. For instance, Stephen sees a push for development in China and Korea. China, in particular, has a 5-year development plan and is self-sufficient in its material supplies. There are trends to develop in Central Asia, i.e. India. There is quite a high level of education than in the EU (large numbers of PhDs in science). Stephen stated that India understood the value of knowledge 40-50 years ago, which prompted its economic growth. However, there is regional corruption; the country is divided due to difference in party and religion, which mean there are issues with policy implementation. Singapore is attractive, as there are tax incentives. The country has advantages over China and India due to better political structuring (regulated and safe) and good supply chains from the rest of Asia – great prospect for industrial growth. On the other hand, Malaysia is not outwardly attractive for investment.
Phone December 2017	Trends in manufacturing	Biologics are low volume and high value. See growth in biosimilars. The life span of CEO tend towards a decade; this is a relatively short time for them to make a lasting impact and/or be motivated to plan beyond their active years. They need to consider risk profiles of drug development, i.e. sunk cost due to drug failures, £3-4 billion can be written off, in the hope to achieve two blockbuster drugs. Corporate policing are developing, the Irish Development Agency (IDA) is restricting tax incentives to develop in Ireland. However, they still have a pool of technical jobs/ job prospect – a great education system.
Phone December 2017	Ways to ensure companies are sustainable.	Need company representatives onboard – hit pressure points and sell reputations. Need mechanism to hold company representative accountable to see change, i.e. mechanisms are needed so people will not go against any initiatives – ultimately change need governmental enforcements. Mentioned: Sir John Bell – Industry Strategy & Alan Milburn – Department of Health - fundamental restructuring

D.2. SITE VISIT DOCUMENTATIONS

D.2.1. Pre-visit information sent to GSK

Penicillin Production at GSK Irvine with Peter Hillier

Charnett Chau Date of visit: 25th August 2016

Project Synopsis:

Life cycle assessment (LCA) is a tool to quantify the environmental impact of a given product, process or system across their whole life cycle, from raw material extraction to end of life. Due to the unknown environmental impact of the biopharmaceutical industry, it is necessary to carry LCA on representable processes to estimate the industry's sustainability. Penicillin and Recombinant Insulin production are being studied. In terms of tonnage, they are the highest produced molecules in their respective manufacturing modes, traditional and modern biotechnology processing. Although there are variations in manufacturing these molecules, a base process for each has been selected and will be used to estimate the environmental impact of the current yearly global supply. Penicillin (G and V) are produced at **$1.5x10^6$t*** per year since they are also converted to other beta-lactam antibiotics, whilst recombinant insulin is produced at 35t per year.

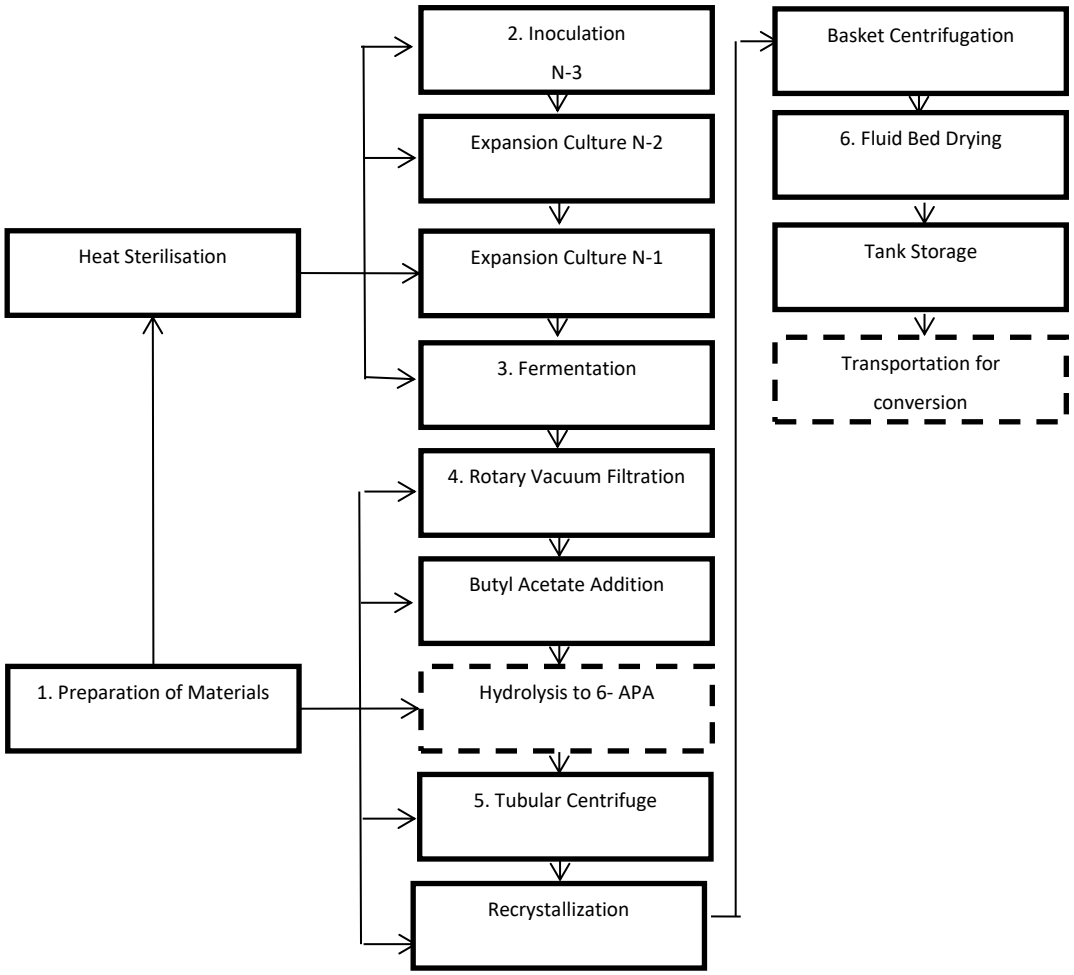
Additionally, the project aims to demonstrate that the utilisation of LCA can help make recommendations in optimizing biopharmaceutical processes. Using environmental and economic metrics in LCA can suggest areas to improve without high cost.

Visit Objectives:

- To understand the Penicillin production process with a focus on the auxiliary and support operations involved. Namely:
    - Heat sterilization
    - Air compression and filtration
    - Reactor temperature control – with glycol / chilled water
    - Preparation of glycol, chilled water, process water (PW) and water for injection (WFI)
    - Cleaning-in-place (CIP) and Steaming-in-place (SIP)
    - Mixing and blending - agitation seal lubrication
    - Centrifugation
    - Fluid bed drying
    - Waste disposal methods
- The key information I would like to obtain on the above operations, to be used as reference figures (the base process will vary from the manufacturing process used at GSK Irvine)
    - Energy consumption
    - Flow rates, i.e. flow of glycol and water
    - Rotational speeds (rpm)
    - Pressure
    - Temperature

Base Process for Penicillin Production Chosen

The following production process cross-references various literature, scientific journals and patents, and the chosen fermentation scale is 100m^3 with 75m^3 final fermentation volume. I am currently carrying out material balances on the process; information can be found in the literature; however, energy input and information on the control processes (e.g. glycol/chill water flow, lubrication and mixing requirements) are not readily published. I hope to obtain some figures which can help me calculate what my process will require.



***This was the original value calculated, assumptions used to calculate this figure was since updated**.

D.2.2. Site visit presentation given

UCL

Using LCA as a Tool to Assess and Make Recommendations for Future Biopharmaceutical Manufacturing

GSK Visit

Charnett Chau
25th August 2016

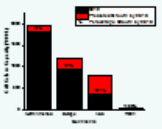
Primary Supervisor: Nigel Titchener -Hooker
Secondary Supervisors: Paola Lettieri & Suzanne Farid

1

UCL

Rationale

- Biopharmaceutical are important for our global health
- Industry's environmental impact unknown
  - Very few studies published
  - Whilst the industry is growing
- Increasing awareness of climate change
  - Public scrutiny on level of sustainability



Need LCA to evaluate and drive sustainable development of the biopharmaceutical industry.

2

UCL

Thesis Aims and Objectives

- To use LCA to form benchmarks and recommendations for future biopharmaceutical manufacturing
  - By estimating the current, and future, environmental impact of the biopharmaceutical industry
  - By comparing manufacturing alternatives
- To create a vision of integrating LCA with the biopharmaceutical industry

3

UCL

Analysis of Key Drivers for Sustainability

4

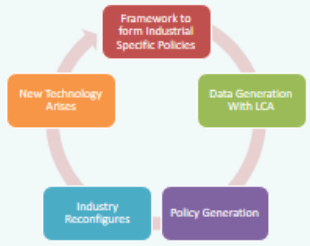
UCL

Collaboration Between Academia, Government and Industry

5

UCL

The Vision to Use LCA to Continually Promote Sustainable Development



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UCL

Process Selection – Demographic Analysis

Manufacture	Traditional Biotechnology	Modern Biotechnology
Age	~100 years	~ 30years
Locations	Worldwide	Mainly: US & EU Growth: China, India & SA
Revenue / Year (2014)	~ $10/kg	$1 - 2000/mg
	$240 billion in 2014[1] / Projected: $390 billion by 2019[2]	
Production / Year (2014)	Single brand	
	> 500Mt	1-10Mt
	Single molecule type / derivative	
	Penicillin: ~1.5x10^3 Mt	Insulin: ~35 Mt

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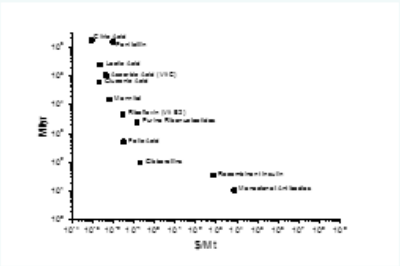
UCL

Biotechnological Products



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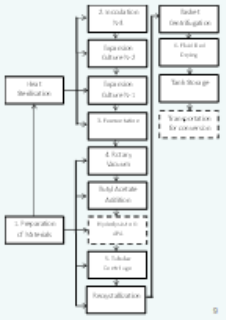
Traditional Process: Penicillin

- First commercialised biopharmaceutical
- Market worth: $15 billion
- Price: $10 / kg
- $1.5x10^9$ kg per annum
- Penicillin G and V are converted to 6 - APA
  - Piperacillin
  - Amoxicillin
  - Ampicillin
  - Other Beta-lactam antibiotics



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Modern Process: Recombinant Insulin

- First recombinant biopharmaceutical
- Highest produced
- Market worth: $24 billion
- Price: $1 / mg
- 35000 kg per annum
- Supplies 20% of global demand
- Increase in non-communicable diseases
- Production capacity predicted to double

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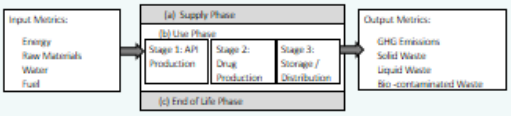
Life Cycle Assessment Overview



- Cradle to grave approach
- Ideally look at all three stages within use phase
- Focus on active pharmaceutical ingredient (API) manufacture
- Will analyse the global supply of the individual molecules

Data Collection
- Use of literature, patent, textbook
- Contacts with industry
- Average data

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Sustainability Indicators / Metrics

Environmental Metrics	Economic Metrics
Calculation from Life Cycle Inventory	
• Energy Intensity ($MAkg^{-1}$) • Process Material Intensity ($kgkg^{-1}$) • Water Consumption (Lkg^{-1}) • GHG Emissions ($kgCO_2$-$eqkg^{-1}$) • Solid Waste ($kgkg^{-1}$) • Wastewater (Lkg^{-1})	• Product Volume (kg) • Raw Material: Product Ratio • Cost of Raw Materials (Skg^{-1}) • Electricity Cost (Skg^{-1}) • Water Cost (Skg^{-1}) • Fuel Cost (Skg^{-1}) • Net Cash Flow Generated (Skg^{-1})
Calculation with Life Cycle Impact Assessment	
• Freshwater Aquatic Toxicity ($kg1,4$- dichlorobenzene $eq.kg^{-1}$) • Global Warming ($kgCO_2eq.kg^{-1}$) • Ozone Layer Depletion ($kgCFC\text{-}11eqkg^{-1}$) • Human Toxicity ($kg1,4$-dichlorobenzene $eq.kg^{-1}$) • Acidification ($kgSO_2eq.kg^{-1}$) • Eutrophication ($kgPO_4eq.kg^{-1}$)	

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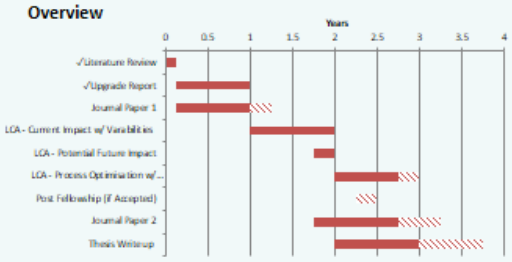
Overview



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Summary

- LCA is necessary to evaluate the biopharmaceutical industry's sustainability
- Collaboration between academia, government and industry can continually support sustainable development
  - A paper draft have been presented
- The project will model the global production of Penicillin and Recombinant Insulin to give a representation of the whole industry
- LCA metrics have been chosen / scoped for the target audience
- Data acquisition is in progress

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Thank you!
Questions?

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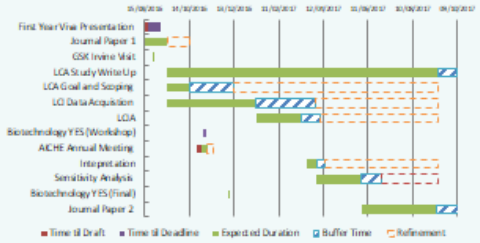
Plans for Next Year



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Background

The manufacture of biologically derived medicine (using biotechnology).

Traditional Biotechnology

The use of natural organisms.

- ~90 years old
- Low value – High production
- First drug being penicillin (antibiotic)

Modern Biotechnology

The use of genetically modified organisms.

- ~30 years old
- High value – Low production
- First biologic being recombinant insulin (hormone)

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Background
Current Status and Trends

Traditional Biotechnology

Established worldwide production

Top selling:
1.5x10⁶Mt of Penicillin worth ~$15billion
1x10⁶Mt of steroids worth $10billion

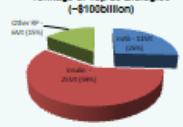
Modern Biotechnology

Production mainly in US and Europe
China, India and South Africa upcoming locations
Various platforms employed

~$230billion revenue in 2014
~$390billion revenue projected for 2019

246 drugs approved but ~6500 drugs in R&D
~1300 are biosimilars and biobetters

Tonnage of Top 20 Biologics (~$100billion)



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Background
Sustainability and the Biopharmaceutical Industry

Governmental Pressure
- Laws and regulations
- UNFCC
- EU Emission Trading System

Social Responsibility
- Established industry set examples
- Show leadership
- Sustainability in CSR
- Scrutiny from the public

Financial Influence
- Cost Intensive
- Competitive advancement

Environmental Impact?
- Was not considered energy intensive
- Few studies
- Comparison between single use and stainless steel production

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Project Drive and Vision
Summary of Background

- Biopharmaceutical production is energy intensive
- Industry utilize a variation of technology
  - A single process is not representative
- Location affects technological efficiencies
- Should always strive for better

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The Project
Processes – Generic

Source: Martins, M.L.; Mata, T.M.;Martins, A.A.; Neto, B.; Costa, C.A.V; Saloedo, R.L.R., 2010. LCA Tool Adaptation to Pharmaceutical Processes. *Faculty of Engineering University of Porto*, (January), pp.1–39

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Current Progress

- Data Acquisition
  - Literature
  - Interviews
- Researching how society influent biopharmaceutical sustainability

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The **goals** of the LCA study are as follows:

- Give estimation of the environmental impact of the whole biopharmaceutical industry.
  - Supply environmental parameter and their ranges, specific to the biopharmaceutical industry.
- Diagnose areas of high environmental impact.
- Compare alternatives and seek changes that can be made to decrease environmental impact without involving excessive costs.

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D.2.3. Post-visit Report

Visit to GSK Irvine Report

Date of Visit: 25th August 2016

Meeting with:

- Peter Hillier, Fermentation Technologist;
- Fiona Reid, Site Environmental Owner;
- Frank Wayman, Wastewater Treatment Plant Contract Manager

Facility Background

GlaxoSmithKline (GSK), as one of the leading pharmaceutical companies globally, provides a variety of products and services. Before the merge between Glaxo and Beechams, they both have a history with the development and production of penicillin. Augmentin, a drug that combines amoxicillin and clavulanic acid, is the top-selling semi-synthetic antibiotic globally. The GSK facility in Irvine manufactures two products: 6-aminopenicillanic acid (6-APA), the precursor to ß-lactam antibiotics, and Potassium Clavulanate, the most common form of clavulanic acid. The site was first used to manufacture Penicillin G (1973), which was then converted to a 6-APA facility in 1998, while Potassium Clavulanate has been in production since 1987. The two products, once formed, are transported offsite for the formulation, a large amount of 6-APA are converted to amoxicillin at the GSK manufacturing facility in Worthing for the formulation of Augmentin.

Over the years, major changes have been implemented on-site, especially in waste treatment; this includes their newest addition of anaerobic digesters as a waste treatment to subsidise their electrical usage as well.

Prior the Visit

Two documents were sent to Dr Peter Hillier, which was circulated to his colleagues, and these were:

- Penicillin Production at GSK Irvine with Peter Hillier.docx

The document outlines the project and the visit objectives. The main objectives of the visit were to understand the penicillin / 6-APA production process with more focus on the auxiliary and support operations and discuss the possibility of obtaining data that can be used as reference figures for the LCA of global Penicillin production.

- 23Aug16_GSK visit.pptx

The PowerPoint presentation details the rationale behind the project as well as the work that has been achieved. In order to gauge the environmental impact of the biopharmaceutical industry, it is necessary to capture a representation of both major classes of production - via traditional biotechnology and modern “recombinant DNA” technology. Penicillin, the precursor of 6-APA, is the highest produced traditional biotechnological molecule. Its conversion to 6-APA, therefore, involves both major techniques used in biotechnology; these are large scale production of secondary metabolites and enzymatic conversion.

The Visit

The afternoon at GSK Irvine was split as follows:

1. 6-APA production tour with Peter Hillier
2. Plant sustainability discussion with Fiona Reid (and Peter Hillier)
3. Waste treatment tour with Frank Wayman (and Peter Hillier)

6-APA Production

The manufacturing facility of 6-APA, spanning over five floors, has been divided into two sections via a central staircase. One side is dedicated to Penicillin production, upstream processing, and the other is used to convert penicillin to 6-APA and subsequent purification, downstream processing. A summary of upstream (Penicillin) production is shown in Figure D.1. A summary of downstream processing (conversion to 6-APA and purification) is shown in Figure D.2.

Upstream Processing

The tour commenced with an explanation of the steps before seed fermentation. Penicillin Chrysogenum spores, provided by DSM, are introduced onto rice to perform static culturing. Moulds appear and are transferred into suspension fluid to separate the rice grains. Once suspended, the fungi are inoculated with media making up 10% of the approximately 500ml in 2L shake flasks. An inoculation can is autoclaved, and the inoculated fungi are then transferred for further expansion before seed fermentation.

There are two seed fermentation steps involved; the first seed fermentation reactor is $8m^3$ in size with a connection to attach the inoculation can. The second seed fermenter is double in size at $16m^3$ capacity; however, the production fermenters used are $100m^3$ and $200m^3$. Depending on the schedule and the amount of product is in demand, the choice of production bioreactor size will vary. The plant holds three of each seed fermenters and five of each production fermenters ($1350m^3$ of total production working volume). This is meant that the plant can schedule production to be staggered, allowing continuous operation at downstream processing.

With the high working volumes, it has been said that the only requirement is to cool the reactors in terms of temperature control. This is because of all the heat given off from the machinery in order to carry out mixing and pumping into materials for cell culturing. Each fermenter has cooling coils within; depending on availability, tower water or chilled water are circulated to maintain culture temperature. Other monitoring includes organic compounds, using mass spectroscopy; pH, where ammonia is used for control; OD to measure the level of dissolved oxygen; and sterile samples are taken to ensure no contaminations are present.

Production of penicillin usually lasts ten days after induction with phenylacetic acid. Once induced, 12 hourly “mini” harvests occurred and replaced with fresh media. This results in 50% more volume of which contains Penicillin. The full harvest is transferred to the $250m^3$ holding tank, ready for processing.

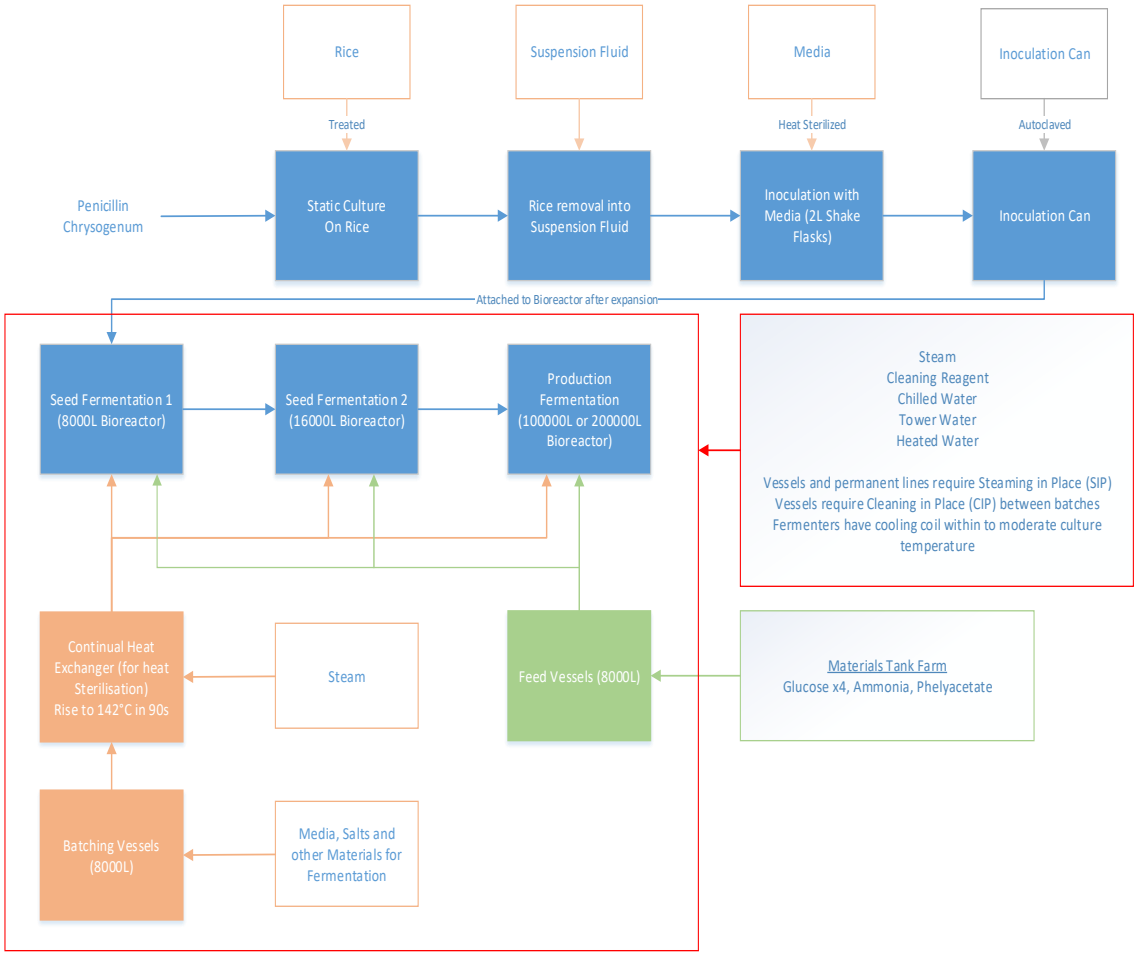

Figure D.1 Penicillin production schematic

For material preparation, there are three 8m^3 batching vessels where media is made up. A Kanban system of salts and nutrients is present is ensure enough materials are available for a full fermentation run. Their plant holds a tank farm where large quantities of materials are stored; this includes Glucose (which occupies four large tanks and are routinely re-filled throughout the day, usually two to three deliveries), Phenylacetate and Ammonia. Glucose consumption is very high; each bioreactor consumes 6Mt each day. The tanks of materials feed into feed vessels before addition to seed and production fermenters.

All vessels and lines require cleaning in place (CIP) and steaming in place (SIP) before materials are passed to ensure sterility. This includes the patch panel system that has been put in place to reduce the number of lines involved. Thus all batch and feed vessels can link to all fermenters, and all seed fermenters can be linked to all production fermenters.

Downstream Processing

Due to time limitations and maintenance work in the facility, a brief overview was shown in Figure 2. Microfiltration is said to be an optional step; depending on time constraints, this may be bypassed, the difference in results is the overall yield. Rotary centrifugation removes biomass. However, a solvent is added to begin the extraction of PenG (although not said on tour, this is typically butyl acetate). For further Penicillin extraction, other (at back extraction stages) typical used are phosphate buffer, chloroform solution and ether solution.

Post penicillin extraction is enzymes hydrolysis from penicillin to 6-APA and phenylacetic acid (PAA). PAA is recycled for inducing the production of penicillin at the production fermentation stage. Similarly, 6-APA is solvent extracted before is it crystallised and dried to powder form ready for bulk transported. The approximate amount of 6-APA produce per year amounts to 2000t.

Like upstream processing, equipment is sterilized before use; for cooling, process glycol is maintained at 5-10°C for downstream equipment.

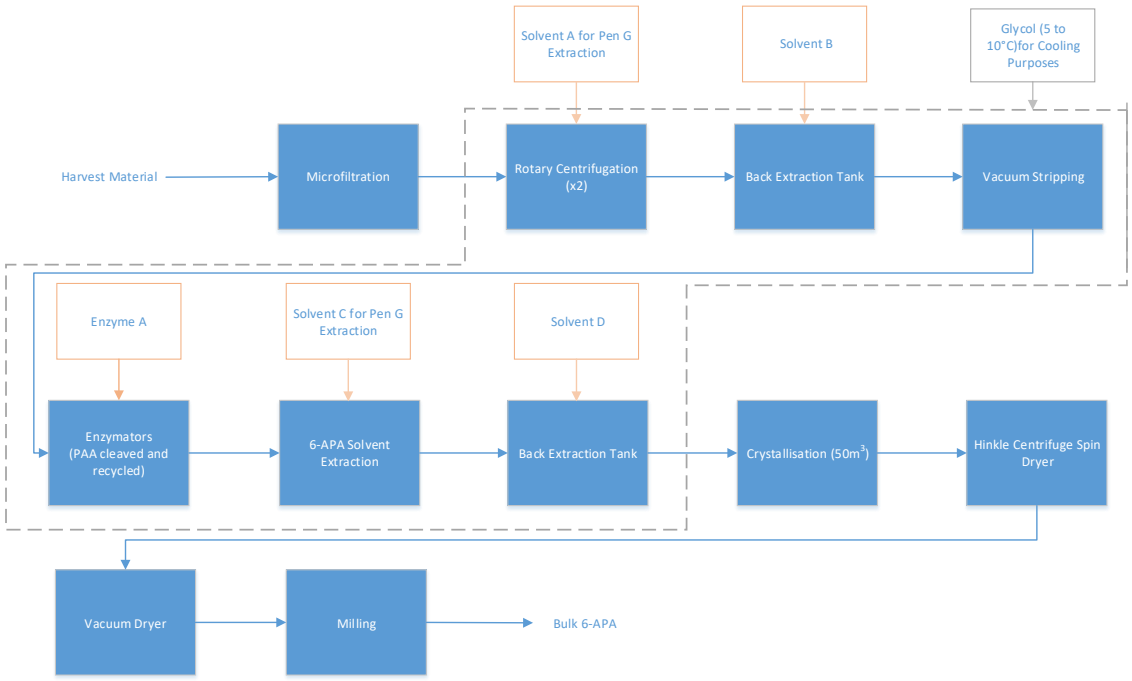

Figure D.2 Downstream processing schematic

Meeting with Fiona

The meeting involved Fiona Reid, Peter Hillier, Shannon – a placement student in Fiona's team and Rachel, who works with Peter in Upstream Processing. The nature of the meeting is for the company to better understand the project by talking through my presentation and discuss what information can be disclosed to aid the LCA study. The implications of allocating steam and electrical usage across the plant were also discussed.

Fiona's team has been working on the electrical and steam efficiency of the manufacturing facility. It was interesting to see that their size has a 40% baseload (percentage of maximum energy loading). This is the amount of steam and electricity required to maintain plant sterility and circulation when production is paused. When analysing the life cycle impact of the yearly production of Penicillin, the baseload for energy consumption should be considered to reflect the environmental burden in order to supply the required tonnage.

Electrical and steam efficiency are calculated as t of CO_2 per t of product. Because a whole manufacturing site itself produces products (6 APA and Clavulanic Acid), assumptions have been made for allocation. The spreadsheet shown by Fiona gave the efficiency per month; the graph shows the electrical efficiency for 6-APA ranges between 6 and 11 t_{CO2}/t_{6APA}, which depends on the intensity of production. As for steam efficiency, it is 6-16 $t_{CO2}/t_{6APA,}$ and 2-4 t_{CO2}/t_{PenG}.

Considering the yearly supply of 6-APA, this means that CO_2 emission could be up to 30000Mt per year purely from steam usage.

The company is particularly interested in steam because steam generation is regarded as the highest energy user of the manufacturing facility. Steam is used for SIP, heat sterilization of raw materials, heating of tank water, solvent recovery and distillation processes. According to the literature, steam generation requires much more energy than HVAC systems, which are usually the highest consumer of energy in smaller production scales.

In tackling climate change, the government has passed legislation to cut down on climate change. GSK Irvine, as a manufacturing facility operating machinery over 25MW, falls in the category that must join the emission trading system (ETS) and limitations to do with the climate change act are required for them to comply. However, discounts on emissions and electricity are given because they are major energy users providing acts are taken to minimise carbon footprint. With this, and since maintaining plant operation is essential and any power cuts can be detrimental to production, one of their energy efficiency projects was to go off the grid. The government backed this, and the company introduced two wind turbines along with three anaerobic digesters, which now produce 10% of the plant's electricity requirement whilst decreasing water consumption. (Dunn, 2016)provides a summary of the energy and water savings; this also indicates yearly site demands on utilities, which can be used as a reference for the LCA study.

The schematic of utility productions and treatment processes were briefly discussed due to time limitations, but it was agreed that an overall schematic could be shared. Due to intellectual property (IP) implications, it was not possible to discuss the materials used during the process or any process parameters on processing the raw materials, such as mixing rate in the blending vessels. However, from speaking with Fiona, it is possible to obtain the spreadsheet detailing the electrical and steam efficiency where they have started to allocate efficiency per unit operation. IP related information must first be taken away, whilst a more specific detail of what is needed for my study is required to be sent to Fiona in order for her to modify the spreadsheet. Please see follow-up actions.

Wastewater Treatment and Energy Generation

The plant was opened in 1973, and as the plant grew, the wastewater treatment plant grew with the increasing requirements before releasing material to the surrounding environment. The story began where the wastewater treatment only consisted of two concrete effluent tanks where materials were directly released after simple primary treatment. A steel effluent tank was commissioned when it was not socially acceptable for direct release, and thus harmful materials were directly the "new" tank for release at night-time; others were directed to the concrete tanks for daytime release. The treatment plant now has a series of 5000m^3 tanks where the effluent is recirculated, and 1MW of air (from the air compressor) is continuously pumped in for secondary treatment. It was said that there are ten 60KW pumps working across the wastewater treatment plant.

Typical hydraulic retention for the old concrete effluent tank is 10days, whereas, for the new tanks, this is 6 to 7 days for aerobic digestion. The newly implemented anaerobic digestion system has a hydraulic retention period of up to 45 days where the concentrated feed is transferred to methane and oxygen; the biogas can be used to generate electricity through the two engines. At 200m^3/h, 500-530KW can be generated. 15KW is used for stirring the three

3000m^3 digesters (a total capacity of 9000m^3 is not reached yet). At current, the energy generated from anaerobic digestion balances the energy used in pumping air into effluent tanks, which reduces the operational cost of the wastewater treatment plant and reduces CO_2 emissions and water consumption.

It may be a good idea to look at power supply separately: power supply purely from the grid, a mixture of own supply and the grid and potential off the grid manufacturing plant. A typical wastewater treatment plant for a penicillin / 6-APA manufacturing site will need to be researched to give the best representation of global norms.

Follow-Up Actions

An email was sent to Peter to thank him and his colleagues for the tour of the facility and the information they provisionally gave. Follow up emails is needed to obtain specific data that have been agreed upon.

Considering IP related implications, it is best to create production blocks to supply energy and steam usage data. For example, firstly split into upstream and downstream, then split into smaller production blocks. For downstream, this could be grouping all processes for penicillin extraction as one block, all processes for 6-APA extraction as another, then final drying and bulking of the product as another. Doing this, GSK will not disclose specific process parameters and will give me starting figures to work with. Scoping of the penicillin manufacturing process will be required as the scoping process may need to be altered to producing 6-APA.

Thus, the current plan is to re-scope the penicillin manufacturing study to 6-APA production and find the typical process to manufacture. The process will then be split into blocks where the company can allocate energy and heat with their current data set. Assumptions can be made for my own allocations to individual processes.

Further research into wastewater treatment in biopharmaceutical manufacturing sites is required. Conversation with Frank may gauge the level of waste involved as well as the electrical usage of the anaerobic digesters themselves. Fiona has mentioned that processes for generation purified water can be disclosed; engaging with Frank wastewater treatment may also be disclosed since they are not part of the production process.

Rough Notes from the day

These are notes made on the day of the tour; anything highlighted has not been worked into the report but may be useful when carrying out LCA.

Learning from the 6APA tour:

- Inoculate on to rice – suspension fluid – media – inoculation can (which is autoclaved)
- Batching vessels, 8ton, 8000kg 8m^3 capacity
- Material tower farms that stores glucose, salts, materials for fermentation
- Continual heat exchanger raise temperature to 142°C in the 90s
- Patch panel, all lines need SIP and feed materials between blending vessel, seed fermenter and production fermenters
- Seed fermenter 8m^3 and 16m^3 has a connection point for the inoculation can
    - Needs SIP, sterile media first
    - Has cooling coils for chilled water/tower water
- Towers from the tank farm feed feed-vessels

    - Four vessels are glucose (2-3 deliveries a day) need to use 6 ton of glucose for feed
    - Use ammonia as pH control
    - Phenylacetate for penicillin induction
- Production reactor 100 and 200m^3
    - 12 hours mini harvest for ten days
    - Result with 50% more volume to extract penicillin
    - 1350m^3 production working volume
- Measurement :
    - Mass spec- off-gas (online)
    - OD
    - pH
    - sterile samples – to check for contamination
- Post-production fermentation – 250ton storage
- Microfiltration
- Rotor centrifuge x 2
- Add solvent to extract pen g during rotary centrifuge
- Back extraction
- Vacuum stripping – need glycol cooling
- Enzymators – PAA cleaved recycled (50ton)
- Extraction of 6APA with solvent - Back extraction
- Crystallisers
- Hinkle Centrifuge spin dryer - Vacuum dryer – mill
- 2000ton/year

Waste Effluent Tour

- Concrete effluent plant (£20mil to build 20years ago, equivalent to £100mil now)
- Overtime cost of electricity increased 2p to 9p to 6.5p/kWh may be up to 8p
- 60kwh x 10 pumps for recirculation of effluent in the tank
- 5000m^3 to add air
- 1MW of air continuously
- 6-7 hydraulic retention (old ones were ten days)
- Anaerobic up to 45 days, feed concentration for the concrete effluent plant, three digesters in total, capacity of 3000m^3 each
- 200m^3 / h engine can get 500kwh output
- 15kw stirring
- Mass + oxygen to get CO_2 but for biomass goes methane from anaerobic digestion which gives oxygen, CO_2 and heat
- CHP engines 30L x2, 500kw each to pump air

Own: Two wind turbines and anaerobic digestion onsite to form 10% of the whole plant's electrical usage.

Talking to Fiona:

- 40% baseload, even when production is off, still need to maintain sterility and air circulation etc.
- Electrical efficiency CO_2 per Mt product 6-11
- Steam efficiency
    - Steam recovery/distillation, heat exchange, SIP
    - 6-16 6APA and 2-4 Pen G fermentation

APPENDIX E: ASSUMPTION AND PROCESS CALCULATIONS

E.1 UPSTREAM PROCESSING

Table E.1: Penicillin chrysogenum growth rates used to calculate cell mass after each cell culture stage.

Cell Growth Phase	Cell Growth Rates (hr-1)	Reference
Static	0.053	(Trinci and Pirt, 1968)
Lag	0.014	(Trinci and Pirt, 1968)
Submerged	0.123	(Trinci, 1969)
Max	0.31	(Ariyo et al., 1998)
Penicillin Production / Growth limited	0.009	(Trinci and Pirt, 1968)

Table E.2: Biomass calculations: duration literature source: (Meštrović and Chow, 2015)*, industry source: (Hillier, 2016; Carleysmith, 2016). Equation used:* $x = x0*exp^{ut}$*, where x= cell mass (kg), x0 = initial cell mass (kg), u= growth rate (hr^{-1}) (Table E.1) and t= time (hr)*

Stage	Volume (L)	Duration (hr)	Growth Phase	Cell Mass IN (kg)	Cell Mass OUT (kg)	Assumptions
Rice Inoculation	0.055	240	Static	5.50E-11	1.84E-05	Rice inoculation procedure and cell mass assumed (Pham et al., 2010)
Flask	0.5	9	Lag	1.84E-05	2.09E-05	
		51	Submerged	2.09E-05	1.11E-02	
Can	150	9	Lag	1.11E-02	1.25E-02	Flask culture was split into two can fermentation processes.
		39	Submerged	1.25E-02	1.52E+00	
N-2	12800	16	Lag	1.52E+00	1.89E+00	Each can fermentation is fed into separate N-2 bioreactors
		32	Submerged	1.89E+00	1.02E+02	
N-1	25600	16	Lag	1.02E+02	1.27E+02	Each N-2 bioreactor out is fed into an N-1 bioreactor
		12	Submerged	1.27E+02	4.78E+02	
Production	240000	15.6	Lag	4.78E+02	5.94E+02	The two N-1 bioreactor output are fed into a 200,000L and a 100,000L bioreactor. Assumed mini harvest every 12 hours - 7500L of fermentation transferred to product harvest tank (total from both production bioreactors, fermentation media assumed to be assed to reactors after mini harvest
		8.4	Max	5.94E+02	8.03E+03	
		216	Growth limited	8.03E+03	1.72E+04	
TOTAL OUTPUT	353500					
Cell Conc. (g/L)	48					

Table E.3: Assumptions for biomass and penicillin production.

Parameters	Value	Range	References
Product Titre (g/L)	57	35 to 100	(Goldrick et al., 2015; Heinzle et al., 2006; Sarafian, 2015)
Biomass yield on glucose Yx/glu (g/g)	0.45	-	(Harding et al., 2007)
Glucose required for maintenance Mglu (g/g/h)	0.022	-	(Harding et al., 2007)
Penicillin yield on phenylacetic acid Ypen/PAA (g/g)	2	-	(Harding et al., 2007)
Oxygen yield on product Yo2/p (mg/g)	160	-	(Harding et al., 2007)

Table E.4: Glucose required for cell growth at each cell culture stage, calculated using the assumptions from Table E.3.

Stage	Cell in (kg)	Growth (kg)	Glucose for Cell Growth (kg)
Rice	5.50E-11	1.84E-05	4.09E-05
Flask	1.84E-05	1.18E-01	2.62E-02
Can	1.11E-02	1.58E+00	3.52E+00
N-2	1.52E+00	1.05E+02	2.35E+02
N-1	1.02E+02	3.93E+02	9.36E+02
Production	4.78E+02	1.75E+04	4.13E+04
	Penicillin (kg)	**PAA for Penicillin Production (kg)**	**Glucose for Penicillin Production (kg)**
Production	2.01E+04	1.00E+04	2.48E+04

Stoichiometry for media components (Doran, 1995):

(Eq. E.1.) $Glucose + Ammonium\ Sulphate + oxygen \rightarrow Biomass + Carbon\ Dioxide + Water$

(Eq. E.2) $Glucose + Ammonium\ Sulphate + Penicilanic\ Acid + Oxygen \rightarrow Penicllin\ G + Carbon\ Dioxide + Water$

Table E.5: Main inputs and outputs of fermentation, calculated using Tables E.2 and E.4., and Equations E.1 and E.2.

	Inputs			Outputs		
Stage	**Ammonia Sulphate (kg)**	**Oxygen (kg)**	**PAA (kg)**	**Carbon Dioxide (kg)**	**Water (kg)**	**Penicillin G (kg)**
Rice	9.88E-06	1.37E-05		2.70E-05	1.64E-05	
Flask	6.33E-03	8.80E-03		1.74E-01	1.06E-02	
Can	8.48E-01	1.19E+00		2.34E+00	1.42E+00	
N-2	5.63E+01	7.98E+01		1.57E+02	9.48E+01	
N-1	2.11E+02	3.59E+02		6.69E+02	3.89E+02	
Production	1.73E+04	1.89E+04	5.54E+03	3.77E+04	2.84E+04	2.01E+04

Table E.6: Fermentation media composition assumptions. These compositions were used to make up the working volumes of each cell culture stage.

Media Components	Standard Media	Production Media	Reference
Corn Steep Liquor (g/L)	100	25	(Bhuyan and Johnson, 1957; Nielsen et al., 1995)
Sucrose/Glucose (g/L)	3	203	(Nielsen et al., 1995) for standard media; value for production media calculated.
KH2PO4 (g/L)	1	2	(Nielsen et al., 1995)
(NH4)2SO4 (g/L)	18	53.1	Stoichiometry-based calculation
CaCl2.2H2O (g/L)	0.06	0.12	(Nielsen et al., 1995)
Pleuronic (ml/L)	0.2	0.4	(Nielsen et al., 1995)
Phenylacetic Acid (g/L)		17	Stoichiometry-based calculation
Purified Water (g/L)	928	981	It was calculated by subtracting the liquid volume of each liquid component (corn steep liquor and pluronic) from 1L.

Table E.7: Assumptions for bioreactor and mixing reactor sizing.

Parameter	Value / Equation	Notes and References
Incubation - Power Consumption (kW)	2.34	Innova 44R – Eppendorf. The model selected based on capacity requirements. (Ependorf, 2019)
Tank Aspect Ratio (H_T : D_T)	3:1 (bioreactor) 3:2 (mixing)	(Jagani et al., 2010)
Impeller Diameter (I_D)	0.4 D_T	D_T – tank diameter, H_T = tank height H_L = Liquid height - is determined by the working volume of the fermentation that was set as 80% of tank capacity. ρ_L = Liquid density – this depended on the cell culture. All fermentation components were added and divided by the volume to obtain the liquid density for each fermentation process. (Doran, 1995; Jagani et al., 2010; Mudde et al., 2016; Richardson et al., 1991; Villadsen, 2015)
Impeller Height (I_H)	0.11 D_T	
Impeller Width (I_W)	0.06 D_T	
Number of Impellers (I_N)	$H_L \times \rho_L / D_T$	
Impeller Spacing	0.45 D_T	
Impeller to Sparger	0.15 D_T	
Impeller to Bottom	0.3 D_T	
Impeller Shaft Width	0.095 D_T	
Impeller Shaft Length	$H_T + 0.25\ D_T$	
Bioreactor Wall thickness (mm)	25	Assumed, adopted from Doran (1995). Used for the calculation of stainless steel requirements for equipment fabrication.
Stainless Steel Density (kg/m^3)	7850	(Mudde et al., 2016)
Mixing Power Number (P_o)	6	(Mudde et al., 2016)
Impeller Tip Speed (u) (m/s)	3	(Doble et al., 2004)
Impeller Speed (N) (rpm)	$(u \times 60) / (\pi \times I_D)$	(Doran, 1995)
Mixing Power (kWh)	$P_o \times I_N \times \rho \times N^3 I_D^5$	(Doran, 1995)

E.2 DOWNSTREAM PROCESSING

Pumping requirements

(Eq. E.3)	P=pQH/367n	Equation: (Doran, 1995)
P=Power	kW	
p=density	kg/dm^3	
Q=flow rate	m^3/hr	Depending on flow rate requirements, different pump specifications were used to obtain H and
H=head	m	n.
n=Efficiency	0 - 1	(Aliasso and Corporation, 1999; KSB, 2005)

Table E.8: Yield assumptions for the harvest tank storage step.

Parameter		Reference
Penicillin yield	0.98	(Sadana, 1998)
liquid yield	0.99	(McAllister, 2009)
Tank capacity (m3)	300	(Hillier, 2016)
Aspect Ratio (HT: DT)	3:2	(Doran, 1995)
Other sizing calculations as Table E.7		

Table E.9: Summary of input of the harvest step. Power consumption considers mixing for ten days from the start of min harvests to all fermentation broth were released to the next unit operation.

	Input	Output	Notes
Penicillin G (kg)	2.01E+04	1.97E+04	2% of penicillin and biomass were assumed degraded during harvest.
Cells (biomass) (kg)	1.80E+04	1.76E+04	
Liquid Broth (kg)	3.86E+05	3.86E+05	
Total (kg)	4.24E+05	4.23E+05	
Volume (L)	3.53.E+05	3.52.E+05	
Power (Mix + Pump (kWh)	1.53E+04		Equation E.3 + Mixing power equation in Table E.7

Table E.10: Parameters used to calculate material requirements, such as wash buffer, filter aid, and subsequent outputs towards waste and the next unit operation.

Process Parameters	Value	Range (if applicable) and Reference
Cycle time (s)	60	(Prasad, 2012)
Area (m^2)	40	(Prasad, 2012)
Filter rate (L/hr)	20000	(Prasad, 2012)
Vacuum (inch Hg)	20	(Prasad, 2012)
(psi)	0.5	
Resistance (s/cm2)	30	(Prasad, 2012)
Cake : Filtrate	0.1	(Prasad, 2012)
Washing efficiency (%)	70	(Prasad, 2012)
Retention (filtrate)	0.01	(Prasad, 2012)
Filtration time (s)	20.8	(Prasad, 2012)
Resistance = (1 - efficiency)^n)		
n	3.8	
Washing time (s)	15.9	(Prasad, 2012)
Wash Area (m^2)	10.6	Calculated based on wash time and size of drum
Airflow (me/hr/ m^2)	20	(Infrigo, n.d.)
Pre-coating Parameters		
Filter aid coat thickness (mm)	85	(Prasad, 2012)
Knife advance rate (mm per rev)	0.07	(Richardson et al., 1991)
Filter aid Conc. (%)	3	2 to 5 (Prasad, 2012)
Cycle time (s)	20	(Prasad, 2012)
Area (m^2)	40	(Prasad, 2012)
Filter rate (L/hr)	40000	(Prasad, 2012)
Vacuum (inch Hg)	20	(Prasad, 2012)
(psi)	1	
Resistance (s/cm2)	1	(Prasad, 2012)
Cake : Filtrate	0.1	(Prasad, 2012)
Retention (filtrate)	0.01	(Prasad, 2012)
Filtration time (s)	2.8	(Prasad, 2012)
Sizing - Drum		
Diameter (m)	1.5	1..2 to 3.6 (Westech, 2018)
Length (m)	8.5	1 to 10 (Westech 2018)
Circumference (m)	4.7	(Westech 2018)

Sizing - Trough		
Length (m)	8.5	(Westech, 2018)
Width (m)	1.5	(Westech, 2018)
Height (m)	0.3	(Westech, 2018)
Volume (m3)	3.83	(Westech, 2018)
Solid Yield	97%	(Harding, 2008; Harding and Harrison, 2016)
Product Yield	97%	(Harding, 2008; Harding and Harrison, 2016)
Liquid Retention	3%	(Harding, 2008; Harding and Harrison, 2016)
Filter aid make up tank sizing calculations as Table E.7		
Equipment Power (kW)	45	(Andritz, 2016)
Vacuum Pump Power (kW)	20.5	(calculated) Pump spec: (Tuthill, 2015)
Other pump calculations using Eq. E.3		
Total Electricity Consumption (kWh)	1012.6	This includes filter aid makeup, pre-coating of drum and the actual operation. Flow rate and volume determined the time, which was multiplied by the power rating of the equipment

Table E.11: Calculated outputs of the rotary vacuum filtration step. Wash was calculated based on the duration of processing the product flow and the area of the drum that requires washing.

	Product Stream		Waste stream	
Component	kg	L	kg	L
Wash Volume	8.81E+03	8.81E+02	4.41E+02	4.41E+02
Fermentation Liquor	3.74E+05	3.42E+05	1.16E+04	1.16E+04
Penicillin	1.91 E+04	-	5.91E+02	-
Cell Debris	5.28E+02	-	1.71E+04	-
Total	4.03E+05	3.51E+05	2.97E+04	1.10E+04

Table E.12: Parameters used for the solvent and back extract unit operations before penicillin hydrolysis to 6APA

Parameters	Value / Assumption	Notes / References
Operational pH	2.5	(Ahuja, 2000; Rai, 2012)
Acid	1M Sulphuric Acid	(Bahloul et al., 2013)
Acid pH	1	
Assumed fermentation pH	6.5	(Liu et al., 2016)
Acid Volume (L)	6.42E+03	$[H+] = 10^{-pH}$ $V_{broth} \times [H+]_{broth} + V_{acid} \times [H+]_{acid} = V_{total} \times [H+]_{target}$
Product Flow rate (L/hr)	20000	From the previous step
Acid Flow rate (L/hr)	370	Acid Flow rate = Acid Volume / Product Volume x Product Flow rate
Solvent Extraction- Product (Aqueous) to Solvent Ratio	7:1	Range 5 to 7:1 (Ahuja, 2000; Rai, 2012)
Solvent Vol. (L)	4.77E+04	Calculated from ratio
Solvent Flow rate (L/hr)	2670	Calculated from ratio
Solvent Extraction – Penicillin Yield	0.96	(Stankiewicz and Moulijn, 2004)
Solvent Extraction – Biomass Removal	0.99	Assumed
Back Extraction – Aqueous (Buffer) to Solvent Ratio	0.2:1	(Ahuja, 2000; Rai, 2012)
Buffer	10mM Phosphate Buffer	Preparation for enzyme hydrolysis (Marconi et al. 2012)
Buffer Volume (L)	9.53E+03	Calculated from ratio
Buffer Flow rate (L/hr)	534	Calculated from ratio
Back Extraction – Penicillin Yield	0.97	(Stankiewicz and Moulijn, 2004)
Back Extraction – Biomass Removal	0.98	Assumed
Solvent Extraction Equipment Power (kW)	15	Two units employed—reference equipment: Rousselet Robatel BXP520. The model was selected based on capacity and flow rate requirements. (Rousselet Robatel, 2019)

Back Extraction Equipment Power (kW)	5.5	One unit employed Reference equipment: Rousselet Robatel BXP320. The model selected based on capacity and flow rate requirements (Rousselet Robatel, 2019)
Total Electricity Consumption (kW)	438	This includes pumping requirements and the running of the equipment, duration of operation, determined by the flow rate, and was multiplied by the power rating of the equipment.

Table E.13: Summary of the combined outputs of solvent and back extraction. Penicillin was assumed extracted into the phosphate phase, ready for enzyme hydrolysis.

	Product Stream		**Waste stream**	
Component	**kg**	**L**	**kg**	**L**
Phosphate Buffer	9.55E+03	9.53E+03		
Fermentation Broth			3.83E+05	3.51E+05
Penicillin	1.78E+04		1.20E+03	
Cell Debris	1.00E-02		5.28E+02	
Butyl Acetate			4.20E+04	4.77E+03
Sulphuric Acid			6.71E+03	6.42E+03
Total	2.73E+04	9.53E+03	4.34E+05	3.62E+05

Table E.14: Parameters and assumptions for enzyme hydrolysis.

Parameters	**Value / Assumption**	**Notes / References**
Pen G to Enzyme Ratio	2:1	(Vélez et al., 2014)
Vessel	4 x CSTR	(de Gooijer et al., 1996; Ghisalba et al., 2010)
Enzyme Life (hrs)	4000	(Poulsen, 1984)
Productivity ($kg_{product}/kg_{enzyme}$)	2000	(Poulsen, 1984)
Substrate (Penicillin) (%w/v)	15	(Poulsen, 1984)
Additional Phosphate buffer (L)	2.38E+04	Calculated to correct the concentration of penicillin
Conversion Yield (%)	98	(Cooney and Acevedo, 1977; Ferreira et al., 2004)
pH maintenance buffer	0.1M sodium hydroxide	(Muzzarelli et al., 1986)
6-APA molar mass (g/mol)	216	(NCBI, 2019)
Penicillin molar mass (g/mol)	334	(NCBI, 2019)
PAA molar mass (g/mol)	136	(NCBI, 2019)
Water molar mass (g/mol)	18	(NCBI, 2019)
Equipment Sizing	Tank as Table E.7.	
Total Energy requirements (kWh)	362	Includes pumping of input materials and mixing CSTRs

Table E.15: Summary of the outputs of enzyme hydrolysis. Note: there is no waste stream.

	Product Stream	
Component	kg	L
Phosphate Buffer	1.04E+05	1.04E+05
Penicillin	3.56E+02	
6-APA	1.13E+04	
Cell Debris	1.00E-02	
0.1M Sodium Hydroxide	4.07E+01	4.05E+01
Total	1.16E+05	1.04E+05

Table E.16: Parameters used for the solvent and back extract unit operations before crystallisation.

Parameters	**Value / Assumption**	**Notes / References**
Operational pH	2.5	(Ahuja, 2000; Rai, 2012)
Acid For pH adjust	1M Sulphuric Acid	(Bahloul et al., 2013)
Acid pH	1	
Assumed fermentation pH	7.99	From previous (phosphate buffer)
Acid Volume (L)	3.43E+03	$[H+] = 10^{-pH}$ $V_{broth} \times [H+]_{broth} + V_{acid} \times [H+]_{acid} = V_{total} \times [H+]_{target}$
Product Flow rate (L/hr)	20000	From the previous step
Acid Flow rate (L/hr)	660	Acid Flow rate = Acid Volume / Product Volume x Product Flow rate

Solvent Extraction-Product (Aqueous) to Solvent Ratio	7.5	(Ahuja, 2000; Rai, 2012)
Solvent Vol. (L)	1.44E+04	Calculated from product to solvent ratio
Solvent Flow rate (L/hr)	2670	Calculated from product to solvent ratio
PAA Removal (%)	99.6	(Bristol Myers Squibb Co., 2004)
Penicillin Removal (%)	98	(Stankiewicz and Moulijn, 2004)
6-APA Transferred (%)	1	(Stankiewicz and Moulijn, 2004) (Bristol Myers Squibb Co., 2004)
Biomass Transferred (%)	5	(Bristol Myers Squibb Co., 2004)
Back Extraction – Solvent to Aqueous (Acid) Ratio	0.1	(Ahuja, 2000; Rai, 2012)
Acid For back extraction	3.2mM Sulphuric Acid	(Bristol Myers Squibb Co., 2004)
Acid Volume (L)	1440	Calculated from solvent to acid ratio
Acid Flow rate (L/hr)	270	Calculated from solvent to acid ratio
PAA Removal	0.4	(Bristol Myers Squibb Co., 2004)
Penicillin Removal	0.01	(Stankiewicz and Moulijn, 2004)
6-APA Transferred	0.99	(Bristol Myers Squibb Co., 2004)
Biomass Transferred	0.95	(Bristol Myers Squibb Co., 2004)
Solvent Extraction Equipment Power (kW)	15	Two units employed. Reference equipment: Rousselet Robatel BXP520 (Rousselet Robatel, 2019)
Back Extraction Equipment Power (kW)	5.5	One unit employed Reference equipment: Rousselet Robatel BXP320 (Rousselet Robatel, 2019)
Total Electricity Consumption	184	This includes pumping requirements and the running of the equipment, duration of the operation. It was determined by the flow rate and was multiplied by the power rating of the equipment.

Table E.17: Summary of the combined outputs of solvent and back extraction. Penicillin should be extracted into the sulphuric acid, ready for crystallisation.

	Product Stream		**Waste stream**	
Component	**kg**	**L**	**kg**	**L**
Penicillin	1.06E+01		6.94E+02	
6-APA	1.12E+04		1.14E+02	
PAA	2.83E+01		1.41E+04	1.23E+04
Biomass	1E-02		6.00E-04	
3.2mM Sulphuric Acid	1.44E+03	1.44E+03		
Phosphate Buffer	1.04E+05	1.04E+05		
0.1 M Sodium Hydroxide	4.07E+01	4.05E+01		
1M Sulphuric Acid	3.58E+03	3.43E+03		
Butyl Acetate			2.54E+04	2.88E+04
Total	1.20E+05	1.09E+05	4.03E+04	4.11E+04

Table E.18: Parameters and assumptions for crystallisation.

Parameters	**Value / Assumption**	**Notes / References**
Operating pH	4.1	(ER Squibb and Sons LLC, 1962)
Vessel Working Volume (m^3)	110	Volume-based
Crystallisation Time (hr)	2	(ER Squibb and Sons LLC, 1962)
Buffer for pH Adjust	0.1M Sodium Hydroxide	(ER Squibb and Sons LLC, 1962)
Buffer Volume (L)	589	Calculated in relation to [OH] in the product stream
Equipment Sizing and Power	As Table E.7.	
Total Energy (kWh)	764	Mixing and pumping materials
Crystallisation Yield	0.99	(Broun et al., 1978; Cao et al., 2001)
Purity	0.99	(Cao et al., 2001)
Penicillin / PAA degradation	0.5	(Cao et al., 2001)
Biomass degradation	0.98	(Cao et al., 2001)

Table E.19: Summary of the outputs of crystallisation. Note: there was no waste stream.

	Product Stream	
Component	**kg**	**L**
Penicillin	5.00E+00	
Degraded Penicillin	5.00E+00	
6-APA	1.13E+02	
Crystallised 6APA	1.12E+04	
PAA	2.80E+01	
Degraded PAA	2.80E+01	
Biomass	N/A	
Cell Debris	1.00 E-02	
1mM Sulphuric Acid	1.44E+03	1.44E+03
Phosphate Buffer	1.04E+05	1.04E+05
0.1M Sodium Hydroxide	6.51E+02	6.34E+02
1M Sulphuric Acid	3.58E+03	3.43E+03
Total	1.21E+05	1.09E+05

Table E.20: Parameters and assumptions for crystallisation.

Parameters	Value / Assumption	Notes / References
Equipment	Basket Centrifuge	(Stanbury et al., 1995)
	Rousselet Robatel Slab 1602 DFR	(Andritz, 2016)
Equipment Power (kWh)	45	(Andritz, 2016)
6-APA on filter	0.98	(Wright, 1993)
Moisture Content after 1st spin	0.1	(Wright, 1993) Solutes (including penicillin and degraded PAA and biomass) assumed to be present in the cake.
Wash with Methanol/Water (L)	3560	Double the liquid volume left within filter cake (Ahuja, 2000; Marconi et al., 1973; Wright, 1993)
Moisture Content after 2nd spin	0.05	(Wright, 1993)
Total Energy Requirements (kWh)	345	Value Includes running the equipment and pumping of materials into the unit operation.

Table E.21: Summary of the outputs of the spin-dry process using a basket centrifuge.

	Product Stream		Waste stream	
Component	**kg**	**L**	**kg**	**L**
Solutes (Aggregated)	3.40E-01		8.05E+00	
Original Process Fluid Liquid	2.56E+01	2.56 E+01	1.09E+05	1.09E+05
WFI/methanol	5.50E+02	6.14E+02	2.63E+03	2.95E+03
Crystallised 6-APA	1.09E+04		223	
Total	1.15E+04	6.39 E02	1.12E+05	1.12E+05

Table E.22: Parameters and assumptions for vacuum drying.

Parameters	Value / Assumption	Notes / References
Equipment	Vacuum Pan Dryer	(Henikel, 2018)
Equipment Power (kWh)	90	(Henikel, 2018)
Moisture content	0.0005	(Parikh, 2015)
Heat Transfer Coefficient (h_c) (J/m^{2}sC)	30	Value for free-flowing granular, powdery products (Doran, 1995).
Air Temperature (ºC)	50	(Henikel, 2018)
Initial Temp of Solid (ºC)	25	Assumed
Surface Area (m^2)	22.5	(Henikel, 2018)
Vapour Heat Transfer Coefficient (h_v) (kj/kg)	2416	(Doran, 1995)
Constant dry rate (Nc) (kg/s)	0.007	$N_c = h_c$ x surface area x dT / h_v (Doran, 1995)
Time (hr)	10.7	Value assumed drying under constant rate.
Electricity consumption (kWh)	1950	Value includes equipment and vacuum pump.
Evaporation		Methanol was assumed to evaporate first.

Table E.23: Summary of the outputs of the spin-dry process using a vacuum dryer.

	Product Stream		Waste stream	
Component	**kg**	**L**	**kg**	**L**
Solutes	3.40E-01			
Original Process Fluid Liquid	5.50E+00	5.50E+00	2.00E+01	2.00E+01
WFI/methanol			5.50E+02	6.14E+02
Crystallised 6-APA	1.09E+04			
Total	1.09E+04	5.50E+00	5.70E+02	6.34E+02

Table E.24: Parameters and assumptions for milling.

Parameters	Value / Assumption	Notes / References
Equipment	HammerWitt	(Frewitt, 2011)
Equipment Power (kWh)	15	(Frewitt, 2011)
Throughput (kg/hr)	4000	(Frewitt, 2011)
Overall Yield	0.99	Assumed.

Table E.25: Product summary – post milling.

	Product Stream	
Component	**kg**	**L**
Solutes	3.30E-01	
Original Process Fluid Liquid	5.40E+00	5.40E+00
Crystallised 6-APA	1.08E+04	
Total	1.08E+04	5.40E+00
Purity (%)	99.9	

E.3 UTILITIES

Table E.26: Equations and assumptions used to calculate cooling water flow rates.

	Parameters /Assumptions	Notes / References
Agitator energy (kW/m³)	1	(Harding, 2008)
Energy per [O2] consumed (kJ/mol)	460	(Harding, 2008)
Heat energy generated (Q)	(Total agitator energy + total energy produced due to O_2 consumption)/cell culture duration	(Harding, 2008)(Harding, 2008)(Harding, 2008)Fermentation volume and duration as assumed Table E.2 and oxygen consumed as calculated in Table E.5)
Equation	$Q=m_c*C_{pc}(Tout-Tin)$	Q = heat energy (kJ) m = mass flow (kg/s) C_p = heat capacity (j/kgC) T= temperature (°C) c = cooling water
Bioreactor temperature (°C)	35	(Goldrick et al., 2015)
Cooling water in (°C)	15	Assumed starting temperature.
Cooling water in (°C)	20	Change in cooling water temperature between 5 to 10 °C (Pratt, 2010)
Mass flow rate (kg/h)	Can – 0.0482 N-2 – 3.75 N-1 – 16.7 100m³ production reactor – 128 200m³ production reactor – 256	The Mass flow rate of cooling water for each fermentation reactor was due to media volume and density differences.
Mass of water not returned to source (consumed) (kg)	Can – 0.116 N-2 – 9.01 N-1 – 22.1 100m³ production reactor – 1460 200m³ production reactor – 2910 Total - 4400	Fermentation duration x mass flow rate x 0.05.

Table E.27: Off-the-shelf requirements for WFI and pure steam generation.

	Parameters /Assumptions	Notes / References
Equipment	Finn Aqua Multiple Effect Water Distiller	(Steris, 2015)
Equipment Spec.	IN: Cooling Water: 1460 L/hr Feed Water: 12,360 L/hr Steam: 2470 kg/hr OUT: WFI: 10750 L/hr	(Steris, 2015)
Power rating (kW)	75	(Steris, 2015)
Equipment	Finn Aqua T Series Pure Steam Generator	(Steris, 2015)
Equipment Spec.	IN: Feed Water: 5035 L/hr Steam: 5460 kg/hr OUT: Pure Steam:4750 kg/hr	(Steris, 2015)
Power rating (kW)	36	(Steris, 2015)

Table E.28: Equations and assumptions used to calculate mass flow rates of steam and cooling water during media sterilisation.

	Parameters /Assumptions	Notes / References
Media flow rate (L/hr)	20000	(Junker et al., 2006)
Heat Capacity - C_p (j/kgC)	Water – 4.184 Steam – 2.198 Glucose at18% concentration – 3.8	Media assumed to be 18% concentration. for calculations
Equation	Q=mc*Cpc(Tout-Tin)=mh*Cph(Tin-Tout)	Q = heat energy (kJ) m = mass flow (kg/s) Cp = heat capacity (j/kgC) T= temperature (°C)
Media Initial Temperature (°C)	20	(Deindoerfer, 1957; Harding, 2008)
Cooling Water Initial Temperature (°C)	15	Output of media sterilisation
Heat Exchange 1 Output Temp. (°C)	80 (heat exchange with heated media)	(Junker et al., 2006)
Heat Exchange 2 Output Temp. (°C)	145 (heat exchange with steam)	(Junker et al., 2006)
Heat Exchange 1 Output Temp. (°C)	85 (heat exchange with fresh media)	(Junker et al., 2006)
Heat Exchange 3 Output Temp. (°C)	35 (heat exchange with cooling water)	(Junker et al., 2006)
Mass flow rate	The mass flow rate of cooling water and steam were calculated using the equation in this table.	

Table E.29: Assumptions used in calculating cleaning requirements: pre-rinse, caustic, acid, water for injection and steam quantities. Equipment cleaning area was calculated based on sizing, and piping width was assumed as per engineering design handbooks. (Doran, 1995; McAllister, 2009; Richardson et al., 1991; Woods, 2007a)

	Parameters /Assumptions	Notes / References
Procedure	1. Pre-rinse 2. Caustic Wash 3. Rinse (WFI) 4. Acid Wash 5. Rinse (WFI) 6. Steam Hold	(Junker et al., 2006; McNulty, 2016; SPX, 2013; Vincent, 2008)
Rinse and Pre-Rinse	Time: 5min (pre-rinse), 15min (rinse) Flow rate: 2.1 m/s (pipe - size 2" diameter)* 18 and 25L/min/m_{Circ}** (spray ball) Temperature: 35°C (pre-rinse) 60°C (rinse)	(Chisti and Moo-Young, 1994; Junker et al., 2006; McNulty, 2016; SPX, 2013; Vincent, 2008) *Although pipe sizes are dependent on individual equipment, 2" pipes were assumed the average. ** m_{circ} = the circumference of the vessel, for tanks < 3m diameter the lower bound flow rate, for which fluid enters tanks through spray balls, was assumed, those > 3m diameter required the upper bound flow rate. ***Recirculation volume ranged 50% to 150%
Caustic and Acid	Recirculation / Vessel Fill Volume: 80% capacity*** Time: 30 min Flow rate: 2.1 m/s (pipe - size 2" diameter)* 18 and 25L/min/m_{Circ}** (spray ball) Temp: 60°C Caustic: 4% Sodium Hydroxide Acid: 1% Nitric Acid	
Steam Hold	Temperature 135°C Amount: 1.5 vessel volume (to include pipes)	(Chisti and Moo-Young, 1994; Junker et al., 2006)

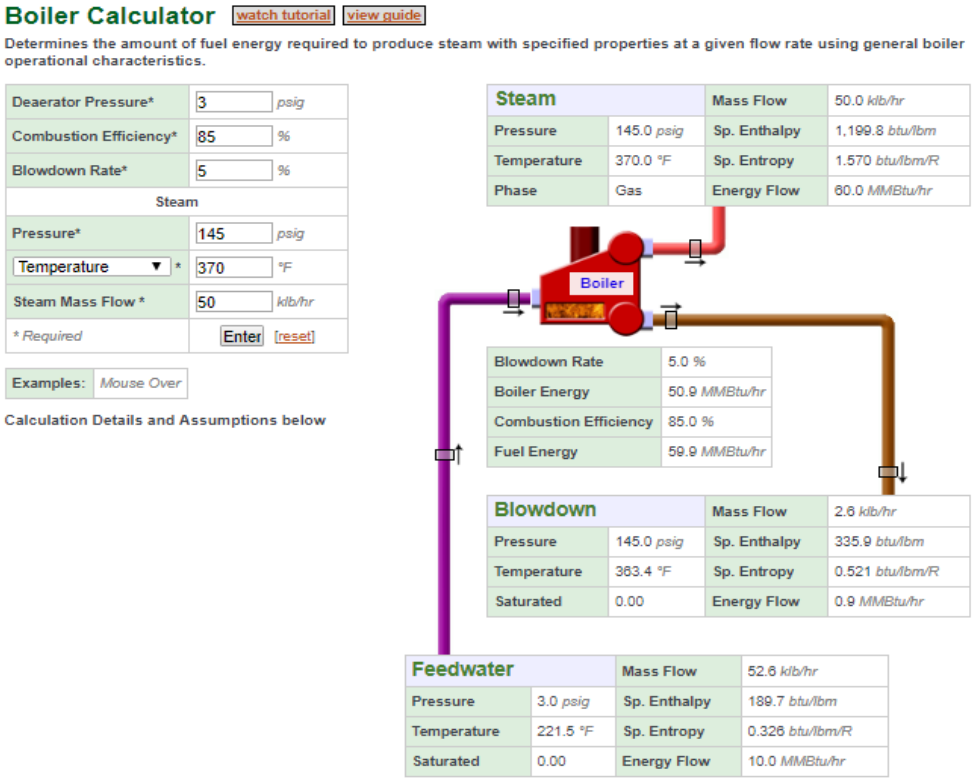

Figure E.1: Using the boiler calculator provided by the US Department of Energy (2015)*, input requirements were assumed. Temperature and pressure were set based on sterilisation requirements and steam pressure requirements for SIP. Sources:* (Deindoerfer, 1957; Doran, 1995; Harding, 2008; Junker et al., 2006; Steris, 2015; Valous et al., 2002; Woods, 2007b)

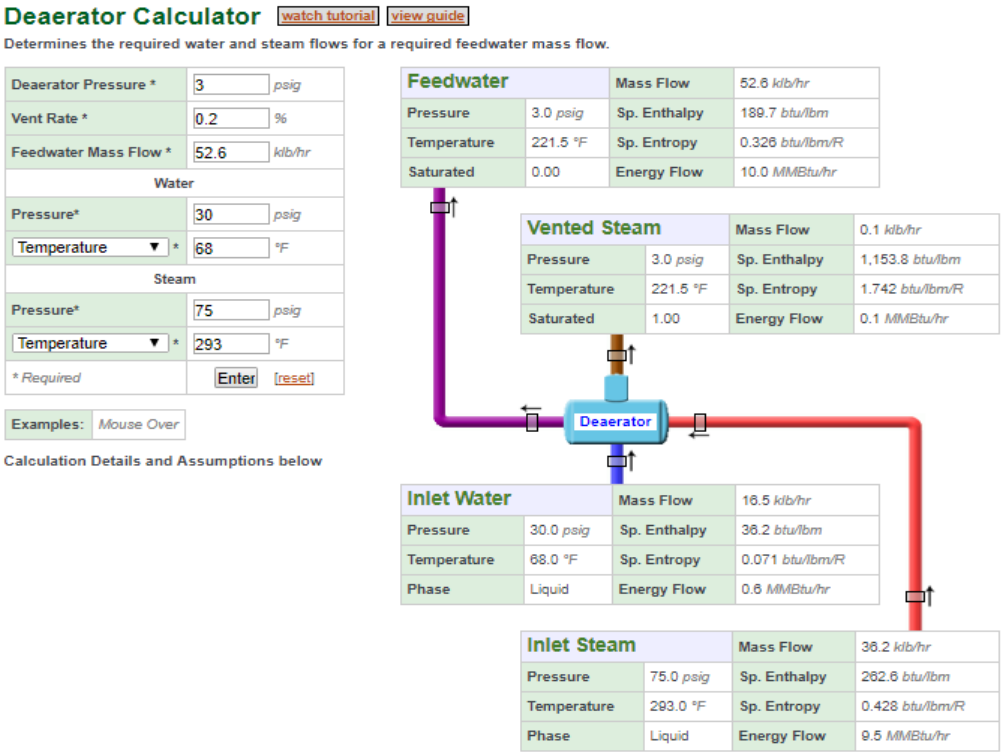

Figure E.2: Using the deaerator calculator provided by the US Department of Energy (2015)*, input requirements were assumed. Temperature and pressure were set based on sterilisation requirements and steam pressure requirements for SIP. Sources:* (Deindoerfer, 1957; Doran, 1995; Harding, 2008; Junker et al., 2006; Steris, 2015; Valous et al., 2002; Woods, 2007b)

Table E.30: Assumptions used to calculated HVAC power requirements, room sizes were calculated, and room specifications were assumed. Air changes requirements were based on room specifications from ISO standards. (Bhatia, 2010; Xu, 2002)

	Parameters /Assumptions	Notes / References
Equipment	The equipment was sized according to its operation; piping capacity/space requirements = 1.5 times of each equipment Life expectancy – 25 years (process equipment) and 50 years (other)	(Albright, 2009; Woods, 2007b) (American Hospital Association (AHA), 1998; UNFCCC/CCNUCC, 2009)
Personnel Space Requirement	Floor area: 47m^2 per FTE Height: 3.3m	Range: 18 to 60 m^2 (Cundall, 2014; Homes & Communities Agency and OffPAT, 2010)
Room Specifications	Inoculation: ISO 5 and 7 Stirred Tank Fermentation – ISO 8 Product Harvest – ISO 8 Product Conversion – ISO 7 Production Purification – ISO 7 Product Condition – ISO 7 Waste Treatment – ISO 9 Utilities – ISO 9 Media and Buffer Preparation ISO 7	(Boehringer Ingelheim Biopharmaceuticals GmbH, 2019; LuinaBio, 2018) ISO 5 (300 air changes/hr) ISO 6 (135 air changes/hr) ISO 7 (45 air changes/hr) ISO 8 (18 air changes/hr) ISO 9 (3 air changes/hr)
HVAC Electricity Requirements	Assumption: 5000 cfm/kW Total Energy $= \sum_{i=5}^{9} \frac{(air\ changes\ per\ hour \times total\ room\ volumes)}{60 \times 5000}$ Where i represents the ISO class. = **2430000 kWh/yr**	Range: 4900 to 10100 cfm/kW (Xu, 2002)

E.4 WASTE TREATMENT

Table E.31: Assumptions used to model the deactivation of solid waste from rotary vacuum filtration.

	Parameters /Assumptions	Notes / References
Equipment / Power Rating	Conveyor to Drum Dryer – 1.7 kW Drum Dryer – 33.5 kW	(Armfield Group, 2014)
Steam	Temperature – 150°C 1.4 kg of steam per kg water evaporated	Range: 1.1 to 1.6kg steam per kg water. (Tang et al., 2003)
Moisture Content (End) (% w/v)	5	Range: 3 to 8% w/v (Tang et al., 2003)
Processing Rate (kg/hr/m^2)	45	Range: 30 to 50 kg/hr/m^2 (Tang et al., 2003)
Drum Area	Waste stream = 1820kg/hr Drum area = 1820 / 45 = 40m^2	Calculated from mass flow rate at the end of rotary vacuum filtration
Steam and Electricity Requirements	Steam = 840 kg/hr Electricity = 620 kWh	Based on duration and the assumed mass of water evaporated during the deactivation process

Table E.32: Assumptions used to model the recovery of butyl acetate.

	Parameters /Assumptions	Notes / References
Equipment	Stripping columns	(Schügerl, 1994; Smallwood, 2002; Sun et al., 2017)
Butyl Acetate	Boiling point: 126.5°C (91°C under vacuum) Yield: 0.98 Water retention: 3 % w/w	(Kerr, 1980; Smallwood, 2002)
Process Steam	Temperature: 180°C Pressure: 10 bar Flow rate = 35 m/s Mass Flow rate: 2357 kg/hr	(Burger et al., 2015; Rasquin et al., 1978)
Cooling water	Temperature: 15°C Flow rate = 5.5 m/s Mass Flow rate: 2261 kg/hr	(Burger et al., 2015; Rasquin et al., 1978)
Total Electricity (kWh)	178	Includes pumping requirements for water for heat exchange

Table E.33: Assumptions used to model the recovery of PAA.

	Parameters /Assumptions	Notes / References
Procedure	1. Add purified water (0.5 x volume) 2. Settle (30min) 3. Purge water 4. Add 0.01M sodium hydroxide 5. Purge solvent butyl acetate	(Shih et al., 2005)
PAA Yield	0.85	(Karyekar and Hegde, 1989; Shih et al., 2005)
Equipment Size / Power	As per Table E.7.	
Total Energy (kWh)	38.5	Includes pumping and mixing of fluids prior to settling phase.

Table E.34: Assumptions used to model the wastewater treatment at the plant.

	Parameters /Assumptions	Notes / References
Procedure	1. pH adjustment 2. Air floatation 3. Primary settling 4. Aeration tank 5. Secondary settling 6. Disinfection	(Singh et al., 2016)
pH Adjustment	Assumed pH 12.9 (phosphate buffer and caustic) Target pH: 6.5	(NPTEL, 2012; Pabby et al., 2009; Singh et al., 2016)
Air Flotation	Average flow rate from processes: 144m^3 / hr Sized equipment – 160 m^3/h (off the shelf) with power 23.5kW (VLT750)	(Ecologix, 2018)
Primary Settling	Retention time: 7 hours Depth: 2.5m Sized as per Table E.7.	(NPTEL, 2012; Singh et al., 2016)
Aeration Tank	Retention: 12 hours Sized as per Table E.7.	(NPTEL, 2012; Singh et al., 2016)
Secondary Settling	Retention time: 12 hours Depth: 2.5m Sized as per Table E.7.	(NPTEL, 2012; Singh et al., 2016)
Disinfection	Sodium hypocrite: 10.5 mg/L Sodium bisulphite: 9.69mg/L	(NPTEL, 2012; Pabby et al., 2009; Singh et al., 2016)

APPENDIX F: PLANT LOCATION AND SUPPLY DISTANCES

F.1 USA (BASE CASE) SCENARIO

Table F.1: Assumed locations for the US (base-case) scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	New Jersey, US	Merck	(Demain, 2004)
Closest Port	New York, US		
Material Supply			
Glucose	New York, US	BOC Sciences	(USCS, 2019)
Sodium Hydroxide	New Jersey, US	Veckridge Chemical	(USCS, 2019)
Phosphate Buffer	New York, US	United Biochemicals	(USCS, 2019)
Butyl Acetate	New Jersey, US	Seidler Chemical Company	(USCS, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	Shanghai, China		
Equipment Fabrication	Hamburg, Germany	Eppendorf	(Bioreactors.net, 2015)
Closest Port	Hamburg, Germany		

Table F.2: Distances between assumed locations set out in Table F.1 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019).

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Port	169	Rail
Port to Port	22737	Ship
Port to/from Equip. Fabrication	23.2	Truck / Lorry
Port to Port	7769	Ship
Port to Plant	67	Road
Material Supply		
Glucose	187	Truck / Lorry
Sodium Hydroxide	64	Truck / Lorry
Phosphate Buffer	627	Truck / Lorry
Butyl Acetate	61	Truck / Lorry
Average	234.75	
Return Journey	469.5	
Model	**450**	

F.2 BRAZIL SCENARIO

Table F.3: Assumed locations for the Brazil scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Sao Paolo	Roche	(Roche, 2019)
Closest Port	N/A		
Material Supply			
Glucose	Sao Paolo	Global Medicom S.A.	(S&P Global, 2019)
Sodium Hydroxide	Sao Paolo	Syngenta Protecao De Cultivos Ltda	(S&P Global, 2019)
Phosphate Buffer	Sao Bernardo do Campo	ICL Brasil	(S&P Global, 2019)
Butyl Acetate	Sao Paolo	Akzo Nobel	(S&P Global, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	N/A		
Equipment Fabrication	Shanghai, China	Bailun Bio	(Bioreactors.net, 2015)
Closest Port	Shanghai, China		

Table F.4: Distances between assumed locations set out in Table F.3 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019).

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Equip. Fabrication	143	Truck / Lorry
Equip. Fabrication to Port	1331	Rail-freight
Port to Port	24407	Ship
Port to Plant	199	Road
Material Supply		
Glucose	16.4	Truck / Lorry
Sodium Hydroxide	30	Truck / Lorry
Phosphate Buffer	38.3	Truck / Lorry
Butyl Acetate	39	Truck / Lorry
Average	30.925	
Return Journey	61.85	
Model	**50**	

F.3 China Scenario

Table F.5: Assumed locations for the China scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Shandong, China	Centrient	(Centrient Pharmaceuticals, 2018)
Closest Port	N/A		
Material Supply			
Glucose	Shandong, China	Luzhou Bio-Chem Technology	(Made-in-China.com, 2019)
Sodium Hydroxide	Shandong, China	Weifang Boteng Chemical	(Made-in-China.com, 2019)
Phosphate Buffer	Shandong, China	Qingzhou Ekato Commerical	(Made-in-China.com, 2019)
Butyl Acetate	Shandong, China	Qingdao Highly Chemical New Materials	(Made-in-China.com, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	N/A		
Equipment Fabrication	Shanghai, China	Bailun Bio	(Bioreactors.net, 2015)
Closest Port	N/A		

Table F.6: Distances between assumed locations set out in Table F.5 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage—distances between supplier companies and shipping port (Google, 2019).

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Equip. Fabrication	143	Truck / Lorry
Equip. Fabrication to Plant	770	Rail-freight
Material Supply		
Glucose	177	Truck / Lorry
Sodium Hydroxide	111	Truck / Lorry
Phosphate Buffer	61.2	Truck / Lorry
Butyl Acetate	260	Truck / Lorry
Average	152.3	
Return Journey	304.6	
Model	**300**	

F.4 GERMANY SCENARIO

Table F.7: Assumed locations for the Germany scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	North-Rhine-Westphalia, Germany	GSK	(GSK, 2019)
Closest Port	N/A		
Material Supply			
Glucose	Munster, Germany	Sanotact GmbH	(Deutscher Medien Verlag GmbH, 2019)
Sodium Hydroxide	Kamp-Lintfort, Germany	Distripark GmbH	Deutscher Medien Verlag GmbH, 2019)
Phosphate Buffer	Waltrop, Germany	Chemische Werke Hommel GmbH & Co. KG	Deutscher Medien Verlag GmbH, 2019)
Butyl Acetate	Bielefeld, Germany	Stockmeier Chemie Eilenburg GmbH & Co. KG	Deutscher Medien Verlag GmbH, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	N/A		
Equipment Fabrication	Hamburg, Germany	Eppendorf	(Bioreactors.net, 2015)
Closest Port	N/A		

Table F.8: Distances between assumed locations set out in Table F.7 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019)

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Port	169	Rail
Port to Port	22737	Ship
Port to Equip Fabrication	23.2	Truck / Lorry
Equip Fabrication to Plant	339	Rail-freight
Material Supply		
Glucose	81	Truck / Lorry
Sodium Hydroxide	107	Truck / Lorry
Phosphate Buffer	48.8	Truck / Lorry
Butyl Acetate	108	Truck / Lorry
Average	86.2	
Return Journey	172.4	
Model	**150**	

F.5 INDIA SCENARIO

Table F.9: Assumed locations for the India scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Rajasthan, India	Dalas Biotech Ltd	(Dalas Biotech, 2002)
Closest Port	N/A		
Material Supply			
Glucose	Jaipur, India	Shyam Enterprises	(Indiamart, 2019)
Sodium Hydroxide	Delhi, India	Vats International	(Indiamart, 2019)
Phosphate Buffer	Delhi, India	Acuro Organics Limited	(Indiamart, 2019)
Butyl Acetate	Delhi, India	Atishay Specialities	(Indiamart, 2019)
Equipment Supply			
Steel Production	Bhadravati, India	Visvesvaraya Iron and Steel Plant	(Sail, 2012)
Closest Port	N/A		
Equipment Fabrication	Delhi, India	Katalyst	(Bioreactors.net, 2015)
Closest Port	N/A		

Table F.10: Distances between assumed locations set out in Table F.9 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage—distances between supplier companies and shipping port (Google, 2019).

	Distance (km) (one way)	Transport Method
Equipment Supply		
Bhadravati to Delhi	2069	Rail-freight
Delhi to Plant	76.6	Truck / Lorry
Material Supply		
Glucose	81.9	Truck / Lorry
Sodium Hydroxide	80.2	Truck / Lorry
Phosphate Buffer	69.3	Truck / Lorry
Butyl Acetate	42.1	Truck / Lorry
Average	68.375	
Return Journey	136.75	
Model	150	

F.6 SINGAPORE SCENARIO

Table F.11: Assumed locations for the India scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Jurong West, Singapore	GSK	(GSK, 2018)
Closest Port	Jurong		
Material Supply			
Glucose	Jurong, Singapore	VWR Singapore Pte Ltd	(Alibaba, 2019)
Sodium Hydroxide	Downtown Core, Singapore	Sinaco Industries Pte Ltd	(Alibaba, 2019)
Phosphate Buffer	Malaysia	BIS CHEMICALS SDN. BHD.	(Alibaba, 2019)
Butyl Acetate	Jalan Ampang, Malaysia	Trad Chem	(Alibaba, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	N/A		
Equipment Fabrication	Shanghai, China	Bailun Bio	(Bioreactors.net, 2015)
Closest Port	Shanghai, China		

Table F.12: Distances between assumed locations set out in Table F.11 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019)

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Plant to Equip Fabrication	143	Truck / Lorry
Equip Fabrication to Port	105	Truck / Lorry
Port to Port	5024	Ship
Port to Plant	6	Truck / Lorry
Material Supply		
Glucose	7	Truck / Lorry
Sodium Hydroxide	23.3	Truck / Lorry
Phosphate Buffer	368	Truck / Lorry
Butyl Acetate	351	Truck / Lorry
Average	187.325	
Return Journey	374.65	
Model	375	

F.7 SOUTH AFRICA SCENARIO

Table F.13: Assumed locations for the South Africa scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Gauteng, South Africa	Aspen Pharmaceuticals	(Aspen Holdings, 2009)
Closest Port	Durban		
Material Supply			
Glucose	Gauteng, South Africa	ScaleAway	(Tuugo, 2019)
Sodium Hydroxide	Wes-Kaap, South Africa	Wetchem	(Tuugo, 2019)
Phosphate Buffer	Gauteng, South Africa	Chemistry Industry	(Tuugo, 2019)
Butyl Acetate	Gauteng, South Africa	ScaleAway	(Tuugo, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	N/A		
Equipment Fabrication	Shanghai, China	Bailun Bio	(Bioreactors.net, 2015)
Closest Port	Shanghai, China		

Table F.14: Distances between assumed locations set out in Table F.13 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019)

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Plant to Equip Fabrication	143	Truck / Lorry
Equip Fabrication to Port	105	Truck / Lorry
Port to Port	15730	Ship
Port to Plant	562	Truck / Lorry
Material Supply		
Glucose	40.6	Truck / Lorry
Sodium Hydroxide	1082	Truck / Lorry
Phosphate Buffer	32	Truck / Lorry
Butyl Acetate	40.6	Truck / Lorry
Average	298.8	
Return Journey	597.6	
Model	600	

F.8 SPAIN SCENARIO

Table F.15: Assumed locations for the Spain scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Madrid, Spain	Reig jofre	(Reif Jofre, 2019)
Closest Port	N/A		
Material Supply			
Glucose	Albacete, Spain	Cargill S.L.U.	(Pharmaoffer, 2019)
Sodium Hydroxide	Guadalajara, Spain	Kluthe Iberica Sau	(S&P GLobal, 2019)
Phosphate Buffer	Barcelona, Spain	Quality Chemicals	(Pharmaoffer, 2019)
Butyl Acetate	València, Spain	Transglory, S.A	(S&P GLobal, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	Shanghai, China		
Equipment Fabrication	Hamburg, Germany	Eppendorf	(Bioreactors.net, 2015)
Closest Port	Hamburg, Germany		

Table F.16: Distances between assumed locations set out in Table F.15 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019)

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Port	169	Rail
Port to Port	22737	Ship
Port to/from Equip. Fabrication	23.2	Truck / Lorry
Equip. Fabrication to Plant	1794	Rail
Material Supply		
Glucose	33	Truck / Lorry
Sodium Hydroxide	55	Truck / Lorry
Phosphate Buffer	567	Truck / Lorry
Butyl Acetate	368	Truck / Lorry
Average	255.75	
Return Journey	511.5	
Model	500	

F.9 UK SCENARIO

Table F.17: Assumed locations for the UK scenario for 6-APA production.

	Locations	Reference Company	Reason / Reference
Plant	Irvine, UK	GSK	(Dunn, 2016)
Closest Port	Dundee		
Material Supply			
Glucose	Perth, UK	Tan International	(UKCS, 2019)
Sodium Hydroxide	Perth, UK	Tan International	(UKCS, 2019)
Phosphate Buffer	Glasgow, UK	Monarch Chemicals	(UKCS, 2019)
Butyl Acetate	Perth, UK	Tan International	(UKCS, 2019)
Equipment Supply			
Steel Production	Jiangsu, China	Wuxi Steel Group	(Basson, 2018)
Closest Port	Shanghai, China		
Equipment Fabrication	Hamburg, Germany	Eppendorf	(Bioreactors.net, 2015)
Closest Port	Hamburg, Germany		

Table F.18: Distances between assumed locations set out in Table F.17 used to model supply distances in the LCA model. The supply distances determine the emissions associated with transport due to fuel usage. Distances were obtained by reviewing distances between shipping ports (Ports.com, 2018) *and distances between supplier companies and shipping port* (Google, 2019)

	Distance (km) (one way)	Transport Method
Equipment Supply		
Steel Production to Port	169	Truck / Lorry
Port to Port	22737	Ship
Port to/from Equip. Fabrication	23.2	Truck / Lorry
Port to Port	1154	Ship
Port to Plant	180	Truck / Lorry
Material Supply		
Glucose	145	Truck / Lorry
Sodium Hydroxide	145	Truck / Lorry
Phosphate Buffer	72.9	Truck / Lorry
Butyl Acetate	145	Truck / Lorry
Average	126.975	
Return Journey	253.95	
Model	250	

APPENDIX G: GABI MODEL (US BASE CASE) AND INPUT EQUATION EXAMPLES

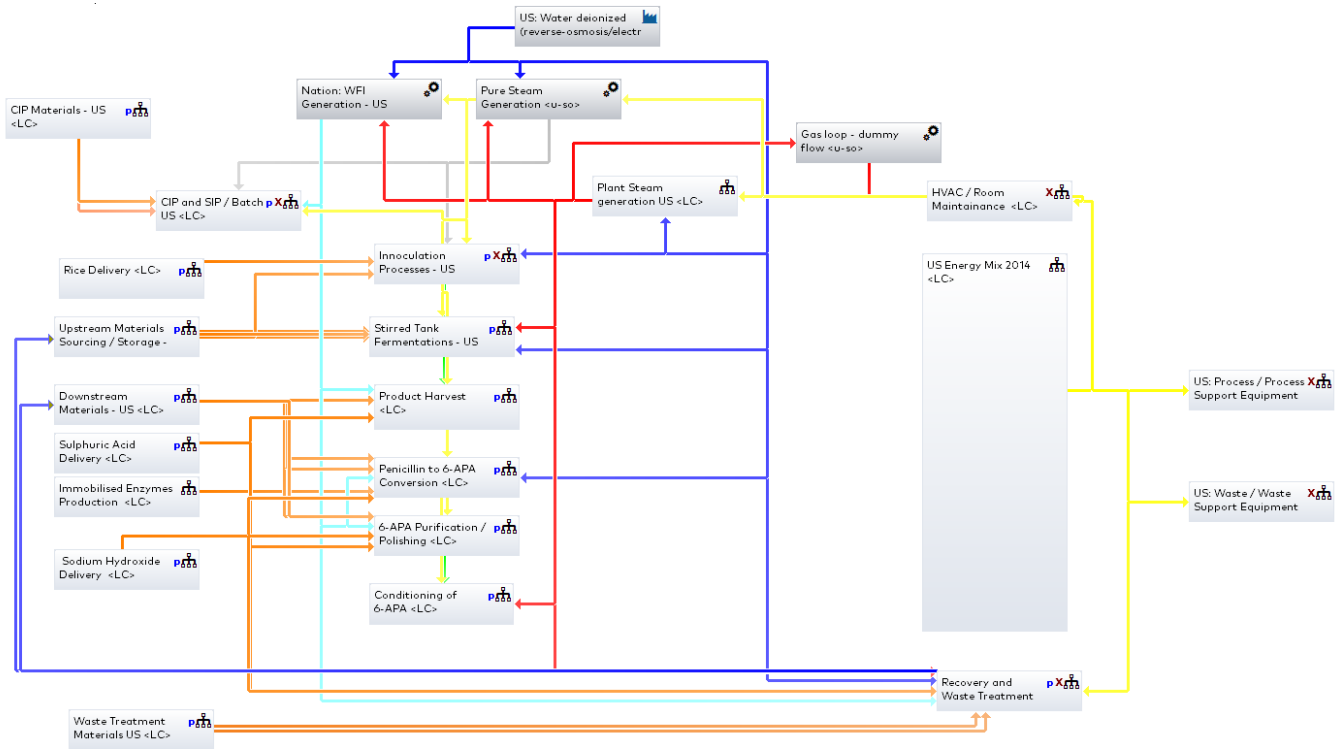

Figure G.1: Screenshot of the LCA model on 6-APA production in the US. indicates that the item is a plan which contains plan(s) and process(es). "p" indicates that the plan/process is parameterised.

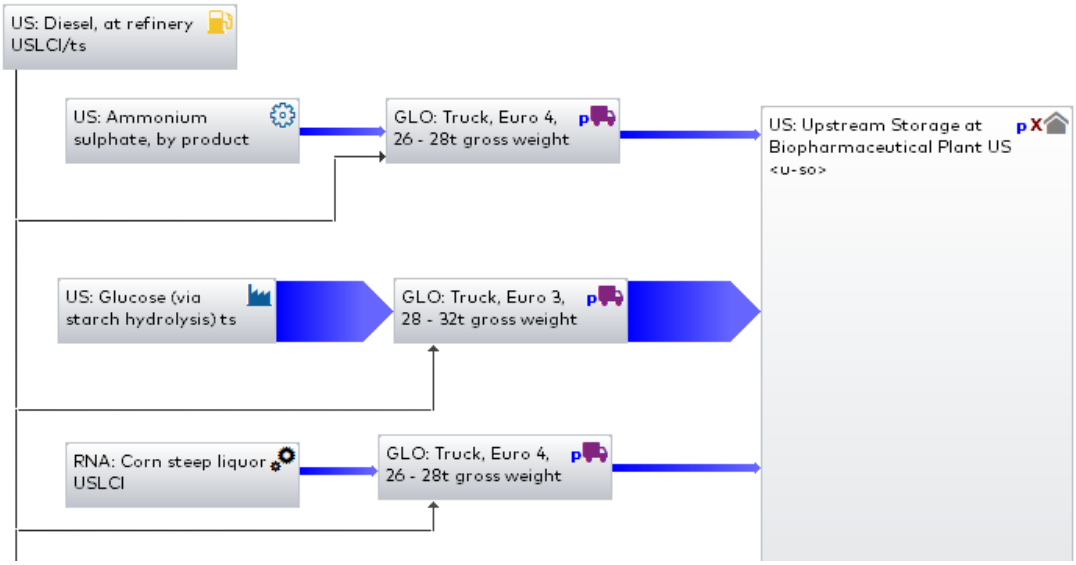

Figure G.2: Example of process material supply, [US], [RNA] and [GLO] regional LCIs were used for the US scenario. "p" parameters for truck transport requires transport distances to be inputted (Appendix F).

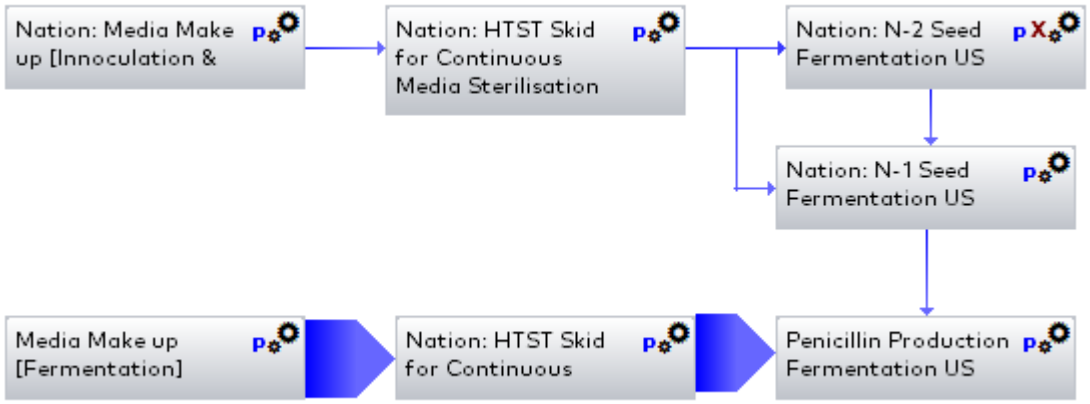

Figure G.3: Processes within the Stirred Tank Fermentation plan. (An example of how each processing block is set up).

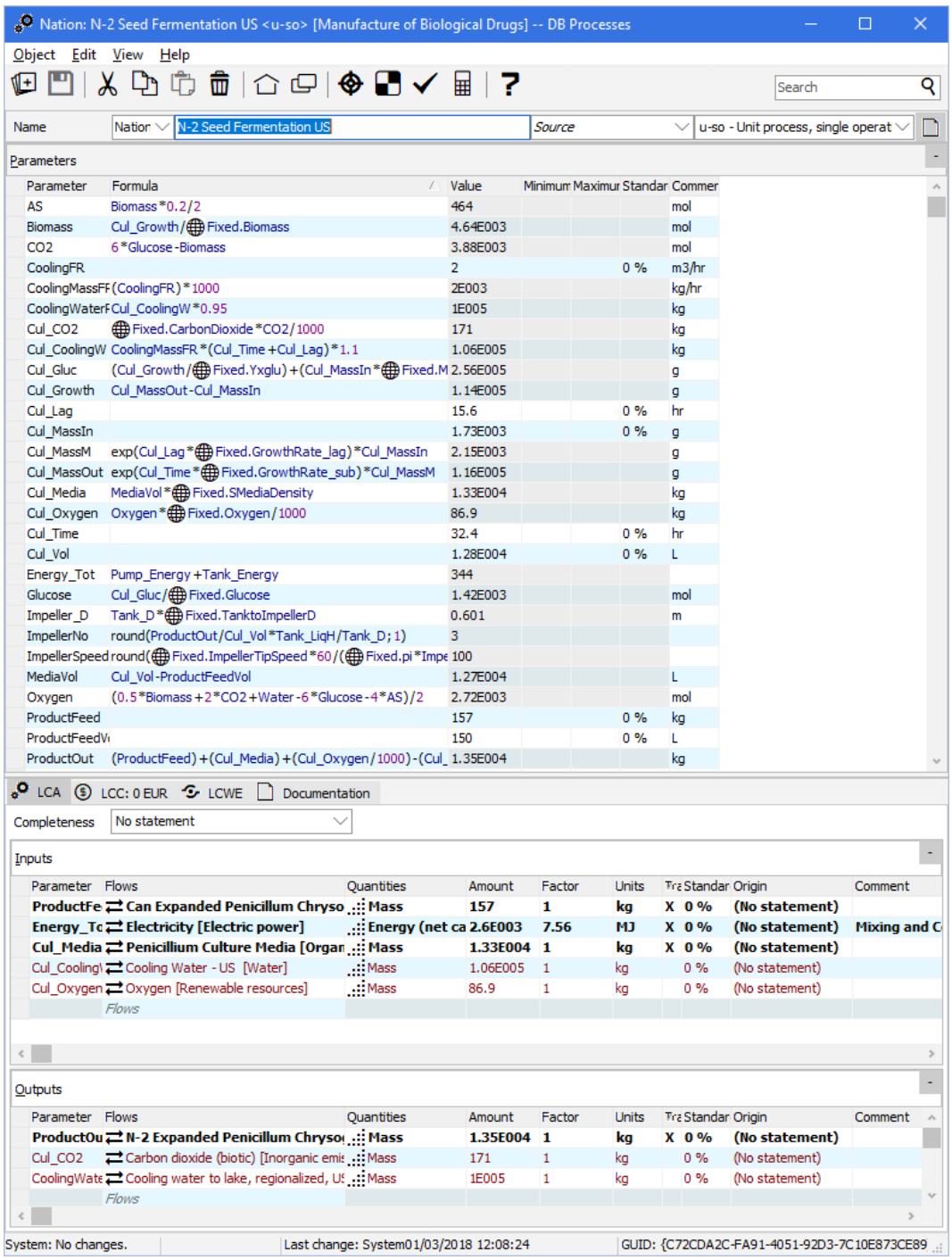

Figure G.4: Process database for N-2 Seed Fermentation, an example of how inputs and outputs are parameterised, examples of equations/formulas used for modelling the 6-APA production process. Equations used to carry out mass balances were transferred to the GaBi model to confirm calculations carried out in Microsoft Excel. For example, "Energy_total" is calculated by summing "Pump_Energy" (electricity requirements for pumping fermentation broth and media into the fermentation tank) and "Tank_Energy" (electricity requirements for mixing during the fermentation process). "Pump_Energy" and "Tank_Energy" are both functions of other parameters. = global/fixed parameters used across processes and plans, including the density of materials, impeller tip speed requirements for all stirred tank fermentation processes.

APPENDIX H: PROCESS CONTRIBUTIONS FOR ALL SCENARIOS

H.1 US (BASE CASE) SCENARIO

Table H.19: Percentage contribution of each life cycle phase of 6-APA manufacture in the US scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	**Supply**	**Use**	**End-of-life**
Acidification - ILCD/PEF (v1.09)	91.5	8.49	0.04
Ecotoxicity (Freshwater) - USEtox 2.1	97.5	2.37	0.12
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	36.0	63.6	0.48
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	89.9	9.99	0.08
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	95.1	3.45	1.43
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	97.2	1.73	1.04
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	83.1	16.9	0.04
Global Warming Potential, excl. biogenic carbon - IPCC AR5	58.5	41.5	0.05
Global Warming Potential, incl. biogenic carbon - IPCC AR5	49.3	50.7	0.07
Human Toxicity, cancer - USEtox 2.1	95.0	2.48	2.47
Human Toxicity, non-cancer - USEtox 2.1	99.0	0.09	0.05
Ionizing Radiation, human health - ILCD PEF (v1.09)	90.7	8.74	0.55
Ozone Depletion - ILCD PEF (v1.09)	93.0	6.86	0.19
Photochemical Ozone Formation, human health - EDIP 2003	98.6	1.33	0.05
Photochemical Ozone Formation, vegetation - EDIP 2003	100	0.00	0.00
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	72.8	27.1	0.08
Total Freshwater Consumption (including rainwater) - [kg]	81.5	18.4	0.05
Water resources - UBP 2013	70.8	29.1	0.08

Table H.2: Percentage contribution of each processing block of 6-APA manufacture in the US scenario towards each environmental impact category. The highest contributing blocks are illustrated to be Stirred Tank Fermentation and Product Harvest.

	Process Contribution (%)						
Environmental Impact Category - Method	**Inoculation**	**Stirred Tank Fermentation**	**Product Harvest**	**Product Conversion**	**Product Purification**	**Product Conditioning**	**Waste Water Treatment**
Acidification - ILCD/PEF (v1.09)	0.26	47.24	31.44	10.97	7.74	0.82	1.53
Ecotoxicity (Freshwater) - USEtox 2.1	0.06	29.12	50.07	2.11	15.70	0.15	2.79
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.80	45.76	26.47	11.74	9.20	0.56	5.47
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.23	31.71	44.01	8.71	11.95	0.68	2.70
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.02	77.22	15.26	0.36	4.90	0.02	2.23
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.04	94.21	3.12	0.75	0.58	0.08	1.22
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.31	50.31	31.91	8.32	5.79	0.89	2.46
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.59	44.21	32.89	10.66	6.66	0.96	4.04
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.63	39.16	35.65	11.56	7.22	1.04	4.73
Human Toxicity, cancer - USEtox 2.1	1.16	44.40	23.27	14.76	8.75	0.67	6.99
Human Toxicity, non-cancer - USEtox 2.1	0.31	36.73	34.36	15.71	10.66	0.87	1.35
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.15	30.77	54.67	5.44	4.36	1.60	3.01
Ozone Depletion - ILCD PEF (v1.09)	0.25	30.18	37.97	15.82	13.55	0.71	1.52
Photochemical Ozone Formation, human health - EDIP 2003	0.40	42.00	28.93	14.93	5.93	0.79	7.02
Photochemical Ozone Formation, vegetation - EDIP 2003	0.39	42.47	29.41	14.37	5.95	0.80	6.61
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.30	34.11	44.89	4.86	12.27	0.48	3.09
Total Freshwater Consumption (including rainwater) - [kg]	0.04	104.83	4.30	0.88	0.83	0.11	-10.98
Water resources - UBP 2013	0.04	66.27	38.21	1.56	11.57	0.16	-17.80

Table H.3: A breakdown of percentage contribution towards acidification of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.01%	0.00%	0.09%	0.13%	0.04%	**0.26%**
Stirred Tank Fermentation	0.00%	24.8%	0.73%	3.38%	13.6%	5.35%	**47.2%**
Harvest	0.00%	4.56%	0.05%	1.71%	12.5%	12.4%	**31.4%**
Conversion	0.00%	0.02%	0.03%	1.14%	8.30%	1.28%	**11.0%**
Purification	0.00%	1.54%	0.02%	0.65%	4.65%	0.79%	**7.74%**
Conditioning	0.00%	0.00%	0.00%	0.05%	0.37%	0.38%	**0.82%**
Waste Treatment	0.00%	0.25%	0.06%	0.54%	0.00%	0.66%	**1.53%**
Column Total	**0.00%**	**31.2%**	**0.89%**	**7.57%**	**39.4%**	**20.9%**	**100%**

Table H.4: A breakdown of percentage contribution towards freshwater ecotoxicity of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.01%	0.00%	0.02%	0.02%	0.01%	**0.06%**
Stirred Tank Fermentation	0.00%	24.2%	0.42%	0.85%	2.25%	1.10%	**28.8%**
Harvest	0.00%	45.2%	0.03%	0.43%	2.05%	2.55%	**50.5%**
Conversion	0.00%	0.18%	0.02%	0.29%	1.37%	0.26%	**2.12%**
Purification	0.00%	14.7%	0.01%	0.16%	0.77%	0.16%	**15.7%**
Conditioning	0.00%	0.00%	0.00%	0.01%	0.06%	0.08%	**0.15%**
Waste Treatment	0.00%	2.41%	0.12%	0.14%	0.00%	0.14%	**2.80%**
Column Total	**0.00%**	**86.7%**	**0.59%**	**1.90%**	**6.52%**	**4.30%**	**100%**

Table H.5: A breakdown of percentage contribution towards marine ecotoxicity of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.73%	0.03%	0.00%	**0.77%**
Stirred Tank Fermentation	0.00%	16.0%	0.37%	27.33%	3.21%	0.56%	**47.5%**
Harvest	0.00%	7.57%	0.02%	13.82%	2.93%	1.30%	**25.6%**
Conversion	0.00%	0.03%	0.01%	9.23%	1.96%	0.13%	**11.4%**
Purification	0.00%	2.46%	0.01%	5.26%	1.10%	0.08%	**8.91%**
Conditioning	0.00%	0.00%	0.00%	0.42%	0.09%	0.04%	**0.54%**
Waste Treatment	0.00%	0.38%	0.49%	4.35%	0.00%	0.07%	**5.29%**
Column Total	**0.00%**	**26.5%**	**0.90%**	**61.1%**	**9.31%**	**2.19%**	**100%**

Table H.6: A breakdown of percentage contribution towards terrestrial ecotoxicity of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.10%	0.10%	0.03%	**0.23%**
Stirred Tank Fermentation	0.00%	11.8%	1.17%	3.84%	10.0%	4.81%	**31.7%**
Harvest	0.00%	21.7%	0.08%	1.94%	9.15%	11.2%	**44.0%**
Conversion	0.00%	0.09%	0.04%	1.30%	6.13%	1.15%	**8.71%**
Purification	0.00%	7.03%	0.03%	0.74%	3.44%	0.71%	**12.0%**
Conditioning	0.00%	0.00%	0.00%	0.06%	0.27%	0.35%	**0.68%**
Waste Treatment	0.00%	1.33%	0.16%	0.61%	0.00%	0.60%	**2.70%**
Column Total	**0.01%**	**42.0%**	**1.48%**	**8.60%**	**29.13%**	**18.8%**	**100%**

Table H.7: A breakdown of percentage contribution towards freshwater eutrophication of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.02%	0.00%	0.00%	0.00%	0.00%	**0.02%**
Stirred Tank Fermentation	0.00%	75.3%	2.80%	0.04%	0.05%	0.09%	**78.2%**
Harvest	0.00%	14.19%	0.18%	0.02%	0.05%	0.22%	**14.7%**
Conversion	0.00%	0.06%	0.11%	0.01%	0.03%	0.02%	**0.23%**
Purification	0.00%	4.60%	0.07%	0.01%	0.02%	0.01%	**4.70%**
Conditioning	0.00%	0.00%	0.01%	0.00%	0.00%	0.01%	**0.01%**
Waste Treatment	0.00%	0.71%	1.40%	0.01%	0.00%	0.01%	**2.13%**
Column Total	**0.00%**	**94.8%**	**4.56%**	**0.10%**	**0.16%**	**0.37%**	**100.00%**

Table H.8: A breakdown of percentage contribution towards marine eutrophication of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.02%	0.00%	0.00%	0.01%	0.01%	**0.04%**
Stirred Tank Fermentation	0.00%	91.3%	1.26%	0.13%	0.77%	0.78%	**94.2%**
Harvest	0.00%	0.46%	0.08%	0.07%	0.70%	1.81%	**3.11%**
Conversion	0.00%	0.00%	0.05%	0.04%	0.47%	0.19%	**0.75%**
Purification	0.00%	0.15%	0.03%	0.02%	0.26%	0.11%	**0.58%**
Conditioning	0.00%	0.00%	0.00%	0.00%	0.02%	0.06%	**0.08%**
Waste Treatment	0.00%	0.04%	1.06%	0.02%	0.00%	0.10%	**1.22%**
Column Total	**0.00%**	**92.0%**	**2.48%**	**0.29%**	**2.23%**	**3.04%**	**100%**

Table H.9: A breakdown of percentage contribution towards terrestrial eutrophication of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.01%	0.00%	0.19%	0.06%	0.06%	**0.31%**
Stirred Tank Fermentation	0.00%	28.3%	0.75%	7.12%	6.26%	8.31%	**50.7%**
Harvest	0.00%	3.00%	0.05%	3.60%	5.71%	19.3%	**31.7%**
Conversion	0.00%	0.01%	0.03%	2.40%	3.82%	1.98%	**8.25%**
Purification	0.00%	0.98%	0.02%	1.37%	2.14%	1.23%	**5.74%**
Conditioning	0.00%	0.00%	0.00%	0.11%	0.17%	0.60%	**0.88%**
Waste Treatment	0.00%	0.21%	0.07%	1.13%	0.00%	1.03%	**2.44%**
Column Total	**0.00%**	**32.5%**	**0.92%**	**15.9%**	**18.1%**	**32.5%**	**100%**

Table H.10: A breakdown of percentage contribution towards global warming potential (excl. biogenic carbon) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.49%	0.04%	0.05%	**0.59%**
Stirred Tank Fermentation	0.00%	13.4%	0.50%	18.3%	4.21%	7.82%	**44.2%**
Harvest	0.00%	1.61%	0.03%	9.24%	3.84%	18.2%	**32.9%**
Conversion	0.00%	0.01%	0.02%	6.18%	2.57%	1.87%	**10.6%**
Purification	0.00%	0.53%	0.01%	3.52%	1.44%	1.15%	**6.65%**
Conditioning	0.00%	0.00%	0.00%	0.28%	0.11%	0.56%	**0.96%**
Waste Treatment	0.00%	0.10%	0.05%	2.91%	0.00%	0.97%	**4.04%**
Column Total	**0.00%**	**15.7%**	**0.62%**	**40.9%**	**12.23%**	**30.6%**	**100%**

Table H.11: A breakdown of percentage contribution towards global warming potential (incl. biogenic carbon) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.53%	0.04%	0.06%	**0.63%**
Stirred Tank Fermentation	0.00%	0.43%	5.91%	19.8%	4.57%	8.47%	**39.2%**
Harvest	0.00%	1.74%	0.05%	10.0%	4.17%	19.7%	**35.7%**
Conversion	0.00%	0.01%	0.03%	6.69%	2.79%	2.02%	**11.5%**
Purification	0.00%	0.57%	0.02%	3.81%	1.57%	1.25%	**7.22%**
Conditioning	0.00%	0.00%	0.00%	0.30%	0.12%	0.61%	**1.04%**
Waste Treatment	0.00%	0.11%	0.42%	3.15%	0.00%	1.05%	**4.73%**
Column Total	**0.00%**	**2.86%**	**6.42%**	**44.3%**	**13.3%**	**33.2%**	**100%**

Table H.12: A breakdown of percentage contribution towards human toxicity (cancer) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	1.16%	0.00%	0.00%	**1.16%**
Stirred Tank Fermentation	0.00%	1.25%	0.04%	43.1%	0.21%	0.06%	**44.6%**
Harvest	0.00%	1.05%	0.00%	21.8%	0.19%	0.14%	**23.2%**
Conversion	0.00%	0.00%	0.00%	14.6%	0.13%	0.01%	**14.7%**
Purification	0.00%	0.34%	0.00%	8.29%	0.07%	0.01%	**8.71%**
Conditioning	0.00%	0.00%	0.00%	0.66%	0.01%	0.00%	**0.67%**
Waste Treatment	0.00%	0.04%	0.05%	6.86%	0.00%	0.01%	**6.96%**
Column Total	**0.00%**	**2.68%**	**0.10%**	**96.4%**	**0.60%**	**0.24%**	**100%**

Table H.13: A breakdown of percentage contribution towards human toxicity (non-cancer) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.07%	0.22%	0.02%	**0.31%**
Stirred Tank Fermentation	0.00%	9.46%	0.72%	2.63%	22.6%	2.73%	**38.1%**
Harvest	0.00%	5.34%	0.05%	1.33%	20.6%	6.33%	**33.6%**
Conversion	0.00%	0.02%	0.03%	0.89%	13.8%	0.65%	**15.4%**
Purification	0.00%	1.75%	0.02%	0.51%	7.73%	0.40%	**10.4%**
Conditioning	0.00%	0.00%	0.00%	0.04%	0.62%	0.20%	**0.85%**
Waste Treatment	0.00%	0.37%	0.19%	0.42%	0.00%	0.34%	**1.32%**
Column Total	**0.00%**	**17.0%**	**1.00%**	**5.88%**	**65.5%**	**10.7%**	**100%**

Table H.14: A breakdown of percentage contribution towards ionising radiation of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.00%	0.00%	0.15%	**0.15%**
Stirred Tank Fermentation	0.00%	8.24%	0.91%	0.13%	0.13%	21.9%	**31.3%**
Harvest	0.00%	3.06%	0.06%	0.06%	0.12%	50.9%	**54.2%**
Conversion	0.00%	0.01%	0.03%	0.04%	0.08%	5.23%	**5.40%**
Purification	0.00%	1.00%	0.02%	0.02%	0.05%	3.23%	**4.32%**
Conditioning	0.00%	0.00%	0.00%	0.00%	0.00%	1.58%	**1.59%**
Waste Treatment	0.00%	0.19%	0.06%	0.02%	0.00%	2.72%	**2.99%**
Column Total	**0.00%**	**12.5%**	**1.09%**	**0.28%**	**0.38%**	**85.7%**	**100%**

Table H.15: A breakdown of percentage contribution towards ozone depletion of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.00%	0.25%	0.00%	**0.25%**
Stirred Tank Fermentation	0.00%	4.37%	0.00%	0.00%	25.8%	0.00%	**30.2%**
Harvest	0.00%	14.5%	0.00%	0.00%	23.5%	0.00%	**38.0%**
Conversion	0.00%	0.06%	0.00%	0.00%	15.8%	0.00%	**15.8%**
Purification	0.00%	4.69%	0.00%	0.00%	8.85%	0.00%	**13.5%**
Conditioning	0.00%	0.00%	0.00%	0.00%	0.70%	0.00%	**0.70%**
Waste Treatment	0.00%	1.52%	0.00%	0.00%	0.00%	0.00%	**1.52%**
Column Total	**0.00%**	**25.1%**	**0.00%**	**0.00%**	**74.9%**	**0.00%**	**100%**

Table H.16: A breakdown of percentage contribution towards photochemical ozone formation (human health) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.30%	0.05%	0.04%	**0.40%**
Stirred Tank Fermentation	0.00%	18.2%	0.68%	11.3%	4.94%	6.66%	**41.8%**
Harvest	0.00%	8.17%	0.04%	5.73%	4.50%	15.5%	**33.9%**
Conversion	0.00%	0.03%	0.03%	3.83%	3.02%	1.59%	**8.49%**
Purification	0.00%	2.66%	0.02%	2.18%	1.69%	0.98%	**7.53%**
Conditioning	0.00%	0.00%	0.00%	0.17%	0.13%	0.48%	**0.79%**
Waste Treatment	0.00%	0.15%	4.27%	1.80%	0.00%	0.83%	**7.05%**
Column Total	**0.00%**	**29.2%**	**5.04%**	**25.3%**	**14.3%**	**26.1%**	**100%**

Table H.17: A breakdown of percentage contribution towards photochemical ozone formation (vegetation) of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.30%	0.05%	0.05%	**0.39%**
Stirred Tank Fermentation	0.00%	18.7%	0.68%	11.0%	5.09%	6.88%	**42.3%**
Harvest	0.00%	7.69%	0.04%	5.57%	4.64%	16.0%	**33.2%**
Conversion	0.00%	0.03%	0.03%	3.72%	3.11%	1.64%	**8.53%**
Purification	0.00%	2.50%	0.02%	2.12%	1.75%	1.01%	**7.39%**
Conditioning	0.00%	0.00%	0.00%	0.17%	0.14%	0.50%	**0.80%**
Waste Treatment	0.00%	0.15%	3.87%	1.75%	0.00%	0.85%	**6.63%**
Column Total	**0.00%**	**29.0%**	**4.64%**	**24.6%**	**14.8%**	**26.9%**	**100%**

Table H.18: A breakdown of percentage contribution towards resource depletion of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.00%	0.00%	0.26%	0.01%	0.03%	**0.30%**
Stirred Tank Fermentation	0.00%	19.6%	-0.15%	9.56%	0.82%	4.28%	**34.1%**
Harvest	0.00%	29.4%	-0.01%	4.83%	0.74%	9.94%	**44.9%**
Conversion	0.00%	0.12%	-0.01%	3.23%	0.50%	1.02%	**4.86%**
Purification	0.00%	9.52%	0.00%	1.84%	0.28%	0.63%	**12.3%**
Conditioning	0.00%	0.00%	0.00%	0.15%	0.02%	0.31%	**0.48%**
Waste Treatment	0.00%	1.06%	-0.02%	1.52%	0.00%	0.53%	**3.09%**
Column Total	**0.01%**	**59.7%**	**-0.19%**	**21.39%**	**2.37%**	**16.7%**	**100%**

Table H.19: A breakdown of percentage contribution towards total freshwater consumption of each processing block of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.02%	0.01%	0.00%	0.00%	0.01%	**0.04%**
Stirred Tank Fermentation	0.00%	87.7%	16.2%	0.12%	0.06%	1.00%	**105%**
Harvest	0.00%	0.93%	0.86%	0.06%	0.06%	2.32%	**4.22%**
Conversion	0.00%	0.00%	0.50%	0.04%	0.04%	0.24%	**0.82%**
Purification	0.00%	0.30%	0.31%	0.02%	0.02%	0.15%	**0.80%**
Conditioning	0.00%	0.00%	0.03%	0.00%	0.00%	0.07%	**0.10%**
Waste Treatment	0.00%	0.02%	-11.2%	0.02%	0.00%	0.12%	**-11.0%**
Column Total	**0.00%**	**89.0%**	**6.67%**	**0.27%**	**0.19%**	**3.91%**	**100%**

Table H.20: A breakdown of percentage contribution towards the impact on water resources of each processing block, of 6-APA manufacture in the US Scenario.

Processing Block	Equipment	Materials - Production	Water	Steam	Materials - Cleaning	Electricity	Row Total
Inoculation	0.00%	0.01%	0.02%	0.00%	0.00%	0.01%	**0.04%**
Stirred Tank Fermentation	0.00%	35.90%	28.91%	0.04%	0.11%	1.47%	**66.42%**
Harvest	0.00%	33.18%	1.53%	0.02%	0.10%	3.41%	**38.25%**
Conversion	0.00%	0.13%	0.90%	0.01%	0.07%	0.35%	**1.46%**
Purification	0.00%	10.75%	0.55%	0.01%	0.04%	0.22%	**11.57%**
Conditioning	0.00%	0.00%	0.05%	0.00%	0.00%	0.11%	**0.16%**
Waste Treatment	0.00%	2.09%	-20.18%	0.01%	0.00%	0.18%	**-17.90%**
Column Total	**0.00%**	**82.06%**	**11.77%**	**0.10%**	**0.31%**	**5.75%**	**100.00%**

H.2 Comparative Study Summary

Table H.21: Environmental impact results per year for each country scenario where 2000 tonnes of 6-APA was assumed produced over this period.

	Brazil	China	Germany	India	Singapore	South Africa	Spain	UK	US
Acidification - ILCD/PEF (v1.09) [Mole of H+ eq.]	5.18E+05	7.05E+05	2.87E+05	1.20E+06	3.98E+05	1.69E+06	3.28E+05	4.00E+05	3.32E+05
Ecotoxicity (Freshwater) - USEtox 2.1 [CTUe]	8.36E+10	1.14E+11	6.26E+10	1.13E+11	8.97E+10	8.22E+10	7.54E+10	7.86E+10	2.29E+10
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	2.68E+05	6.06E+05	1.86E+05	3.69E+05	2.65E+05	2.63E+05	2.26E+05	2.56E+05	2.98E+05
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	1.15E+08	3.76E+08	5.32E+07	1.69E+08	6.97E+07	9.24E+07	6.09E+07	6.02E+07	2.32E+07
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H) [kg P eq.]	8.82E+03	1.13E+04	7.58E+03	1.09E+04	9.72E+03	9.22E+03	8.48E+03	8.86E+03	5.18E+03
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H [kg N eq.]	1.40E+04	1.27E+04	1.37E+04	1.21E+04	1.25E+04	1.21E+04	1.20E+04	1.23E+04	1.22E+04
Eutrophication (Terrestrial) - ILCD/PEF (v1.09) [Mole of N eq.]	6.62E+05	1.38E+06	5.70E+05	2.17E+06	1.06E+06	2.48E+06	6.24E+05	8.04E+05	6.95E+05
Global Warming Potential, excl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	1.18E+08	1.85E+08	1.45E+08	1.89E+08	1.66E+08	2.22E+08	1.23E+08	1.44E+08	1.61E+08
Global Warming Potential, incl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	1.04E+08	1.73E+08	1.33E+08	1.78E+08	1.54E+08	2.09E+08	1.09E+08	1.18E+08	1.48E+08
Human Toxicity, cancer - USEtox 2.1 [CTUh]	1.23E+00	1.03E+01	9.52E-01	1.73E+00	1.39E+00	1.37E+00	1.13E+00	1.23E+00	1.38E+01
Human Toxicity, non-cancer - USEtox 2.1 [CTUh]	1.03E+01	1.96E+01	1.01E+01	1.79E+01	1.01E+01	1.29E+01	9.82E+00	1.13E+01	7.94E+00
Ionizing Radiation, human health -ILCD PEF (v1.09) [kBq U235 eq.]	1.59E+06	2.66E+06	5.61E+06	2.61E+06	1.44E+06	2.86E+06	6.38E+06	7.26E+06	6.04E+06
Ozone Depletion - ILCD PEF (v1.09) [kg CFC-11 eq.]	2.37E+00	3.91E+00	2.89E+00	3.83E+00	3.62E+00	3.37E+00	3.20E+00	3.24E+00	1.86E+00
Photochemical Ozone Formation, human health - EDIP 2003 [pers*ppm*hours]	3.23E+04	5.44E+04	2.96E+04	7.26E+04	4.38E+04	9.27E+04	3.22E+04	3.15E+04	3.70E+04
Photochemical Ozone Formation, vegetation - EDIP 2003 [m2 UES*ppm*hours]	4.45E+08	7.60E+08	4.04E+08	1.04E+09	6.06E+08	1.32E+09	4.41E+08	4.92E+08	5.05E+08
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09) [kg Sb eq.]	1.29E+03	2.04E+03	1.30E+03	2.52E+03	1.81E+03	1.76E+03	1.36E+03	1.61E+03	1.01E+03
Total Freshwater Consumption (including rainwater) [kg]	1.01E+10	9.38E+09	8.18E+09	9.07E+09	8.41E+09	8.22E+09	8.48E+09	7.89E+09	8.04E+09
Water resources - UBP 2013 [UBP]	4.76E+08	8.56E+08	7.41E+08	1.41E+09	4.21E+08	9.52E+08	1.40E+09	4.47E+08	6.51E+08

Table H.22: Environmental impact results per kilogram of 6-APA produced for each country scenario, where 2000 tonnes of 6-APA was assumed produced over a year.

	Brazil	China	Germany	India	Singapore	South Africa	Spain	UK	US
Acidification - ILCD/PEF (v1.09) [Mole of H+ eq.]	2.58E-01	3.51E-01	1.43E-01	5.97E-01	1.99E-01	9.09E-01	1.63E-01	1.99E-01	1.65E-01
Ecotoxicity (Freshwater) - USEtox 2.1 [CTUe]	4.17E+04	5.70E+04	3.12E+04	5.61E+04	4.47E+04	4.43E+04	3.76E+04	3.92E+04	1.14E+04
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	1.34E-01	3.02E-01	9.29E-02	1.84E-01	1.32E-01	1.42E-01	1.13E-01	1.27E-01	1.49E-01
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H) [kg 1,4-DB eq.]	5.71E+01	1.88E+02	2.65E+01	8.41E+01	3.47E+01	4.98E+01	3.03E+01	3.00E+01	1.16E+01
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H) [kg P eq.]	4.39E-03	5.66E-03	3.78E-03	5.41E-03	4.84E-03	4.97E-03	4.23E-03	4.42E-03	2.58E-03
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H [kg N eq.]	6.98E-03	6.31E-03	6.81E-03	6.04E-03	6.22E-03	6.52E-03	5.98E-03	6.14E-03	6.10E-03
Eutrophication (Terrestrial) - ILCD/PEF (v1.09) [Mole of N eq.]	3.30E-01	6.87E-01	2.84E-01	1.08E+00	5.28E-01	1.34E+00	3.11E-01	4.01E-01	3.46E-01
Global Warming Potential, excl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	5.87E+01	9.20E+01	7.23E+01	9.43E+01	8.26E+01	1.20E+02	6.12E+01	7.20E+01	8.01E+01
Global Warming Potential, incl. biogenic carbon - IPCC AR5 [kg CO2 eq.]	5.20E+01	8.61E+01	6.65E+01	8.88E+01	7.67E+01	1.13E+02	5.45E+01	5.86E+01	7.39E+01
Human Toxicity, cancer - USEtox 2.1 [CTUh]	6.11E-07	5.14E-06	4.75E-07	8.61E-07	6.95E-07	7.36E-07	5.65E-07	6.11E-07	6.89E-06
Human Toxicity, non-cancer - USEtox 2.1 [CTUh]	5.12E-06	9.79E-06	5.05E-06	8.94E-06	5.02E-06	6.96E-06	4.89E-06	5.63E-06	3.95E-06
Ionizing Radiation, human health -ILCD PEF (v1.09) [kBq U235 eq.]	7.94E-01	1.33E+00	2.80E+00	1.30E+00	7.18E-01	1.54E+00	3.18E+00	3.62E+00	3.01E+00
Ozone Depletion - ILCD PEF (v1.09) [kg CFC-11 eq.]	1.18E-06	1.95E-06	1.44E-06	1.91E-06	1.80E-06	1.82E-06	1.60E-06	1.61E-06	9.25E-07
Photochemical Ozone Formation, human health - EDIP 2003 [pers*ppm*hours]	1.61E-02	2.71E-02	1.48E-02	3.62E-02	2.18E-02	5.00E-02	1.60E-02	1.57E-02	1.84E-02
Photochemical Ozone Formation, vegetation - EDIP 2003 [m2 UES*ppm*hours]	2.22E+02	3.79E+02	2.01E+02	5.21E+02	3.02E+02	7.10E+02	2.20E+02	2.45E+02	2.51E+02
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09) [kg Sb eq.]	6.41E-04	1.01E-03	6.47E-04	1.26E-03	9.04E-04	9.47E-04	6.77E-04	8.04E-04	5.02E-04
Total Freshwater Consumption (including rainwater) [kg]	5.03E+03	4.67E+03	4.08E+03	4.52E+03	4.19E+03	4.43E+03	4.23E+03	3.93E+03	4.01E+03
Water resources - UBP 2013 [UBP]	2.37E+02	4.27E+02	3.69E+02	7.04E+02	2.10E+02	5.13E+02	6.96E+02	2.23E+02	3.24E+02

H.3 BRAZIL SCENARIO

Table H.23: Percentage contribution of each life cycle phase of 6-APA manufacture in the Brazil scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	99.75	0.20	0.05
Ecotoxicity (Freshwater) - USEtox 2.1	98.41	1.54	0.05
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	98.26	0.91	0.83
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	99.68	0.30	0.02
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	97.68	0.93	1.38
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	98.55	0.05	1.40
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	99.67	0.24	0.09
Global Warming Potential, excl. biogenic carbon - IPCC AR5	63.89	36.00	0.11
Global Warming Potential, incl. biogenic carbon - IPCC AR5	50.90	48.95	0.15
Human Toxicity, cancer - USEtox 2.1	97.09	2.01	0.90
Human Toxicity, non-cancer - USEtox 2.1	99.28	0.52	0.20
Ionizing Radiation, human health - ILCD PEF (v1.09)	98.63	1.02	0.34
Ozone Depletion - ILCD PEF (v1.09)	97.01	2.99	0.00
Photochemical Ozone Formation, human health - EDIP 2003	95.08	4.76	0.16
Photochemical Ozone Formation, vegetation - EDIP 2003	95.55	4.30	0.15
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	99.51	0.51	-0.01
Total Freshwater Consumption (including rainwater) - [kg]	107.00	-5.43	-1.57
Water resources - UBP 2013	101.96	0.12	-2.09

Table H.24: Percentage contribution of each processing block of 6-APA manufacture in the Brazil scenario towards each environmental impact category. The highest contributing block is illustrated to be Stirred Tank Fermentation.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.19	45.01	40.00	6.20	4.77	1.13	2.71
Ecotoxicity (Freshwater) - USEtox 2.1	0.04	72.97	19.86	0.70	4.77	0.20	1.46
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.08	66.78	23.08	2.58	4.48	0.43	2.58
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.13	49.75	38.35	4.33	4.08	1.05	2.32
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.02	84.54	9.90	0.05	2.94	0.02	2.52
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.05	85.74	9.67	1.02	0.76	0.29	2.48
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.20	52.69	35.20	4.55	3.85	0.98	2.53
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.57	51.35	28.50	8.84	5.83	0.80	4.10
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.64	44.51	32.19	9.98	6.58	0.91	5.19
Human Toxicity, cancer - USEtox 2.1	0.05	71.42	19.48	1.61	4.98	0.21	2.25
Human Toxicity, non-cancer - USEtox 2.1	0.19	52.79	26.83	10.80	7.57	0.67	1.15
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.09	54.83	34.18	2.44	5.19	0.70	2.56
Ozone Depletion - ILCD PEF (v1.09)	0.19	44.58	30.28	12.06	10.74	0.54	1.61
Photochemical Ozone Formation, human health - EDIP 2003	0.29	45.57	28.72	12.90	4.52	0.76	7.23
Photochemical Ozone Formation, vegetation - EDIP 2003	0.28	46.06	29.51	12.12	4.50	0.79	6.76
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.02	85.26	10.12	-0.10	3.06	0.01	1.62
Total Freshwater Consumption (including rainwater) - [kg]	0.08	85.23	19.77	2.11	1.53	0.59	-9.31
Water resources - UBP 2013	0.02	73.41	20.78	0.12	6.67	0.01	-1.00

H.4 China Scenario

Table H.25: Percentage contribution of each life cycle phase of 6-APA manufacture in the China scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	**Supply**	**Use**	**End-of-life**
Acidification - ILCD/PEF (v1.09)	98.10	1.81	0.10
Ecotoxicity (Freshwater) - USEtox 2.1	86.18	13.73	0.09
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	94.35	4.75	0.90
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	98.90	1.08	0.02
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	88.81	8.84	2.35
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	95.62	0.68	3.70
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	98.45	1.43	0.12
Global Warming Potential, excl. biogenic carbon - IPCC AR5	74.45	25.39	0.16
Global Warming Potential, incl. biogenic carbon - IPCC AR5	66.79	32.97	0.24
Human Toxicity, cancer - USEtox 2.1	96.83	2.91	0.26
Human Toxicity, non-cancer - USEtox 2.1	96.37	3.34	0.29
Ionizing Radiation, human health - ILCD PEF (v1.09)	92.05	7.50	0.45
Ozone Depletion - ILCD PEF (v1.09)	77.84	22.16	0.00
Photochemical Ozone Formation, human health - EDIP 2003	95.62	4.15	0.22
Photochemical Ozone Formation, vegetation - EDIP 2003	95.93	3.86	0.21
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	95.75	4.27	-0.01
Total Freshwater Consumption (including rainwater) - [kg]	99.38	4.80	-4.18
Water resources - UBP 2013	97.00	6.77	-3.78

Table H.26: Percentage contribution of each processing block of 6-APA manufacture in the China scenario towards each environmental impact category. The highest contributing block is illustrated to be Stirred Tank Fermentation.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.20	41.90	41.05	7.60	5.65	1.15	2.45
Ecotoxicity (Freshwater) - USEtox 2.1	0.06	69.35	20.01	2.84	6.60	0.14	1.01
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.32	50.94	31.47	6.93	6.01	0.78	3.55
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.16	34.14	50.67	6.36	4.52	1.50	2.65
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.04	77.02	13.67	1.75	4.49	0.08	2.95
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.03	92.13	2.74	0.62	0.58	0.06	3.82
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.25	41.88	40.01	8.58	5.79	1.16	2.33
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.45	43.19	35.49	8.96	5.90	1.02	4.98
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.48	39.22	37.93	9.58	6.31	1.09	5.39
Human Toxicity, cancer - USEtox 2.1	0.99	48.49	22.70	12.92	8.04	0.62	6.24
Human Toxicity, non-cancer - USEtox 2.1	0.19	42.29	39.04	9.04	6.39	1.09	1.98
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.10	47.61	38.89	4.14	6.01	0.86	2.39
Ozone Depletion - ILCD PEF (v1.09)	0.17	50.97	27.13	10.47	9.59	0.47	1.20
Photochemical Ozone Formation, human health - EDIP 2003	0.26	38.90	38.11	11.11	5.11	1.07	5.44
Photochemical Ozone Formation, vegetation - EDIP 2003	0.26	39.13	38.59	10.68	5.12	1.08	5.12
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.07	77.95	13.59	2.67	4.38	0.14	1.20
Total Freshwater Consumption (including rainwater) - [kg]	0.05	93.86	12.23	1.73	1.44	0.34	-9.66
Water resources - UBP 2013	0.04	70.06	30.20	1.58	8.57	0.20	-10.65

H.5 GERMANY SCENARIO

Table H.27: Percentage contribution of each life cycle phase of 6-APA manufacture in the Germany scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	95.32	4.44	0.24
Ecotoxicity (Freshwater) - USEtox 2.1	74.71	25.13	0.16
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	81.60	15.45	2.94
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	92.23	7.63	0.14
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	83.13	13.33	3.54
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	95.88	0.64	3.48
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	96.25	3.46	0.28
Global Warming Potential, excl. biogenic carbon - IPCC AR5	67.34	32.46	0.20
Global Warming Potential, incl. biogenic carbon - IPCC AR5	57.08	42.61	0.31
Human Toxicity, cancer - USEtox 2.1	65.62	31.53	2.85
Human Toxicity, non-cancer - USEtox 2.1	92.96	6.47	0.57
Ionizing Radiation, human health - ILCD PEF (v1.09)	96.22	3.57	0.22
Ozone Depletion - ILCD PEF (v1.09)	69.99	30.01	0.00
Photochemical Ozone Formation, human health - EDIP 2003	91.67	7.92	0.41
Photochemical Ozone Formation, vegetation - EDIP 2003	92.07	7.53	0.40
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	92.19	7.84	-0.02
Total Freshwater Consumption (including rainwater) - [kg]	103.07	1.73	-4.79
Water resources - UBP 2013	93.28	13.69	-6.97

Table H.28: Percentage contribution of each processing block of 6-APA manufacture in the Germany scenario towards each environmental impact category. The highest contributing block is illustrated to be Stirred Tank Fermentation.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.22	54.64	29.50	7.57	5.18	0.82	2.06
Ecotoxicity (Freshwater) - USEtox 2.1	0.05	75.52	16.38	1.93	3.44	0.29	2.38
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.08	74.79	13.50	3.20	3.66	0.24	4.53
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.11	61.96	26.30	4.65	4.11	0.68	2.19
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.03	86.27	6.52	0.71	1.73	0.08	4.67
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.04	86.51	7.48	1.11	0.74	0.22	3.90
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.18	54.05	33.13	5.46	3.81	0.97	2.40
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.51	44.95	33.21	9.04	5.50	1.00	5.79
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.55	39.90	36.21	9.85	6.01	1.08	6.40
Human Toxicity, cancer - USEtox 2.1	0.06	72.30	15.79	2.74	4.61	0.20	4.29
Human Toxicity, non-cancer - USEtox 2.1	0.21	47.48	30.40	11.72	7.60	0.84	1.75
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.15	36.46	49.80	5.20	3.75	1.49	3.15
Ozone Depletion - ILCD PEF (v1.09)	0.18	59.31	19.71	11.14	7.23	0.51	1.92
Photochemical Ozone Formation, human health - EDIP 2003	0.31	45.10	26.90	14.43	4.31	0.77	8.18
Photochemical Ozone Formation, vegetation - EDIP 2003	0.30	45.72	27.60	13.64	4.29	0.79	7.66
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.05	80.18	13.37	0.98	3.13	0.17	2.13
Total Freshwater Consumption (including rainwater) - [kg]	0.05	103.45	9.06	1.19	0.89	0.27	-14.90
Water resources - UBP 2013	0.03	98.38	17.44	1.28	4.84	0.15	-22.13

H.6 INDIA SCENARIO

Table H.29: Percentage contribution of each life cycle phase of 6-APA manufacture in the India scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	98.92	1.06	0.02
Ecotoxicity (Freshwater) - USEtox 2.1	86.04	13.94	0.02
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	91.81	7.79	0.40
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	97.59	2.40	0.01
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	89.97	9.34	0.69
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	98.20	0.73	1.07
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	99.07	0.91	0.02
Global Warming Potential, excl. biogenic carbon - IPCC AR5	76.52	23.44	0.04
Global Warming Potential, incl. biogenic carbon - IPCC AR5	69.30	30.64	0.06
Human Toxicity, cancer - USEtox 2.1	82.20	17.38	0.42
Human Toxicity, non-cancer - USEtox 2.1	96.27	3.64	0.09
Ionizing Radiation, human health - ILCD PEF (v1.09)	92.22	7.66	0.13
Ozone Depletion - ILCD PEF (v1.09)	77.41	22.59	0.00
Photochemical Ozone Formation, human health - EDIP 2003	96.73	3.23	0.05
Photochemical Ozone Formation, vegetation - EDIP 2003	97.05	2.91	0.04
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	96.43	3.57	0.00
Total Freshwater Consumption (including rainwater) - [kg]	99.14	2.02	-1.15
Water resources - UBP 2013	91.65	9.30	-0.94

Table H.30: Percentage contribution of each processing block of 6-APA manufacture in the India scenario towards each environmental impact category. The highest contributing block is illustrated to be Stirred Tank Fermentation.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.22	36.42	46.93	7.27	4.72	1.41	3.03
Ecotoxicity (Freshwater) - USEtox 2.1	0.08	77.18	13.25	3.17	4.38	0.16	1.78
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.11	66.43	21.51	4.87	4.56	0.49	2.02
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.17	46.97	37.82	6.84	4.82	1.09	2.30
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.04	84.23	9.03	1.83	2.98	0.09	1.80
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.03	95.99	1.71	0.58	0.46	0.04	1.19
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.22	36.40	46.78	7.70	4.84	1.42	2.65
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.44	43.47	36.88	8.98	5.59	1.10	3.54
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.47	39.67	39.15	9.54	5.94	1.17	4.06
Human Toxicity, cancer - USEtox 2.1	0.07	74.81	15.56	3.31	4.37	0.24	1.64
Human Toxicity, non-cancer - USEtox 2.1	0.20	45.57	35.78	9.43	6.11	1.03	1.89
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.12	52.74	34.69	4.44	4.45	0.90	2.65
Ozone Depletion - ILCD PEF (v1.09)	0.17	56.40	22.17	10.67	8.00	0.48	2.11
Photochemical Ozone Formation, human health - EDIP 2003	0.24	36.02	43.19	9.91	4.41	1.29	4.94
Photochemical Ozone Formation, vegetation - EDIP 2003	0.24	35.90	43.99	9.49	4.41	1.32	4.66
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.05	84.50	8.62	2.15	2.79	0.11	1.76
Total Freshwater Consumption (including rainwater) - [kg]	0.06	94.32	11.76	1.82	1.30	0.34	-9.60
Water resources - UBP 2013	0.09	102.62	26.23	4.36	6.16	0.47	-39.93

H.7 SINGAPORE SCENARIO

Table H.31: Percentage contribution of each life cycle phase of 6-APA manufacture in the Singapore scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	96.88	2.94	0.17
Ecotoxicity (Freshwater) - USEtox 2.1	83.75	16.13	0.11
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	87.92	10.01	2.07
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	94.53	5.36	0.11
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	87.75	9.50	2.75
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	95.54	0.64	3.82
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	98.13	1.71	0.15
Global Warming Potential, excl. biogenic carbon - IPCC AR5	71.57	28.26	0.17
Global Warming Potential, incl. biogenic carbon - IPCC AR5	63.00	36.74	0.27
Human Toxicity, cancer - USEtox 2.1	78.23	19.82	1.95
Human Toxicity, non-cancer - USEtox 2.1	93.44	5.99	0.57
Ionizing Radiation, human health - ILCD PEF (v1.09)	85.83	13.30	0.87
Ozone Depletion - ILCD PEF (v1.09)	77.94	22.06	0.00
Photochemical Ozone Formation, human health - EDIP 2003	94.51	5.21	0.28
Photochemical Ozone Formation, vegetation - EDIP 2003	94.86	4.87	0.26
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	94.87	5.15	-0.02
Total Freshwater Consumption (including rainwater) - [kg]	100.15	4.51	-4.66
Water resources - UBP 2013	105.52	0.15	-5.67

Table H.32: Percentage contribution of each processing block of 6-APA manufacture in the Singapore scenario towards each environmental impact category. The highest contributing blocks are illustrated to be Stirred Tank Fermentation and Product Harvest.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.20	48.68	32.33	8.85	7.18	0.78	1.97
Ecotoxicity (Freshwater) - USEtox 2.1	0.07	61.42	25.10	3.59	8.39	0.16	1.27
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.12	60.45	22.96	5.49	7.66	0.27	3.06
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.15	54.85	26.12	8.77	8.64	0.44	1.02
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.04	73.22	15.95	2.02	5.23	0.10	3.44
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.03	92.34	2.47	0.60	0.58	0.05	3.92
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.21	43.17	38.56	8.89	6.18	1.09	1.91
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.44	42.51	35.85	9.05	6.03	1.03	5.09
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.48	38.00	38.61	9.75	6.50	1.10	5.56
Human Toxicity, cancer - USEtox 2.1	0.07	64.51	21.70	3.70	7.06	0.19	2.76
Human Toxicity, non-cancer - USEtox 2.1	0.21	48.62	27.58	12.48	9.28	0.62	1.20
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.06	60.50	26.00	2.89	8.43	0.15	1.97
Ozone Depletion - ILCD PEF (v1.09)	0.18	47.25	29.21	11.25	10.32	0.51	1.28
Photochemical Ozone Formation, human health - EDIP 2003	0.19	37.99	38.12	11.67	5.09	1.04	5.90
Photochemical Ozone Formation, vegetation - EDIP 2003	0.19	38.44	38.46	11.20	5.13	1.05	5.51
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.07	72.16	17.12	3.34	5.65	0.16	1.49
Total Freshwater Consumption (including rainwater) - [kg]	0.05	99.97	8.19	1.38	1.26	0.21	-11.05
Water resources - UBP 2013	0.01	56.37	35.64	0.17	11.54	0.00	-3.73

H.8 SOUTH AFRICA SCENARIO

Table H.33: Percentage contribution of each life cycle phase of 6-APA manufacture in the South Africa scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	99.27	0.69	0.04
Ecotoxicity (Freshwater) - USEtox 2.1	82.41	17.47	0.12
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	87.91	10.00	2.09
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	95.91	4.01	0.08
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	87.32	9.82	2.86
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	95.69	0.62	3.69
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	99.21	0.73	0.07
Global Warming Potential, excl. biogenic carbon - IPCC AR5	78.88	20.99	0.13
Global Warming Potential, incl. biogenic carbon - IPCC AR5	73.13	26.67	0.20
Human Toxicity, cancer - USEtox 2.1	78.01	20.02	1.98
Human Toxicity, non-cancer - USEtox 2.1	94.93	4.62	0.45
Ionizing Radiation, human health - ILCD PEF (v1.09)	93.23	6.35	0.42
Ozone Depletion - ILCD PEF (v1.09)	76.78	23.22	0.00
Photochemical Ozone Formation, human health - EDIP 2003	97.42	2.45	0.13
Photochemical Ozone Formation, vegetation - EDIP 2003	97.65	2.23	0.12
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	94.80	5.22	-0.02
Total Freshwater Consumption (including rainwater) - [kg]	100.44	4.34	-4.78
Water resources - UBP 2013	93.39	11.46	-4.85

Table H.34: Percentage contribution of each processing block of 6-APA manufacture in the South Africa scenario towards each environmental impact category. The highest contributing blocks are illustrated to be Stirred Tank Fermentation and Product Harvest.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.20	31.76	52.20	6.94	4.51	1.58	2.81
Ecotoxicity (Freshwater) - USEtox 2.1	0.08	68.88	19.92	3.96	6.59	0.20	0.37
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.11	63.47	21.62	5.70	6.21	0.38	2.51
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.16	49.56	33.42	8.32	6.56	0.85	1.13
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.05	79.02	11.79	2.13	3.92	0.10	2.99
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.03	93.33	1.85	0.55	0.46	0.04	3.74
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.19	33.61	49.99	7.37	4.69	1.52	2.62
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.42	39.65	39.79	8.72	5.48	1.19	4.74
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.45	35.66	42.38	9.30	5.84	1.27	5.11
Human Toxicity, cancer - USEtox 2.1	0.08	67.73	19.74	3.94	5.88	0.26	2.38
Human Toxicity, non-cancer - USEtox 2.1	0.20	44.69	34.11	11.17	7.51	0.93	1.38
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.13	44.49	42.14	4.87	4.95	1.11	2.31
Ozone Depletion - ILCD PEF (v1.09)	0.19	50.89	26.55	12.04	9.52	0.55	0.26
Photochemical Ozone Formation, human health - EDIP 2003	0.26	33.40	46.40	9.26	4.55	1.39	4.73
Photochemical Ozone Formation, vegetation - EDIP 2003	0.25	33.27	47.13	8.92	4.52	1.42	4.49
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.08	75.62	15.40	3.60	4.92	0.20	0.18
Total Freshwater Consumption (including rainwater) - [kg]	0.04	104.79	4.67	1.04	0.89	0.12	-11.54
Water resources - UBP 2013	0.06	82.75	28.85	2.98	7.80	0.31	-22.76

H.9 SPAIN SCENARIO

Table H.35: Percentage contribution of each life cycle phase of 6-APA manufacture in the Spain scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	96.05	3.90	0.06
Ecotoxicity (Freshwater) - USEtox 2.1	79.05	20.92	0.04
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	86.58	12.77	0.65
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	93.29	6.68	0.03
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	87.27	11.86	0.87
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	98.23	0.72	1.05
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	96.74	3.19	0.07
Global Warming Potential, excl. biogenic carbon - IPCC AR5	63.67	36.26	0.06
Global Warming Potential, incl. biogenic carbon - IPCC AR5	51.44	48.46	0.10
Human Toxicity, cancer - USEtox 2.1	72.84	26.53	0.64
Human Toxicity, non-cancer - USEtox 2.1	93.17	6.68	0.16
Ionizing Radiation, human health - ILCD PEF (v1.09)	96.80	3.15	0.05
Ozone Depletion - ILCD PEF (v1.09)	72.90	27.10	0.00
Photochemical Ozone Formation, human health - EDIP 2003	92.67	7.23	0.10
Photochemical Ozone Formation, vegetation - EDIP 2003	93.05	6.86	0.10
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	94.02	5.98	0.00
Total Freshwater Consumption (including rainwater) - [kg]	96.32	4.91	-1.23
Water resources - UBP 2013	86.74	14.31	-1.05

Table H.36: Percentage contribution of each processing block of 6-APA manufacture in the Spain scenario towards each environmental impact category. The highest contributing block is illustrated to be Stirred Tank Fermentation.

Environmental Impact Category - Method	Process Contribution (%)						
	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.26	55.76	29.00	7.64	5.13	0.82	1.39
Ecotoxicity (Freshwater) - USEtox 2.1	0.03	85.05	8.53	1.08	2.53	0.09	2.69
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.06	81.57	10.27	2.47	2.91	0.17	2.55
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.08	74.89	16.22	3.44	3.20	0.38	1.79
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.02	90.86	4.61	0.51	1.46	0.03	2.51
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.03	96.23	1.99	0.28	0.25	0.05	1.16
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.27	56.26	30.87	6.11	4.11	0.90	1.48
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.60	51.39	28.31	9.62	5.84	0.84	3.40
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.67	44.75	31.91	10.82	6.59	0.94	4.32
Human Toxicity, cancer - USEtox 2.1	0.05	78.19	13.02	2.29	3.87	0.16	2.43
Human Toxicity, non-cancer - USEtox 2.1	0.19	56.23	23.25	11.25	7.32	0.61	1.15
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.18	38.15	49.76	5.65	3.94	1.49	0.82
Ozone Depletion - ILCD PEF (v1.09)	0.16	62.02	17.77	10.04	6.52	0.46	3.01
Photochemical Ozone Formation, human health - EDIP 2003	0.36	46.95	26.53	14.07	4.45	0.76	6.87
Photochemical Ozone Formation, vegetation - EDIP 2003	0.35	47.56	27.14	13.36	4.45	0.78	6.37
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.04	83.71	10.34	0.77	2.39	0.14	2.62
Total Freshwater Consumption (including rainwater) - [kg]	0.04	103.06	6.00	0.88	0.69	0.17	-10.83
Water resources - UBP 2013	0.06	99.44	20.83	2.29	3.69	0.43	-26.74

H.10 UK SCENARIO

Table H.37: Percentage contribution of each life cycle phase of 6-APA manufacture in the UK scenario towards each environmental impact category. The highest contributing phase is illustrated to be Supply.

	Phase Contribution (%)		
Environmental Impact Category - Method	Supply	Use	End-of-life
Acidification - ILCD/PEF (v1.09)	96.64	3.19	0.17
Ecotoxicity (Freshwater) - USEtox 2.1	79.80	20.07	0.13
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	86.55	11.30	2.15
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	93.12	6.76	0.12
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	88.79	8.86	2.35
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	97.21	0.43	2.36
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	97.34	2.46	0.20
Global Warming Potential, excl. biogenic carbon - IPCC AR5	67.15	32.65	0.20
Global Warming Potential, incl. biogenic carbon - IPCC AR5	50.95	48.70	0.35
Human Toxicity, cancer - USEtox 2.1	73.31	24.48	2.21
Human Toxicity, non-cancer - USEtox 2.1	93.68	5.81	0.51
Ionizing Radiation, human health - ILCD PEF (v1.09)	97.28	2.57	0.15
Ozone Depletion - ILCD PEF (v1.09)	73.20	26.80	0.00
Photochemical Ozone Formation, human health - EDIP 2003	93.04	6.62	0.34
Photochemical Ozone Formation, vegetation - EDIP 2003	93.49	6.18	0.32
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	94.82	5.20	-0.02
Total Freshwater Consumption (including rainwater) - [kg]	99.53	3.54	-3.07
Water resources - UBP 2013	103.16	2.12	-5.28

Table H.38: Percentage contribution of each processing block of 6-APA manufacture in the UK scenario towards each environmental impact category. The highest contributing blocks are illustrated to be Stirred Tank Fermentation and Product Harvest.

	Process Contribution (%)						
Environmental Impact Category - Method	Inoculation	Stirred Tank Fermentation	Product Harvest	Product Conversion	Product Purification	Product Conditioning	Waste Water Treatment
Acidification - ILCD/PEF (v1.09)	0.17	58.68	28.47	5.98	4.07	0.82	1.80
Ecotoxicity (Freshwater) - USEtox 2.1	0.03	86.98	7.90	0.99	2.40	0.07	1.62
Ecotoxicity (Marine) - ReCiPe 2016 v1.1 (H)]	0.06	83.38	8.55	2.13	2.54	0.14	3.21
Ecotoxicity (Terrestrial) - ReCiPe 2016 v1.1 (H)	0.07	78.44	13.68	3.09	3.00	0.30	1.41
Eutrophication (Freshwater) - ReCiPe 2016 v1.1 (H)	0.02	92.13	3.33	0.37	1.08	0.02	3.05
Eutrophication (Marine) - ReCiPe 2016 v1.1 (H)	0.02	96.41	0.86	0.13	0.12	0.02	2.43
Eutrophication (Terrestrial) - ILCD/PEF (v1.09)	0.17	58.96	30.00	4.65	3.18	0.89	2.17
Global Warming Potential, excl. biogenic carbon - IPCC AR5	0.46	53.02	27.69	7.85	4.80	0.83	5.37
Global Warming Potential, incl. biogenic carbon - IPCC AR5	0.56	42.06	34.10	9.64	5.93	1.01	6.70
Human Toxicity, cancer - USEtox 2.1	0.05	79.31	11.65	2.04	3.53	0.14	3.29
Human Toxicity, non-cancer - USEtox 2.1	0.18	54.76	25.83	10.33	6.70	0.71	1.49
Ionizing Radiation, human health - ILCD PEF (v1.09)	0.15	38.24	48.54	5.11	3.54	1.47	2.96
Ozone Depletion - ILCD PEF (v1.09)	0.16	63.74	17.57	9.93	6.44	0.45	1.71
Photochemical Ozone Formation, human health - EDIP 2003	0.18	50.81	27.15	11.29	3.20	0.79	6.58
Photochemical Ozone Formation, vegetation - EDIP 2003	0.18	51.30	27.76	10.61	3.23	0.81	6.11
Resource Depletion, mineral, fossils and renewables - ILCD PEF (v1.09)	0.03	88.01	8.02	0.52	1.99	0.09	1.34
Total Freshwater Consumption (including rainwater) - [kg]	0.02	105.17	1.61	0.31	0.29	0.04	-7.43
Water resources - UBP 2013	0.02	91.83	10.12	0.20	3.19	0.02	-5.37

APPENDIX I: SENSITIVITY ANALYSIS

I.1 DOWNSTREAM PROCESS PARAMETERS

Table I.1: Percentage variation (%) of each environmental impact category when all downstream process parameters were varied by ±1%. AP=acidification. FETP=freshwater ecotoxicity. METP= marine ecotoxicity. TETP=terrestrial ecotoxicity. FEP=freshwater eutrophication. MEP=marine eutrophication. TEP=terrestrial eutrophication. GWP=global warming potential (exclude or include biogenic carbon). HTP=human toxicity (cancer or non-cancer). IRP=ionising radiation. ODP=ozone depletion. POF=photochemical ozone formation (human health or vegetation). RDP=resource depletion. IWR=impact on water resources (water scarcity). TWC=total water consumption. No figure = ≤ ±0.01%, White = ≤ ±0.10%, Blue = ±0.11-0.20%, Green = ±0.21-0.30%, Yellow =±0.31-0.40%, Orange= ±0.41-0.50%, Red = ≥ ±0.51%.

Processing Block	Parameter Description	AP	FETP	METP	TETP	FEP	MEP	TEP	GWP (excl)	GWP (incl)	HTP (C)	HTP (NC)	IRP	ODP	POF (HH)	POF (VEG)	RDP	IWR	TWC
Product Harvest	Harvest tank storage - degradation %																		
Product Harvest	Harvest tank storage - line lost																		
Product Harvest	Rotary vacuum filtration - biomass and impurities yield																		
Product Harvest	Rotary vacuum filtration - liquid yield	0.01	0.52	0.12	0.22	0.17		0.05	0.15	0.01	0.33	0.04	0.04	0.2	0.04	0.03	0.28	0.07	0.09
Product Harvest	Rotary vacuum filtration - penicillin yield	0.02	0.15	0.03	0.09	0.05		0.01				0.02		0.06	0.01	0.01	0.05		0.04
Product Harvest	Solvent extraction - aqueous to solvent ratio	0.06	0.54	0.1	0.27	0.18	0.01	0.04	0.02	0.01	0.02	0.08	0.04	0.2	0.15	0.14	0.38		0.15
Product Harvest	Solvent extraction - biomass yield																		
Product Harvest	Solvent extraction - penicillin yield	0.02	0.15	0.03	0.09	0.05		0.01				0.02		0.06	0.01	0.01	0.05		0.04
Product Conversion	Back extraction process - aqueous to solvent ratio	0.02	0.18	0.04	0.1	0.06		0.01	0.01		0.01	0.03	0.02	0.06	0.09	0.09	0.23		0.05
Product Conversion	Back extraction process - biomass yield																		
Product Conversion	Back extraction process - penicillin yield	0.02	0.15	0.03	0.09	0.05		0.01				0.02		0.06	0.01	0.01	0.05		0.04
Product Conversion	Enzyme hydrolysis process - biomass yield																		
Product Conversion	Enzyme hydrolysis process - buffer to penicillin ratio	0.02	0.16	0.03	0.09	0.06		0.01	0.01		0.01	0.03	0.01	0.06	0.02	0.02	0.21		0.05
Product Conversion	Enzyme hydrolysis process - conversion percentage	0.02	0.15	0.03	0.09	0.05		0.01				0.02		0.06	0.01	0.01	0.05		0.04
Product Conversion	Enzyme hydrolysis process - enzyme to product ratio														0.07	0.06			
Product Conversion	Enzyme hydrolysis process - reaction time																		

Product Conversion & Product Purification	Back extraction process - number of units																			
Product Purification	Back extraction process - 6-APA yield																			
Product Purification	Solvent extraction - 6-APA yield																			
Product Purification	Solvent extraction - aqueous to solvent ratio	0.01	0.14	0.03	0.07	0.04		0.01	0.01		0.01	0.02	0.01	0.05	0.02	0.02	0.06		0.04
Product Purification	Solvent extraction - biomass yield																			
Product Purification	Solvent extraction - PAA yield	0.03	0.15	0.04	0.1	0.05		0.02	0.01			0.02	0.02	0.06	0.02	0.02	0.06	0.01	0.05
Product Purification	Solvent extraction - penicillin yield																			
Product Purification	Back extraction process - aqueous to solvent ratio																			
Product Purification	Back extraction process - biomass yield																			
Product Purification	Back extraction process - PAA yield																			
Product Purification	Back extraction process - penicillin yield																			
Product Purification	Crystallisation process - 6-APA Yield	0.01						0.01	0.01				0.01		0.01	0.01	0.01	0.01	0.01
Product Purification	Crystallisation process - biomass degradation %																			
Product Purification	Crystallisation process - penicillin and PAA degradation %																			
Product Conditioning	Milling process - 6-APA yield																			
Product Conditioning	Spin dry process - 6-APA yield (spinning stage)							0.01	0.01						0.01	0.01	0.01		
Product Conditioning	Spin dry process - 6-APA yield (wash stage)																			
Product Conditioning	Spin dry process - biomass yield (spinning stage)																			
Product Conditioning	Spin dry process - biomass yield (wash stage)																			
Product Conditioning	Spin dry process - impurities yield (wash stage)																			
Product Conditioning	Spin dry process - wash to cake ratio (liquid)							0.01	0.01						0.01	0.01	0.01		
Product Conditioning	Spin dry process - wash to cake ratio (solid)							0.01	0.01						0.01	0.01	0.01		
Product Conditioning	Spin dry process - water to product ratio																			

I.2 Cleaning Parameters

Table I.2: Percentage variation (%) of each environmental impact category due to varying cleaning parameters (part 1). Process cleaning parameters were varied by their uncertainty range L = negative deviation % and H = positive deviation %. AP=acidification. FETP=freshwater ecotoxicity. METP= marine ecotoxicity. TETP=terrestrial ecotoxicity. FEP=freshwater eutrophication. MEP=marine eutrophication. TEP=terrestrial eutrophication. GWP=global warming potential (exclude or include biogenic carbon). No figure = < ±0.01%, White = ≤ ±1.00%, Blue = ±1.01-2.00%, Green = ±2.01-3.00%, Yellow =±3.01-4.00%, Orange =±4.01-5.00%, Red =±>5.01%

		AP		FETP		METP		TETP		FEP		MEP		TEP		GWP (excl)		GWP (incl)	
Parameter	**Variation (L to H %)**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**
Acid volume	**-12 to 25**	-1.36	0.34	-0.50	0.13	-4.35	1.09	-1.26	0.32	-0.45	0.11	-0.61	0.15	-2.38	0.60	-4.21	1.06	-3.98	1.00
Caustic volume	**-20 to 5**	-7.50	1.88	-1.29	0.32	-4.45	1.11	-5.80	1.45	-0.28	0.07	-0.22	0.05	-3.88	0.97	-3.64	0.91	-3.43	0.86
Energy requirements	**-20 to 5**	-0.04	0.01	-0.01				-0.04	0.01			-0.01		-0.06	0.02	-0.06	0.02	-0.06	0.01
Pre-rinse volume	**-20 to 5**		0.02	-0.02	0.05	-0.01	0.02	-0.01	0.04	-0.02	0.04	-0.01	0.02		0.03		0.02	-0.01	0.03
Steam requirements	**-20 to 5**	-0.02				-0.08	0.02	-0.02						-0.03	0.01	-0.06	0.02	-0.06	0.01
WFI volume	**-12 to 25**	-0.23	0.48	-0.05	0.11	-0.91	1.91	-0.24	0.49	-0.06	0.12	-0.04	0.08	-0.40	0.83	-0.73	1.53	-0.69	1.44

Table I.3: Percentage variation (%) of each environmental impact category due to varying cleaning parameters (part 2). Process cleaning parameters were varied by their uncertainty range L = negative deviation % and H = positive deviation %. HTP=human toxicity (cancer or non-cancer). IRP=ionising radiation. ODP=ozone depletion. POF=photochemical ozone formation (human health or vegetation). RDP=resource depletion. IWR=impact on water resources (water scarcity). TWC=total water consumption. No figure = < ±0.01%, White = ≤ ±1.00%, Blue = ±1.01-2.00%, Green = ±2.01-3.00%, Yellow =±3.01-4.00%, Orange =±4.01-5.00%, Red =±>5.01%

		HTP (C)		HTP (NC)		IRP		ODP		POF (HH)		POF (VEG)		RDP		IWR		TWC	
Parameter	**Variation (L to H %)**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	H
Acid volume	**-12 to 25**	-6.48	1.62	-0.94	0.24	-2.13	0.54	-0.14	0.03	-2.66	0.67	-2.65	0.67	-1.86	0.47	-2.12	0.54	-1.74	0.44
Caustic volume	**-20 to 5**	-4.37	1.09	-9.68	3.23	-1.38	0.35	-14.16	3.54	-3.60	0.90	-3.65	0.91	-1.46	0.36	-1.39	0.35	-1.13	0.28
Energy requirements	**-20 to 5**			-0.02	0.01	-0.17	0.04			-0.05	0.01	-0.05	0.01	-0.03	0.01	-0.14	0.04	-0.11	0.03
Pre-rinse volume	**-20 to 5**			-0.01	0.02	-0.01	0.06	-0.02	0.03		0.02		0.02	-0.01	0.03	-0.03	0.10	-0.03	0.09
Steam requirements	**-20 to 5**	-0.13	0.03	-0.01		-0.03	0.01			-0.04	0.01	-0.04	0.01	-0.03	0.01	-0.03	0.01	-0.02	0.01
WFI volume	**-12 to 25**	-1.40	2.92	-0.16	0.34	-0.43	0.90			-0.49	1.02	-0.48	1.01	-0.36	0.75	-0.39	0.81	-0.31	0.64

I.3 OTHER PARAMETERS

Table I.4: Percentage variation (%) of each environmental impact category due to uncertainties with HVAC requirements and equipment life span. AP=acidification. FETP=freshwater ecotoxicity. METP= marine ecotoxicity. TETP=terrestrial ecotoxicity. FEP=freshwater eutrophication. MEP=marine eutrophication. TEP=terrestrial eutrophication. GWP=global warming potential (exclude or include biogenic carbon). HTP=human toxicity (cancer or non-cancer). IRP=ionising radiation. ODP=ozone depletion. POF=photochemical ozone formation (human health or vegetation). RDP=resource depletion. IWR=impact on water resources. TWC=total water consumption. No figure = ≤ ±0.01%, White = ≤ ±0.10%, Blue = ±0.11-0.20%, Green = ±0.21-0.30%, Yellow =±0.31-0.40%, Orange= ±0.41-0.50%, Red = ≥ ±0.51%.

Parameter Description	% Variation	AP	FETP	METP	TETP	FEP	MEP	TEP	GWP (excl)	GWP (incl)	HTP (C)	HTP (NC)	IRP	ODP	POF (HH)	POF (VEG)	RDP	IWR	TWC
HVAC energy requirements	25	0.15	0.03	0.02	0.13	0.00	0.02	0.22	0.21	0.21		0.08	0.57		0.18	0.18	0.11	0.49	0.38
Process equipment life	10																		
Waste equipment life	10																		

APPENDIX J: ADDITION INFORMATION FOR SCENARIO ANALYSIS

J.1 Parameter Changes for Production Scales Scenario Analysis

Table J.1: Parameters that were changed due to a change in annual production throughput and changes in the production fermentation capacity.

			Scenarios - Annual Throughput (tonnes / year)				
Process Block	**Parameter Description**	**Units**	**Base-case 2000**	**Scenario 3 1000**	**Scenario 4 500**	**Scenario 5 100**	**Scenario 6 20**
Inoculation	Inoculation processing block - fermentation media make up, power consumption	kWh	114	76	45	14	9
Inoculation	Can fermentation - cooling jacket flow rate (both reactors combined)	m3/hr	0.05	0.025	0.0125	0.0025	0.0005
Inoculation	Can fermentation - fermentation working volume (both reactors combined)	L	150	75	38	8	2
Inoculation	Can fermentation - fermentation bioreactor capacity	m^3	0.1	0.05	0.025	0.005	0.001
Inoculation	Can fermentation - fermentation bioreactor working volume	m^3	0.075	0.038	0.019	0.004	0.001
Inoculation	Flask fermentation - fermentation working volume	L	0.5	0.25	0.125	0.025	0.005
Inoculation	Incubator power rating for dry and flask fermentations	kW	2	2	2	2	2
Inoculation	Cell suspension volume	L	0.0562	0.0562	0.0141	0.0028	0.0006
Inoculation	Inoculation onto rice, cell concentration in	mg/L	0.001	0.0005	0.0005	0.0005	0.001
Inoculation	Inoculation onto rice, total volume	L	0.055	0.055	0.0275	0.0055	0.0006
Inoculation	Inoculation onto rice, rice mass in	kg	0.05	0.05	0.25	0.005	0.005
Inoculation	Inoculation onto rice, trace solution in	kg	0.05	0.05	0.25	0.005	0.005
Inoculation	Inoculation processing block - benchtop fermentation media make up, power consumption	kWh	0.212	0.281	0.179	0.0587	0.0172
Stirred Tank Fermentation	Cooling jacket flow rate	m^3/hr	90	45	23	5	1
Stirred Tank Fermentation	Production fermentation - mini harvest volumes	m^3	7.5	3.75	1.86	3.75	0.075
Stirred Tank Fermentation	**Production fermentation - fermentation working volume (both reactors combined)**	**m^3**	**240**	**120**	**60**	**12**	**2.4**
Stirred Tank Fermentation	Production fermentation - fermentation media input flow rate	m^3/hr	6.4	3.2	1.6	0.32	0.064
Stirred Tank Fermentation	Production fermentation - large production fermentation bioreactor capacity	m^3	200	100	50	10	2
Stirred Tank Fermentation	Production fermentation - large production fermentation bioreactor working volume	m^3	160	80	40	8	1.6

Stirred Tank Fermentation	Production fermentation - small production fermentation bioreactor capacity	m^3	100	50	25	5	1
Stirred Tank Fermentation	Production fermentation - small production fermentation bioreactor working volume	m^3	80	40	20	4	0.8
Stirred Tank Fermentation	N-1 fermentation - cooling jacket flow rate	m^3/hr	20	10	5	1	0.2
Stirred Tank Fermentation	N-1 fermentation - fermentation working volume	m^3	25.6	12.8	6.4	1.28	0.256
Stirred Tank Fermentation	N-1 fermentation - fermentation media input flow rate	m^3/hr	6.4	3.2	1.6	0.32	0.064
Stirred Tank Fermentation	N-1 fermentation - fermentation bioreactor capacity	m^3	16	8	4	0.8	0.16
Stirred Tank Fermentation	N-1 fermentation - fermentation bioreactor working volume	m^3	13	6	3.2	0.64	0.128
Stirred Tank Fermentation	N-2 fermentation - cooling jacket flow rate	m^3/hr	2	1	0.5	0.1	0.02
Stirred Tank Fermentation	N-2 fermentation - fermentation working volume	L	12800	6400	3200	640	128
Stirred Tank Fermentation	N-2 fermentation - fermentation bioreactor capacity	m^3	8	4	2	1	0.08
Stirred Tank Fermentation	N-2 fermentation - fermentation bioreactor working volume	m^3	6	3	2	1	0.06
Stirred Tank Fermentation	Stirred Tank Fermentation processing block - fermentation media makeup, power consumption	MWh	12.6	8.71	3.36	8.91	3.96
Product Harvest	Production harvest - flow rate	m^3/hr	20	10	5	1	0.2
Product Harvest	Product harvest tank volume	m^3	300	150	75	15	3
Product Harvest	product harvest tank working volume	m^3	270	135	68	14	3
Product Harvest	Rotary vacuum filtration - fermentation broth input flow rate	m^3/hr	20	10	5	1	0.2
Product Harvest	Rotary vacuum filtration - filter aid broth input flow rate	m^3/hr	40	20	10	2	0.5
Product Harvest	Rotary vacuum filtration - filter aid volume	m^3	40	20	10	2	0.4
Product Harvest	Rotary vacuum filtration - filter area	m^2	40	20	10	2	0.4
Product Harvest	Rotary vacuum filtration - filter circumference	m	5	5	5	2	1
Product Harvest	Rotary vacuum filtration - filter length	m	9	4	2	1	1
Product Harvest	Rotary vacuum filtration - power rating (motor)	kW	45	22	19	4	2
Product Harvest	Filter aid make up tank capacity	m^3	60	30	15	3	1
Product Harvest	Filter aid make up tank working volume	m^3	45	25	12	3	0.45
Product Harvest	Acid make up - water for injection flow rate	m^3/hr	3	1.5	0.75	0.15	0.03
Product Harvest	Acid make up tank capacity	m^3	12	6	3	1	0.12
Product Harvest	Solvent extraction - fermentation broth input flow rate	m^3/hr	20	10	5	1	0.2

Product Harvest	Solvent extraction - equipment power rating	kWh	7.5	5.5	5.5	0.75	0.24
Product Conversion	Back extraction - equipment power rating	kWh	5.5	1.1	1.1	0.75	0.04
Product Conversion	Enzyme hydrolysis tank capacity	m^3	40	20	10	5	0.4
Product Conversion	Enzyme hydrolysis - phosphate buffer flow rate	m^3/hr	10	5	2.5	0.5	0.1
Product Conversion	Phosphate buffer tank power consumption (mixing)	kWh	23	15	9	3	1
Product Conversion	Enzyme hydrolysis - Immobilised enzyme input flow rate	m^3/hr	5	2.5	1.25	0.25	0.05
Product Purification	Solvent extraction product stream flow rate	m^3/hr	20	10	5	1	0.2
Product Purification	Solvent extraction - equipment power rating	kWh	7.5	5.5	5.5	1.1	0.24
Product Purification	Back extraction - equipment power rating	kWh	1.1	1.1	1.1	1.1	0.2
Product Purification	6-APA crystallisation tank capacity	m^3	120	60	30	10	2
Product Purification	Spin dry centrifuge power rating	kW	90	45	30	8	4
Product Purification	Spin dry centrifuge - input flow rate	m^3/hr	15	15	7.5	1.5	3.17
Product Purification	Product Purification processing block - buffer make up tank capacity	m3	1	0.5	0.25	0.05	0.01
Product Purification	Product Purification processing block - buffer make up - water for injection input flow rate	m^3/hr	3	1.5	7.5	0.15	0.03
Product Purification	Product Purification processing block - acid make up tank capacity	m^3	2	1	0.5	0.1	0.02
Product Purification	Product Purification processing block - acid make up - water for injection input flow rate	m^3/hr	3	1.5	7.5	0.15	0.03
Product Purification	Product Purification processing block - methanol and water input power consumption	kWh	4	2	1	1	1
Product Conditioning	Vacuum drying - equipment bowl volume	m^3	3.95	3.95	2.15	0.38	0.073
Product Conditioning	Vacuum drying - equipment heating area	m2	23	23	14	4	1
Product Conditioning	Vacuum drying - equipment power rating	kW	90	90	45	15	6
Product Conditioning	Vacuum drying - vacuum pump power rating	kW	1	1	1	1	1
Product Conditioning	Milling equipment power rating	kW	15	15	8	8	5
Product Conditioning	Milling equipment throughput	kg/h	4000	2000	1000	200	40
Waste Treatment	Acid make up tank capacity	L	250	125	63	20	3
Waste Treatment	Filter cake drying area	m^2	44	24	13	2	1
Waste Treatment	Filter cake drying power consumption	kwh	35	17	13	8	6
Waste Treatment	PAA recovery - sodium hydroxide flow rate	m^3/hr	18	9	4.5	0.9	0.18
Waste Treatment	PAA recovery -purified water flow rate	m^3/hr	18	9	4.5	0.9	0.18
Waste Treatment	PAA recovery - tank capacity	m^3	30	15	8	2	1
Waste Treatment	Vacuum stripping - steam flow rate	kg/hr	2360	1690	1130	683	349
Waste Treatment	Air flotation power rating	kW	16	8	6	2	2

Waste Treatment	Mass flow rate	kg/hr	44000	24100	14100	5960	3890
Waste Treatment	Primary settler energy requirements (mixing)	kWh	0.0279	0.0234	0.0119	0.0125	0.00642
Waste Treatment	Primary settler tank capacity	m^3	280	150	90	30	20
Waste Treatment	Aeration equipment power rating	kW	12	6.1	3.42	1.21	0.723
Waste Treatment	Aeration equipment capacity	m^3	480	250	150	50	40
Waste Treatment	Aeration - scum output	kg	13700	6780	342	687	141
Waste Treatment	Primary settler energy requirements	kWh	13	6.28	3.6	1.26	0.792
Waste Treatment	Secondary settler tank capacity	m^3	480	250	150	50	40
ALL	CIP - acid and caustic make up energy requirements	kWh	426	157	55	9	3
ALL	CIP - Acid requirements	tonnes	1810	955	531	205	127
ALL	CIP - Caustic requirements	tonnes	1230	638	341	111	573
ALL	CIP - energy requirements, pumping and cleaning of all equipment	MWh	3.94	2.59	1.82	1.04	0.74
ALL	CIP – pre-rinse requirements	tonnes	356	294	254	190	159
ALL	CIP - SIP requirements	tonnes	7.66	3.85	1.95	0.482	0.133
ALL	CIP - water for injection requirements	tonnes	652	544	473	359	305
ALL	HVAC electricity requirements	MWh	10.8	5.84	3.05	0.424	1.08
ALL	Total process equipment mass	tonnes	4500	2870	1853	771	356
ALL	Total waste equipment mass	tonnes	1000	633	465	255	181

J.2 Product Titre Impact Corrections

Table J.2: Environmental impact values at each production titre scenario as a fraction of the base case scenario (impact values were divided by values generated from the base case). Correlation models were fitted for every environmental impact category. y = m x + C, y = environmental impact as a fraction of base case, x = the change in product titre, C = 1 to reflect that base-case as the most reliable, and m values are represented in the table.

	Scenario 1 35 g/L	Base-case 57 g/L	Scenario 2 100 g/L	y = mx + 1	
% Change in Product Titre (x)	-0.386	0	0.754		
Impact values normalised to fraction of base case (y)				m	R-square
Acidification	0.95	1.00	1.06	0.09	0.94
Ecotoxicity (Freshwater)	0.96	1.00	1.08	0.11	1.00
Ecotoxicity (Marine)	0.97	1.00	1.05	0.07	1.00
Ecotoxicity (Terrestrial)	0.95	1.00	1.06	0.09	0.94
Eutrophication (Freshwater)	0.89	1.00	1.21	0.28	1.00
Eutrophication (Marine)	0.87	1.00	1.25	0.33	1.00
Eutrophication (Terrestrial)	0.92	1.00	1.09	0.14	0.94
Global Warming Potential, excl. biogenic carbon	0.94	1.00	1.06	0.09	0.89
Global Warming Potential, incl. biogenic carbon	0.95	1.00	1.03	0.06	0.72
Human Toxicity, cancer	0.99	1.00	1.02	0.02	1.00
Human Toxicity, non-cancer	0.98	1.00	1.03	0.04	0.93
Ionizing Radiation, human health	0.88	1.00	1.06	0.13	0.62
Ozone Depletion	0.99	1.00	1.02	0.03	1.00
Photochemical Ozone Formation, human health	0.94	1.00	1.07	0.11	0.94
Photochemical Ozone Formation, vegetation	0.94	1.00	1.07	0.11	0.94
Resource Depletion, mineral, fossils and renewables	0.95	1.00	1.06	0.09	0.96
Total Freshwater Consumption (including rainwater)	0.88	1.00	1.24	0.32	1.00
Water resources	0.94	1.00	1.10	0.13	1.00

J.3 Production Scale Impact Correlations

Table J.3: Environmental impact values at each production scale as a fraction of the base case scenario (impact values were divided by values generated from the base case). Correlation models were fitted for every environmental impact category. y = m x + C, y = environmental impact as a fraction of base case, x = production scale as a fraction of base-case, m and C are represented in the table.

	Scenarios					y = mx + C		
Production Scale - Fermentation Capacity (m3)	**300 Base-case**	**150 Scenario 3**	**75 Scenario 4**	**15 Scenario 5**	**3 Scenario 6**	**m**	**C**	**R-square**
Production Scale / Base-Case (x)	**1**	**0.5**	**0.25**	**0.05**	**0.1**			
Impact values normalised to % of base case (2000 tonnes)								
Acidification	1.00	0.53	0.30	0.09	0.05	0.96	0.05	1.00
Ecotoxicity (Freshwater)	1.00	0.51	0.26	0.06	0.02	0.99	0.01	1.00
Ecotoxicity (Marine)	1.00	0.55	0.31	0.12	0.07	0.93	0.07	1.00
Ecotoxicity (Terrestrial)	1.00	0.52	0.29	0.09	0.04	0.96	0.04	1.00
Eutrophication (Freshwater)	1.00	0.50	0.25	0.06	0.01	0.99	0.01	1.00
Eutrophication (Marine)	1.00	0.50	0.26	0.06	0.02	0.99	0.01	1.00
Eutrophication (Terrestrial)	1.00	0.54	0.32	0.10	0.06	0.95	0.06	1.00
Global Warming Potential, excl. biogenic carbon	1.00	0.55	0.33	0.12	0.08	0.93	0.08	1.00
Global Warming Potential, incl. biogenic carbon	1.00	0.56	0.34	0.13	0.08	0.92	0.09	1.00
Human Toxicity, cancer	1.00	0.57	0.34	0.15	0.10	0.90	0.11	1.00
Human Toxicity, non-cancer	1.00	0.52	0.29	0.09	0.05	0.96	0.05	1.00
Ionizing Radiation, human health	1.00	0.56	0.37	0.12	0.08	0.92	0.09	0.99
Ozone Depletion	1.00	0.51	0.27	0.08	0.04	0.97	0.03	1.00
Photochemical Ozone Formation, human health	1.00	0.54	0.31	0.10	0.06	0.95	0.06	1.00
Photochemical Ozone Formation, vegetation	1.00	0.54	0.31	0.10	0.06	0.95	0.06	1.00
Resource Depletion, mineral, fossils and renewables	1.00	0.53	0.29	0.09	0.04	0.96	0.04	1.00
Total Freshwater Consumption (including rainwater)	1.00	0.51	0.26	0.06	0.02	0.99	0.01	1.00
Water resources	1.00	0.51	0.27	0.07	0.03	0.98	0.02	1.00

APPENDIX K: GLOBAL 6-APA PRODUCTION DISTRIBUTION

The OCE dataset on the export of penicillin and derivatives were used to assume the proportion of 6-APA that countries annually produce (OEC; 2018). To sense-check whether export proportions of penicillin product can reflect production volumes of 6-APA in a country, documentations that were available for China were used. China was found to export, and therefore potentially produce, 43% of all penicillin and its derivatives. As calculated in Appendix C, global 6-APA production could amount to 103,000 tonnes/yr. and therefore, China may produce up to 44,300 tonnes by considering the percentage contribution calculated. Since China was quoted to have a capacity of producing > 100,000 tonnes of penicillin salts (PA International, 2017), the estimate for 6-APA was considered viable and in line with the weight ratio between 6-APA and beta-lactam antibiotics and the percentage of penicillin that are converted (Appendix C).

For the number of 6-APA facilities within China, Spain and India, the sources for their assumed quantities in Chapter 5 are as follows. Reports stated that there are at least seven pharmaceutical companies producing penicillin and its derivatives in China (Business Wire, 2008) and that plant throughputs have been quoted up to 7500 tonnes of product intermediates (NCPC, 2019) and over 10,000 tonnes of penicillin salts years per year (Xiaobo, 2013). Assuming seven 6-APA manufacturing plants in China, this meant that each plant would produce 6,300 tonnes per year. Similarly, it was noted from The Pharma Letter (1997) that there are six penicillin producers in India, and so this value was assumed the number of facilities in this country. Spain was assumed to have five manufacturing plants with the knowledge that Uquifa (Uquifa 2019), Reig Jofre (Reif Jofre, 2019), Antibiotios (ADL Biopharma, 2019) and Centrient (Centrient Pharmaceuticals, 2018) operate in the country. Uquifa holds three sites in Spain and 1 in Mexico (Uquifa 2019). Centrient was found to have a manufacturing plant in Mexico as well; this meant that assuming two facilities situated in this country was correct.