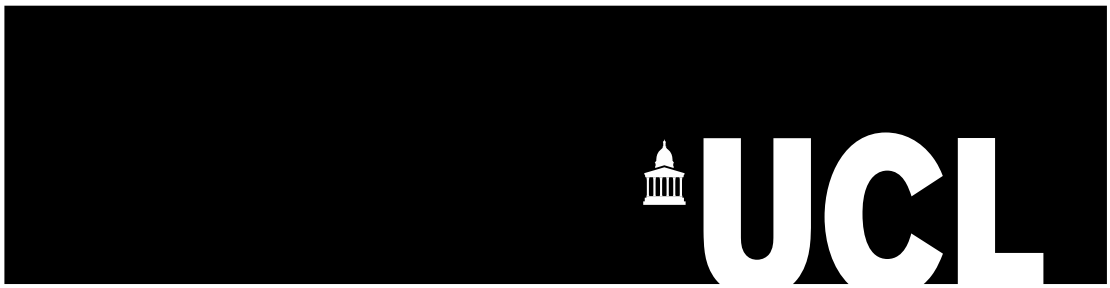


A Framework to Support Environmentally-Based Decision-Making in the Biopharmaceutical Industry

Sri Vaitheki Ramasamy, *MEng*

Department of Biochemical Engineering

A Thesis Submitted for the

Degree of Doctor of Philosophy

University College London

February 2018

To my dearest parents

Declaration

'I, Sri Vaitheki Ramasamy, 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.'

Abstract

The past decade has seen an increasing focus on the issues surrounding climate change and this has triggered governments internationally to develop environmental legislation and policies for the energy-intensive industries (EIIs) that can help reduce their anthropogenic greenhouse gases (GHGs) emissions. The biopharmaceutical industry is a relatively new EII. As the industry matures, the level of environmental scrutiny is increasing. Therefore, there is a need for the development of a framework specific to this industry to help guide the selection of manufacturing and disposal routes that reflect the potential environmental impact.

In this doctorate, a framework based on the life cycle assessment (LCA) tool was developed. The application of the framework for evaluating manufacturing and solid waste management alternatives is demonstrated via case studies that focus on production of therapeutic monoclonal antibodies using mammalian cell culture process at 200 L operational scale using either traditional or a hybrid based on a mix of traditional and disposable modes of production. The framework was employed to identify the process (whether traditional or hybrid) that contributes least to environmental impact, and also to identify the most suitable solid waste management method (landfill, incineration and pyrolysis). The life cycle inventory of the manufacturing processes, and the methodology used to obtain the inventory are presented. It is expected that this information will be beneficial for future studies in this area of research. The analysis also utilised sensitivity analysis studies to assess critically the uncertainties in the assumptions made in the case study. Finally, the application of the framework in evaluating the cumulative environmental impact, from manufacture in support of clinical stages up to production was assessed. Here, the focus was not only to evaluate the cumulative environmental impact, but also to explore the benefits of employing single-use technologies during clinical phase manufacture when developing a monoclonal antibody for therapeutic use.

The work in this thesis highlights the benefits of adopting a consistent engineering framework to guide process and technology selections in the biopharmaceutical industry by improving the overall quality of decision-making. This in turn will help the industry to predict and to control their environmental performance.

Acknowledgement

I would like to thank my primary supervisor, Professor Nigel Titchener-Hooker and my secondary supervisor, Professor Paola Lettieri for their continuous guidance and support throughout the course of my PhD. I would not have been able to complete this thesis without your encouragement.

I would also like to thank Dr Sara Evangelist (Dept. of Chemical Engineering, UCL), Dr Olga Parkes (DECC, UK) and Dr Sally Hassan for valuable discussions on the project.

I would like to express my deepest gratitude to my dear parents, Ramasamy and Kalaiarasi, sister Gayatri and brother Vijay who have encouraged and inspired me throughout my life. Without your love and infinite support, I would not have been able to come all this way.

Finally, I would like to thank all my friends and colleagues who made my time in UCL an unforgettable and enjoyable experience, particularly Aminat, Banjo, Carla, and Kate.

Table of Contents

List of Figures

List of Tables

Abbreviations

ALCA	Attributional Life Cycle Assessment
CH_4	Methane
CHO	Chinese Hamster Ovary
CIP	Cleaning-in-place
CLCA	Consequential Life Cycle Assessment
CO_2	Carbon Dioxide
DNA	Deoxyribonucleic acid
EAA	European Environmental Agency
EIA	Environmental Impact Assessment
EII	Energy-Intensive Industry
EMA	European Medicines Agency
EPA	Environmental Protection's Agency
EU	European Union
FDA	Food and Drug Administration
GHG	Greenhouse Gases
GWP	Global Warming Potential
HFCs	Hydrofluorocarbons
IET	International Emission Trading Mechanism
IPPC	Intergovernmental Panel on Climate Change
ISO	International Organisation for Standardisation
JI	Joint Implication
LCA	Life-Cycle Assessment
LCI	Life Cycle Inventory

LCIA	Life Cycle Impact Assessment
MFA	Material Flow Analysis
N_2O	Nitrous Oxide
OECD	Organisation for Economic Co-operation and Development
PFCs	Perfluorocarbons
PW	Pure Water
RA	Risk Assessment
SF_6	Sulphur Hexafluoride
SIP	Sterilising-in-place
TA	Technology Assessment
UN	United Nations
UNEP	United Nations Environmental Programme
UNFCCC	United Nations Framework Convention on Climate Change
WFI	Water for Injection
WCED	World Commission on Environmental and Development

Chapter 1. Project Vision

1.1 Project Vision-Introduction to the Project

Sustainable development has been carefully defined as one that meets present needs without compromising the ability of future generations to meet their own needs (WCED, 1987; Drexhage and Murphy, 2010). With the establishment of the sustainable development framework by the Brundtland Report and the Earth Summit, sustainable development has transitioned from being simply an idea to being a widely accepted and used concept by international institutions, governments, and society (Drexage and Murphy, 2010). Sustainable development comprises three key aspects; environment, social and economic aspects (Giddings *et al*., 2002).

It is claimed that achieving sustainable development would bring immense benefits to organisations, governments and society through better environmental, economic and social development (Drexage and Murphy, 2010). However, sustainable development will not be achieved if climate change is associated with negative development activities. Internationally, governments are focusing on developing legislation, policies and initiatives for organisations to adopt in order to address climate change issues. These are being developed especially for the energy-intensive industries (EIIs) because these are seen as one of the major contributors to greenhouse gases (GHGs) emissions. These industries include iron and steel, chemical, petroleum, automotive, and potentially the biotechnology industries. Collectively, they are responsible for 45% of all business and public sector GHG emissions (Bullock, 2009 and POST, 2012).

The introduction of legislation such as "The Climate Change Act 2008" by the UK government, and the introduction of environmental programmes such as the Responsible Care by the intergovernmental agencies require the Ells to reduce and manage their environmental impacts (POST, 2012). Therefore, the increasing concerns on sustainable development and climate change amongst the public, investors, and the international governments are driving the EIIs to implement low carbon technologies and green innovations for their manufacturing processes to reduce their carbon footprints and achieve sustainable development.

The biopharmaceutical industry is increasingly recognised as being an EII though historically it has been perceived as less energy intensive than others (Accenture, 2012). The industry focuses on healthcare and it is an important contributor to improved global health (Mehta, 2008). The main products of this industry include monoclonal antibodies, biologics drugs such as growth factors, hormones, fusion proteins, cytokines, blood factors and therapeutic enzymes, and vaccines (Aggarwal, 2010; Mehta, 2008). Worldwide sales of biologics in 2010 were estimated to be in excess of $100 billion, with over 200 biologics currently on the market (Walsh, 2010). As the number of biologics emerging from clinical development rises, manufacturers are now being prompted to find flexible, cost-efficient and environmentally feasible solutions for global scales of production.

The biopharmaceutical industry uses a range of manufacturing operations to achieve the exacting standards needed for therapeutic drugs, run in either a traditional mode where equipment is cleaned in-between batches or in single-use mode where no such cleaning is required (Sinclair *et al*., 2008; Farid *et al*., 2005). Although traditional

manufacturing processes employing stainless steel equipment are well-established, several issues can be identified including high levels of water consumption (mainly for CIP and SIP operations), long process times and high capital investments (attributed to stainless steel equipment manufacture and assembly) (Mirasol, 2008). These issues are driving the industry to seek alternative manufacturing routes. One such alternative is the adoption of single-use manufacturing processes. The single-use equipment can be disposed at the end of every production batch. The single-use equipment arrives pre-sterilised, operation consumes less water (CIP and SIP operations can be eliminated), reduces process time, and reduces the chances of process cross-contamination (Mirasol, 2008; Pierce and Shahbram, 2004; Rodrigues *et al*., 2009). However, several limitations associated with this type of manufacturing process have been identified and they include limited production scale, the generation of leachables and extractables from the single-use bags that could contaminate the product, and potential adverse effects to the environment due to the increased solid waste levels inherent in operation with single-use components (Sinclair *et al.*, 2008; Eibl *et al*., 2010a; Shukla and Gottschalk, 2012).

Presently, the most significant challenge faced by the decision makers in the biopharmaceutical industry is selecting between the available manufacturing technologies. The efficiency and benefits of these manufacturing alternatives have been studied and compared through process economics modelling using process economic decision-support tools (Farid *et al.*, 2005). Decision-support tools are beneficial for the industries coping with limited finances and pressing timelines, as they can aid capital investment decisions, cost-of-goods analysis, project management analysis and risk assessments, all which are functions of the mode of

manufacturing selected (Farid, 2007). The ability of these tools to evaluate the process economics of manufacturing alternatives has encouraged biopharmaceutical manufacturers to access them more widely during the decision-making process. There are a number of commercially available software tools that are being used extensively in the biopharmaceutical industry including Aspen Batch Plus (Aspen Technology Inc, Cambridge, MA, USA), SuperPro Designer (Intelligen Inc, Scotch Plains, NJ, USA), BioSolve Process Enterprise (Biopharm Services Limited, Amersham, UK) (Sinclair *et al*., 2008; Pietrzykowski *et al*., 2011; Farid, 2007).

Whilst there are many established tools to determine the process economics and efficiencies of alternative technologies and strategies, the environmental impact of these processes remain ambiguous, and despite some preliminary work, this has not been the subject of significant and rigorous engineering analysis (Ramasamy *et al*., 2015; Sinclair *et al*., 2008; Rawlings and Pora, 2009; Mauter, 2009). It is increasingly important for the biopharmaceutical industry to measure the environmental impacts produced by their manufacturing processes, because only by measuring the environmental impacts can the industry take the necessary steps to control and reduce them.

The relative and absolute environmental impacts produced by the traditional and single-use manufacturing processes are unclear. In principle, a single-use manufacturing process could produce higher levels of environmental impact than would a traditional manufacturing process due to the production of plastics (consumables) necessary for every manufacturing batch. The solid waste produced by the single-use manufacturing process will also be significantly higher than the

solid waste produced by the traditional manufacturing option due to the use and disposal of consumables after every manufacturing batch in the single-use manufacturing option. Conversely, it would be expected that the traditional manufacturing process could also produce higher environmental impacts than the single-use manufacturing alternative due to the large amount of water consumption, stainless steel equipment fabrication and disposal of stainless steel equipment at the end of the plant life (5-15 years). The solid waste produced from the traditional and single-use manufacturing processes are currently disposed as landfill (Guldager, 2009). However, landfill does not offer an environmentally favourable solution in the long-term due to drawbacks such as tax introduced by the UK Environmental Agency on landfill disposal methods, noise produced by vehicles on the landfill sites, and odor produced by the waste dumped at the landfill sites (Guldager, 2009; Rawlings and Pora, 2009). Other disposal options that can be considered by the biopharmaceutical industry include incineration and pyrolysis (Rawlings and Pora, 2009).

Presently, whilst the environmental impacts of the competing alternatives for biopharmaceuticals manufacturing remain unclear, it is certain that as the biopharmaceutical industry grows, so the potential environmental impacts of the industry will rise unless measures to control and reduce the environmental impact are instituted. In addition, the industry will be open to pressure from government, investors and society if it is perceived that inadequate efforts are made to reduce the environmental impact. There is hence, an urgent need for the development of an engineering framework that can be utilised by the industry to quantify and compare the environmental impacts produced by manufacturing alternatives. This is a primary

goal of the studies reported in this doctoral thesis.

The ability to determine the actual environmental impacts of each manufacturing alternative and the ability to compare these, lie in the use of life cycle assessment (LCA) tool. These abilities make LCA an ideal tool to be used in this doctoral project to be applied in the decision-support framework. Such a framework can be used to help in process selection and to identify the best waste disposal options (Beaver, 2000). This doctoral project will examine how such a decision-support framework can aid the biopharmaceutical industry to quantify the overall environmental impact and make decisions based on facts rather than depending on "rule of thumb" methods (Baumann and Tillman, 2004).

Within this context, the key technical questions that the studies in this doctoral project addresses include:

1. The environmental burden savings that could be achieved by the industry were there a shift from the established traditional technology to single use technologies for the manufacture of biopharmaceuticals.
2. Is it possible to identify the best solid waste management practice for the biopharmaceutical industry, and if so what is it?
3. How robust are the assumptions made to develop the decision-support framework and the influence of key process parameters on the overall estimated environmental impacts produced?
4. What are the cumulative environmental impacts of monoclonal antibodies manufacture from development phase up to production scale?

5. And finally, are the biopharmaceutical manufacturing processes energy-intensive?

These questions will be addressed in turn within Chapters 5, 6 and 7 of the thesis where the results of the analyses are discussed.

1.2 Aims and Organisation of Thesis

The aim of this doctoral project was to investigate the possibility of capturing the environmental perspective of biopharmaceutical processes using a consistent framework. This can facilitate more informed decision-making when evaluating manufacturing and solid waste management alternatives. A generic process for the production of monoclonal antibodies, based on CHO cell culture, is used for the development and application of the framework. In order to achieve this goal, a set of objectives has been identified which form the basis of each of the following chapters.

Chapter 2 presents an introduction to the background of the research. A general introduction to sustainable development, climate change and GHGs, and sustainable manufacturing practices within the major EIIs is provided. The remainder of Chapter 2 focuses on the biopharmaceutical industry and its best-selling product, monoclonal antibodies. The technologies used to manufacture monoclonal antibodies are highlighted in this chapter. The analysis also highlights that the environmental aspect of manufacturing processes should be considered before selecting a manufacturing process.

In Chapter 3, a decision-support framework based on the LCA tool is presented to facilitate the environmental impacts evaluation of biopharmaceutical processes. A systematic approach for the development of the framework and the application of the LCA tool in this framework are provided. A hypothetical case study is set up to assess the environmental impacts of manufacturing and solid waste management alternatives at 200 L scale of operation. The case study was established to demonstrate the functionality of the framework developed.

Chapter 4 presents the life cycle inventory of the environmental analysis carried out in this research. In this chapter, the phases and activities evaluated by the framework in the hypothetical case study (described in Chapter 3) to evaluate the environmental impacts of the manufacturing and solid waste disposal alternatives are described, and the life cycle inventory of the activities evaluated by the framework in the case study are provided. The methods employed to obtain the life cycle inventory are also presented. Finally, the assumptions made to obtain the necessary life cycle inventory are listed. The results of the case study are presented in Chapter 5.

The results of a hypothetical case study are presented in Chapter 5. An assessment of the suitable manufacturing process based on the environmental performance is provided in this chapter. The analysis is taken a step further in this chapter by carrying out a solid waste management analysis at different scales of operation to identify the best possible solid waste management approach for the biopharmaceutical industry.

Chapter 6 presents sensitivity analysis studies to assess critically the parameters/assumptions made to carry out the environmental assessments. This chapter's main aim was to test the robustness of the assumptions made and evaluate the influence of the parameters/assumptions on the overall environmental impacts produced. The information generated from this analysis was used to help determine the critical parameters of this analysis.

The final analysis using the framework developed in this research is presented in Chapter 7. In this chapter, a hypothetical case study was developed to evaluate the cumulative environmental impacts of monoclonal antibodies manufacture over a period of time. The cumulative environmental impacts of monoclonal antibodies manufacture in the clinical development phase and the benefits of applying single-use technologies in this phase are highlighted. The chapter also provides a comparison of environmental impacts produced per annum between the clinical development phase and the production stage. Finally, this chapter provides an example of how process intensification of biopharmaceutical processes are useful to reduce the difference in environmental impacts between the clinical development phase and the production stage.

Chapter 8 summarises the main contribution of this work and presents suggestion for future work. Finally, the list of papers by the author, published through the course of this work, are provided in Appendix 1.

Chapter 2. Background to Research

2.1 Introduction

This chapter describes the background against which this doctoral project is set. Sustainable development is a concept that is gaining wide recognition by governments, organisations, and consumers worldwide due to its ability to lead to better environmental, economic, and social development (Drexage and Murphy, 2010). However, the increasing concentration of GHGs in the atmosphere is widely believed to be causing increments in the earth's temperature, and it is unlikely that sustainable development can be achieved without significant interventions taking place to reduce these GHGs.

The EIIs are believed to be one of the significant contributors to GHG emissions. These industries are facing increasing pressure from governments worldwide to reduce their emissions. Due to that, EIIs are adopting sustainable manufacturing practices to reduce their environmental contributions. The biopharmaceutical industry is a relatively new EII and the environmental burdens of their manufacturing processes remain unclear. Therefore, there is a strong need for environmental studies in this industry and this provided the impetus for this research. The process to manufacture monoclonal antibodies will be used to explore the environmental impacts of biopharmaceutical processes.

In section 2.2, an introduction to the sustainable development concept and climate change is provided. Section 2.3 focuses on sustainable manufacturing within the

EIIs. In this section, the initiatives taken by some of the EIIs are also presented. In section 2.4, a general introduction to the biopharmaceutical industry and monoclonal antibodies is provided. This section highlights one of the main issues faced by the industry, that is deciding between traditional and single-use manufacturing processes. The future trends in biopharmaceutical manufacturing are provided in this section. Finally, a summary of the chapter is provided in section 2.5.

2.2 Sustainable Development and Climate Change

This section describes in detail the relationship between sustainable development, GHGs, climate change, and the measures that have been taken by governmental agencies to combat the climate change issue.

2.2.1 Introduction to sustainable development

Sustainable development is a concept that has been carefully defined as a development that meets the present without compromising the ability of future generations to meet their needs (WCED, 1987; Drexhage and Murphy, 2010). This definition originated from a publication released in 1987 known as "**Our Common Future"** or the Brundtland Report for the World Commission on Environment and Development (WCED). Although other definitions exist for sustainable development, this one is the most widely used (Zeijil-Rozema *et al.,* 2008).

Sustainable development focuses on three key aspects; economic, environmental, and social aspects (Figure 2.1) (Giddings *et al*., 2002). The economic aspect emphasises the ability to achieve a defined level of economic growth with low environmental burden (EEA and Norwegian Financial Mechanism, 2006). The

environmental aspect emphasises satisfying the present needs while reducing the burden of human activity without harming the environment (EEA and Norwegian Financial Mechanism, 2006). The social aspect places emphasis on a comfortable life for human beings with a balance between health, wealth and safety (EEA and Norwegian Financial Mechanism, 2006). To achieve sustainability, all three aspects should be given importance.

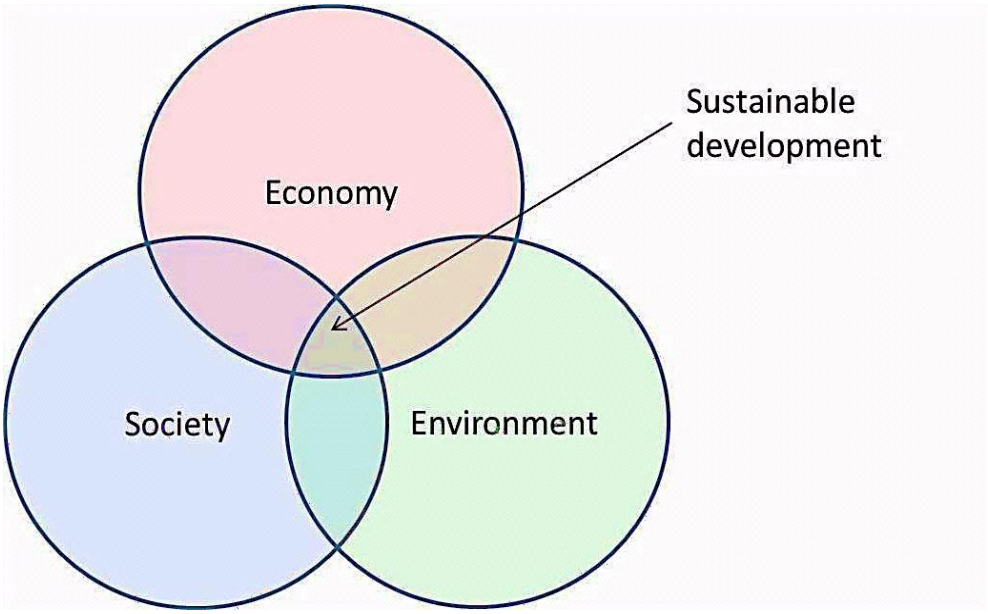


Figure 2.1 *Three important aspects of sustainable development. Adapted from OECD, 1998.*

The initiative for sustainable development started with the United Nation's Conference on the Human Environment and Development (UNCED), held in Stockholm in 1972 (Drexhage and Murphy, 2010). This was followed by the assembly of the WCED at the request of the UN in 1983, chaired by former Norwegian Prime Minister Gro Harlem Brundtland. The Brundtland Report was published in 1987 by WCED, and was prepared by a team consisting of ministers, civil servants, and environmental experts from all parts of the world (WCED, 1987).

The main aim of the report was to educate the public of the importance of making progress towards sustaining economic development without damaging the environment (Shepard and Donlon, 2007).

The importance of the Brundtland Report is that it established the foundations for the Earth Summit, which took place in Rio de Janeiro, Brazil (Shepard and Donlon, 2007). Among the summit's outcomes were the establishment of the UN Framework Convention on Climate Change (UNFCCC), the Convention on Biological Diversity (CBD), and the non-legally binding Statement of Forest Principles by UNCED, under the administration of UNEP (Drexhage and Murphy, 2010; OECD, 1998). The framework for sustainable development was established with the Brundtland Report and the Earth Summit. Since then, sustainable development has transitioned from being an idea to a widely accepted concept adopted by international institutions, governments, and society to improve the overall health of their organisations through better economic, social and environmental development (Drexage and Murphy, 2010).

2.2.2 Importance of achieving sustainable development

Sustainable development should be recognised as a route towards better global technological, economic and social development. Unsustainable development arises when any one of the three aspects of sustainable development is ignored. Unsustainable development is not desirable as it would affect the world through depletion of natural resources, food crisis, and increased prevalence of life-threatening diseases (UNEP, 2009). Moreover, the actions taken today affect the current generation, but also the generations to come. According to an ancient Native

American proverb, "*We did not inherit the world from our ancestors, we borrowed it from our children*". This proverb clearly stresses the importance of leaving the world in good shape for the future generations to meet their own needs.

2.2.3 Sustainable development and climate change

Sustainable development is not achievable as long as climate change issues persist (Cohen *et al*., 2008). The fourth report by the Intergovernmental Panel on Climate Change (IPCC) in 2007 identified the inter-relationship between sustainable development and climate change (Drexage and Murphy, 2010). The report also stated the policies needed to achieve sustainable development and to solve climate change issues are mutually reinforcing (Drexage and Murphy, 2010; IPPC, 2007).

The relationship between sustainable development and climate change is known to be close because addressing climate change issues requires abatements and adaptations, which are linked to sustainable development (Drexage and Murphy, 2010). Abatement measures to limit climate change can help sustain the ecosystem and improve the quality of life for society, thus improving overall social and economic development (IPPC, 2007). Sustainable development pathways on the other hand have the potential to reduce GHG emissions, and hence, lessen the impact of climate change (IPPC, 2007). Therefore, to achieve sustainable development, climate change and its impact on the environment, society and economic development should all be recognised.

2.2.4 Reality of climate change

2.2.4.1 Greenhouse gases (GHGs)

GHGs are gases that trap heat in the atmosphere. This phenomenon is known as the greenhouse effect. There are six different GHGs: Carbon Dioxide ($C0_2$), Methane (CH_4), Nitrous Oxide (N_2O), Hydrofluorocarbons (HFCs), Perfluorocarbons (PFCs), Ozone and Sulphur Hexafluoride (SF_6) (UNFCC, 2008). GHGs such as CO_2 and CH_4 are either emitted naturally or through human activities, and GHGs such as fluorocarbons, ozone and sulphur hexafluoride are emitted solely through human-related activities (Reilly *et al.*, 2003). CO_2 is the biggest contributor and CH_4 is the second biggest contributor to the overall anthropogenic GHG emissions (Pandey *et al.*, 2010).

The concentration of GHGs in the atmosphere has increased significantly over the past years. The worldwide rate of emission of GHGs increased by 26% from 1990 to 2005, with CO_2 accounting for most of the increase (EPA, 2010). The rate of increase of CO_2 concentration in the atmosphere is believed to be 1.9ppm/year (EPA, 2010). Due to the increasing concentration of these gases in the atmosphere, scientist and politicians from different parts of world are now raising concern over the ensuing dangers of climate change.

2.2.4.2 Climate change

The first identification of the role of gases in trapping heat close to the earth was made in 1827 by Jean-Baptiste Fourier, a French scientist (Mcginess, 2001). However, it was Svante Arrhenius, a Swedish scientist who formally stated that CO_2 emissions from the combustion of fossil fuels could lead to the greenhouse effect,

contributing to climate change and global warming events (McKibben and Wilcoxen, 2002). Upon the identification of the CO_2's role in the greenhouse effect, G.S Callendar, a British scientist, drew two conclusions; that the planet's temperature is rising and that there has been a 10% increase of CO_2 levels in the atmosphere from 1850-1940 (Mcginess, 2001).

Climate change is a phenomenon in which significant changes to the climate (temperature, wind and precipitation) occur over a long period of time (EPA, 2010). Temperature is just one aspect to climate change but it is also a significant one. Climate change occurs when GHGs trap heat close to the earth causing a change in the earth's temperature. When the energy from the sun passes through the atmosphere, some of the energy is reflected back, and the rest is absorbed by GHGs (McKibbin and Wilcoxen, 2002). Whilst GHGs are important to sustain the ecosystem, creating more of these gases in the atmosphere will trap more heat, eventually leading to an increase in the earth's temperature.

GHGs have different heat absorption potentials (or global warming potential (GWP)). GWP is a measure of the relative radioactive effect of a given gas compared to CO_2 over a period of time, and the GWP potential of GHGs depend on their radiative forcing and the period of time the GHGs stays in the atmosphere (Pandey *et al*., 2010; Lashof and Ahuja, 1990). Some GHGs have a higher GWP than the others, for an example, CH_4 has a 3.7 times higher GWP potential than that of CO_2 per mole (Lashof and Ahuja, 1990). The climate of the earth is based on the GWP of each GHG (Oberthur and Ott, 1999). The GWP can be calculated and the unit of GWP is CO_2 equivalent (CO2-Eq) (Pandey *et al.*, 2010).

Climate change is a phenomenon primarily caused by human activities. According to Ban Ki-Moon, Secretary-General to the United-Nations (UN), climate change is considered as the most defining challenge faced by the world population today, and he also made climate change as one of the three priorities for the United Nations (UNEP, 2009). Therefore, there is a strong need for governments internationally to take action to combat climate change issues.

2.2.5 A first step towards combating climate change issue: Kyoto Protocol

The Kyoto protocol was introduced by the United Nations Framework Convention (UNFCC) on Climate Change in 1997 and came into force in 2005 (UNFCC, 2008). This protocol was introduced as a first step to stabilise the GHGs concentrations in the atmosphere (UNFCC, 2008). This protocol was negotiated in Kyoto, Japan and it is a legally binding agreement involving the highly industrialised countries (Wigley, 1998).

The main aim of the Kyoto Protocol was to reduce the emission of GHGs of industrialised countries and to stabilise the concentration of GHGs in the atmosphere at a level that would avoid harmful interference with the climate system (Wigley, 1998; Roger *et al*., 2007). The Kyoto Protocol's primary focus was on reducing fossil fuel emissions, which have been identified as the main source of CO_2 (Schulze *et al*., 2002).

The Kyoto Protocol was the first international protocol that aimed to address the issues of climate change. However, the Kyoto Protocol failed to achieve its targets, mainly due to the refusal of the United States and Canada to ratify the protocol

(Feldon, 2007). According to the United States, the protocol is fundamentally flawed and ratifying the protocol would further burden their economy (Mcginess, 2001). The United States further added that the exclusion of India and China from the protocol clearly showed the protocol was flawed as India and China may be responsible for the greatest level of GHG emissions in the future (Feldon, 2007). Canada too agreed with America and added that following the Kyoto Protocol would not be economically feasible for them. With the expiry of the Kyoto Protocol in 2012, there is an urgent need for a further international agreement on climate change to be established.

2.2.6 Other agreements on climate change

A number of climate change conferences have been organised by the UN to develop a new international climate change protocol. The UN is currently negotiating for a global climate change agreement. The negotiations for a new climate change agreement started with the Durban Climate Change Conference and continued with the Warsaw Climate Change Conference (2013), where a strong message was provided to all countries to start preparing their contributions to reducing or limiting emissions in order to comply with a new climate change agreement that will be adopted in 2015 (European Commission, 2014). The Lima Climate Change Conference (2014) continued the negotiations for the new climate change agreement.

A first universal legally binding global climate deal was adopted in 2015 at the Paris Climate Change Conference, involving 165 countries. The agreement sets out a global action plan to avoid dangerous climate change by limiting global warming to well below 2°C (European Commission, 2014). The Paris Agreement entered into

force on 4 November 2016, and it is an agreement with UNFCCC dealing with greenhouse gas emissions mitigation, adaptation and finance starting in year 2020. The aims of the Paris Agreement are to strengthen the global response to the threats of climate change and enhance the ability of countries to deal with the impacts of climate change (UNFCCC, 2016).

As discussed above, sustainable development can only be achieved when climate change issues are solved. The power to reduce GHG emissions lies principally with government whose policies can encourage business investments in low carbon technologies. In response to such policies, a number of EIIs are now focusing on sustainable manufacturing by employing technologies and manufacturing routes that will produce lower environmental impacts. The next section provides an introduction to sustainable manufacturing within the EIIs, and discusses the measures taken by a large majority of EIIs to reduce their environmental impacts and process waste in order to achieve industrial sustainability.

2.3 Sustainable Manufacturing in the Energy-Intensive Industries

This section provides an introduction to sustainable manufacturing and describes the measures taken by the EIIs to adopt this practice in their production.

2.3.1 Sustainable manufacturing

The concept "sustainable manufacturing" was defined by the US Department of Commerce as the creation of manufactured products that use processes that reduce negative environmental impacts, conserve energy, and natural resources, are safe for employees, communities, and consumers and are economically viable (Jayal *et al.*,

2010). The sustainable manufacturing concept focuses on products or processes that use less energy and materials, and produce less waste (Jayal *et al.*, 2010). A manufacturing process targeted at sustainability does not focus solely on environmental benefits, but also on economic and social objectives (Zhang *et al.*, 2013).

As stated above, the EIIs are seen as one of the major contributors to GHG emissions. The EIIs include the steel and iron, petroleum, chemical and the automotive industry. Collectively, they are responsible for 45% of all business and public sector GHG emissions (Bullock, 2009; POST, 2012). With increasing environmental legislation, policies and governmental initiatives associated with climate change, the EIIs are driven to adopt sustainable manufacturing practices (Post, 2012). Pressure from governmental bodies is not the only factor generating this interest. Other reasons include the need to achieve process waste reduction, resource reduction and achieving customer satisfaction. For example, in a short study carried out in 2008, out of 71% of consumers who were reducing the carbon footprint, 33% of them were doing so to improve the environment, 24% were doing so to cut costs, and the rest were motivated to cut costs whilst helping the environment. Such a study shows consumers today have high levels of environmental awareness. Together these play crucial roles in driving the EIIs to manufacture their product sustainably (Morrow and Rondinelli, 2002; Lash and Wellington, 2007; Miettinen and Hamalainen, 1997; William *et al.*, n.d).

It is clear that there are real incentives for the EIIs to consider sustainable manufacturing. By adopting sustainable manufacturing practices, not only can the

industry improve their environmental performance, but also achieve significant cost-savings. The following sections highlight the measures taken by the chemical, pharmaceutical and automotive industries to manufacture sustainably.

2.3.2 Sustainable manufacturing practices within the chemical industry

The chemical industry is a large EII, and contributes towards improving the quality of life in areas such as health, agriculture, construction and transport (ICCA, 2009). The products of the chemical industry are used extensively and globally. The chemical industry was the first industry to adopt sustainable manufacturing practices to reduce the GHG emissions using environmental tools (ICCA, 2012). In 2005, McKinsey & Company commissioned carbon footprint evaluation studies to determine emissions attributed to the chemical industry, from extraction feedstock and fuel, through production, to disposal (ICCA, 2012). The study not only identified the carbon footprint, which amounted to 3.3 Gigatonnes CO_2-eq, but also identified that the major source of carbon produced were a result of the production of chemicals from feedstock delivered to the plant (ICCA, 2009). This drove the chemical industry to take immediate measures such as setting environmental targets in an effort to reduce their carbon footprint (Jenck *et al.*, 2004).

Several initiatives have been established to monitor the environmental performance of the chemical industry. Responsible Care, European Responsible Care Security Code, REACH (The Registration, Evaluation and Authorization and Restriction of Chemicals Regulation) legislation, and SUSTECH (Collaborative Research and Development in Sustainable Technologies for the Process Industries) are among the

initiatives taken by the industry to help reduce GHG emissions and achieve industrial sustainability (Jenck *et al*., 2004).

2.3.3 Sustainable manufacturing practices within the pharmaceutical industry

The pharmaceutical industry manufactures small molecules or synthetic organic molecules for therapeutic applications. Small molecules are manufactured via synthetic chemistry processes or naturally occurring compounds that have been isolated or re-synthesized in the laboratory (Mehta, 2008). The pharmaceutical industry is a part of the wider chemical industry, however for this thesis, the pharmaceutical industry is reviewed separately. The pharmaceutical industry is faced with major environmental problems because of the nature of synthetic methodologies, which require high number of synthesis steps to manufacture many small molecules (Dunn *et al*., 2010). Some small molecule manufacture requires large amounts of solvent use and the waste attributed to solvent use by the pharmaceutical industry alone was reported to be 88 million kilograms in 2008 (Raymond *et al*., 2010). The high number of synthesis steps in small molecules manufacture contributes to higher energy usage, higher raw material usage, increased waste and increased carbon dioxide emissions, and thus a higher carbon footprint than the biopharmaceutical industry (Woodley, 2008).

Many environmental impact studies performed by the pharmaceutical industry focus on the use of solvents; the contributions of the solvents to the environment have been studied (Raymond *et al*., 2010). The environmental studies in the pharmaceutical industry were used in process selection, understanding and development. For example, Pfizer carried out environmental impact studies on the synthesis of

Sertraline (antidepressant) using an environmental tool to determine the best synthesis process, and Novozymes used an environmental tool to understand better the enzyme production from an environmental perspective (Nielsen *et al*., 2006). The synthesis of small molecules requires large amounts of raw materials and energy usage, however by employing environmental studies, efficient synthesis routes (with fewer synthesis steps) for small molecule manufacturing can be determined, essentially reducing the environmental impact produced by the manufacturing processes.

2.3.4 Sustainable manufacturing practices within the automotive industry

The automotive industry design, develops and manufactures motor vehicles. Due to increasing legislation and consumers pressure to reduce the environmental impacts of manufacturing, the automotive industry has conducted several in-depth environmental studies (Bonollo *et al*., n.d). The in-depth studies incorporated carbon footprint evaluation studies using environmental tools.

The automotive industry has been taking great initiatives to reduce the environmental impact of their production processes. One such initiative was developing cars that avoid or reduce the use of gasoline fuel such as electric cars (Yuan and Dornfeld, 2009). Electric cars, or green cars, are manufactured in sustainable ways using recycled materials and do not require the use of gasoline, instead they use rechargeable batteries (Anon, 2012a). Electric cars are claimed to be of zero carbon emissions however this claim is only valid for the use-phase and not for the entire life cycle of the car. Hybrid cars on other hand are cars that operate on gasoline and re-chargeable batteries and they have lower carbon emission than the cars that

operate on gasoline only (Anon, 2012b). They represent a halfway having between fully electric and fully petrol driven cars.

The automotive industry worldwide is facing tremendous pressure (Freshfields Bruckhaus Deringer, 2010; KPMG, 2010). For example, the European Union required manufacturers to reduce average CO_2 emissions from 159g/km to 120g/km by 2012 (KPMG, 2010). The European Union has also produced a directive which defines new standards for end-of-life disposal of vehicles (Bonollo *et al.*, n.d). The pressure from individual governments is driving the automotive industry to adopt green manufacturing practices in an attempt to achieve industrial sustainability.

Previously, the efforts taken by the chemical, pharmaceutical and automotive industries to manufacture sustainably were highlighted. The biopharmaceutical industry is a relatively new industry. This industry should be recognised as an EII as their manufacturing processes contribute to significant environmental impacts. The industry's best-selling products, monoclonal antibodies, employ high levels of energy and water during production. As the number of monoclonal antibodies emerging from clinical development rises, manufacturers are now being prompted to find flexible, cost-efficient and environmentally feasible solutions for global scales of production. In an attempt to achieve this, manufacturers are striving to implement new manufacturing technologies and strategies. The environmental benefits of these technologies and strategies are not yet easily ascertained, therefore studies evaluating the environmental impacts of manufacturing alternatives will be valuable. In this research, the environmental impacts of a platform monoclonal antibody manufacturing process will be examined.

2.4 General Introduction to the Biopharmaceutical Industry and Monoclonal Antibodies

This section initially provides an introduction to the biopharmaceutical industry. An overview of the industry and drug development process is also given. The section then presents an investigation of the production methods employed to manufacture monoclonal antibodies. A general introduction to antibodies, monoclonal antibodies and manufacturing processes is provided. The key manufacturing issues faced by the industry is also highlighted in this section.

2.4.1 Overview of the biopharmaceutical industry

The biopharmaceutical industry employs biological processes to create useful commercial drugs most often through the genetic manipulation of living organisms. Biologics are manufactured in living systems such as microorganisms, plant or animal cells (Puppo *et al*., 2005 and Mehta, 2008). Since the discovery of recombinant DNA and the monoclonal antibody technologies in the 1970s, there has been tremendous interest in the area of biopharmaceuticals (Pharmahorizons, 2001).

The biopharmaceutical industry is a relatively new industry. The industry is characterised by a high growth rate and strong pipeline of drugs (Mehta, 2008). The worldwide sales of biologics in 2010 were estimated to be in excess of $100 billion, with over 200 biologics currently on the market (Walsh, 2010). As more biologic drugs emerge from clinical development, manufacturers are prompted to find solutions for flexible, cost-efficient and environmentally feasible bio-manufacturing.

2.4.2 Products of the biopharmaceutical industry

The three categories of biologics include antibodies (with monoclonal antibodies being the most established class of antibody), biologics drugs such as growth factors, hormones, fusion proteins, cytokines, blood factors and therapeutic enzymes and vaccines (Aggarwal, 2010; European Commission, 2009).

Monoclonal antibodies are highly specific proteins that are made from a single clone of B-lymphocytes, which recognises a "foreign molecule" or antigen at an antigenic site (Walsh, 2004). Biologic drugs are large-molecular proteins and manufactured using cells, blood or blood components or within other living organisms (Mehta, 2008). Vaccines are typically manufactured in chicken eggs or engineered cell lines (Patriarca, 2007).

Amongst biologics, monoclonal antibodies have the highest annual compound growth rate; an annual compound growth rate of 30% (Zhou and Kantardjieff, 2014). Other biologics such as vaccines and growth hormones are predicted to have an annual average growth rate of 15% (Zhou and Kantardjieff, 2014). This indicates that the monoclonal antibodies have a huge market demand and commercial potential. The high popularity of monoclonal antibodies make them ideal candidates to be explored in this doctoral project.

2.4.3 Drug development in the biopharmaceutical industry

The development of a biopharmaceutical is a lengthy and costly process. The total development time of a biopharmaceutical can range from five to twelve years, with the estimated cost ranging from $0.8-$2 billion (Collier, 2009). The manufacture of

biopharmaceuticals is also heavily regulated and controlled (Walsh, 2004). In order to obtain a market approval, a biopharmaceutical must fulfill its safety, quality and efficacy aspects.

A biopharmaceutical has to undergo a number of stages before reaching the market. Once a molecule has been identified, it is subjected to pre-clinical (studies using animal subjects) and clinical studies (studies using human subjects) before gaining market approval (ABPI, 2007). The clinical studies stage can be categorised into Phase I, Phase II and Phase III. The stage itself can take up to 10 years (Dimasi and Grabowski, 2007). A summary of the features of Phase I-III clinical studies is provided in Table 2.1. The data produced by the pre-clinical and clinical stages of a drug will be analysed by government appointed regulatory agencies such as the Food and Drug Administration (FDA) in the United States and European Medicines Agency (EMA) in the United Kingdom or Europe before gaining marketing approval.

Table 2.1 *The features of clinical study phases for a biopharmaceutical drug (ABPI, 2007).*

	Phase I	**Phase II**	**Phase III**
Timeline (Year)	0.5-2	1-3	1-5
Number of Subjects	50-100	100-400	1000-5000
Type of Subjects	Healthy volunteers	Patient volunteers	Patient volunteers
Purpose of the Study	Safety and dosage study	Dosage and effectiveness study	Studies to confirm effectiveness

2.4.4 General introduction to antibodies

Antibodies are protein molecules or serum Immunoglobulins (Igs) secreted by B-lymphocytes (B-cells). Antibodies have a general "Y" shaped structure comprising of four polypeptides. The four polypeptides consist of 2 heavy chains (-55 KD) and 2 light chains (-25 KD), held together by disulphide and non-covalent bonds (Lipman *et al*., 2005). The light chains have 2 domains; a variable region domain (V_L) and a constant region domain (C_L). Each heavy chain contains 4 domains; a variable region domain (V_H) and 3 constant region domains (C_H^1, C_H^2, and C_H^3). Both the variable region domains (V_Ls and V_Hs) have complementarity determining regions (CDRs), which form the antigen-binding site (Lipman *et al*., 2005). A schematic representation of an antibody molecule is given in Figure 2.2.

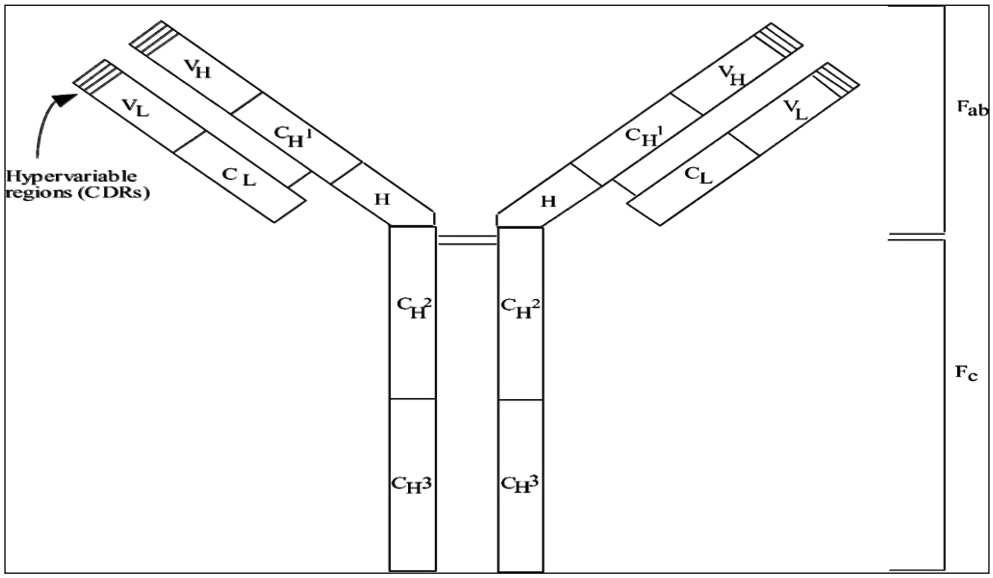


Figure 2.2 *A general structure of an antibody molecule. Adapted from Walsh, 2004. An antibody molecule consists of two identical heavy chains and two identical light chains, held together by disulphide and non-covalent bonds. The heavy (H) chains have a variable (V) domain and three constant (C) domains. The light chains have a variable (V) domain and a constant (C) domain. The hypervariable regions (complementarity-determining regions) are shown in the diagram.*

2.4.5 Monoclonal antibodies

More than ten decades ago, Paul Ehrlich, the founder of chemotherapy, received a Nobel Prize for his hypothesis on "*the use of monoclonal antibodies as magic bullets for therapeutic applications*" which inspired many scientists to conduct research in an attempt to actualise the hypothesis (Strebhardt and Ullrich, 2008). The hypothesis became a reality in 1975 through the development of the hybridoma technology by Kohler and Milstein (Adams and Weiner, 2005). The hybridoma technology is a technique to create hybridoma cells by fusion of cancerous immune cells known as myeloma tumour cells with antibody-producing cells taken from rodents (Pandey, 2010). The hybridoma technology allowed the production of monoclonal antibodies specific to the antigen of choice.

However, the monoclonal antibodies produced from the hybridoma technology suffer a major drawback. The first generation monoclonal antibodies (monoclonal antibodies that are produced through hybridoma technology) triggered immunogenic responses when administered to humans due to their murine origin (Walsh, 2004). This led to the development of second generation monoclonal antibodies; antibodies produced using genetic engineering which do not trigger immunogenic responses when administered into humans. These antibodies can be classified as "chimeric" and "humanised" monoclonal antibodies, and they are ideally suited for therapeutic applications (Walsh, 2004). The examples of first and second generation monoclonal antibodies are provided in Table 2.2.

As discussed above, monoclonal antibodies have huge potential applications in therapeutics and diagnostics. All therapeutic monoclonal antibodies belong to the

IgG class (Shukla *et al*., 2007). The therapeutic applications of monoclonal antibodies include inhibition of allo-immune and auto-immune reactivity, antitumor therapy, antiplatelet therapy, and antiviral therapy (Bredveld, 2000). The monoclonal antibodies for anti-tumour therapy (cancer) and the monoclonal antibodies for allo or auto-immune reactivity therapy (inflammatory diseases) are the best sellers (Aggarwal, 2010).

Table 2.2 *Therapeutic monoclonal antibodies produced using hybridoma and genetic engineering technologies (Walsh, 2004).*

Product	Manufacturer	Technology	Year Approved
Orthoclone OKT3	Ortho Biotech	Hybridoma	1986
Zevalin	Biogen Idec	Hybridoma	2002
Bexxar	GlaxoSmithKline	Hybridoma	2003
Rituxan	Biogen Idec	Genetic Engineering	1997
Synagic	MedImmune	Genetic Engineering	1998
Herceptin	Genentech/Roche	Genetic Engineering	1998

The top five best-selling monoclonal antibodies in 2015 (Table 2.3) included Humira (AbbVie) for the treatment of cancer, Rituxan (Roche) for the treatment of inflammatory diseases, Avastin (Roche) for the treatment of cancer, Herceptin (Roche) for the treatment of cancer and Remicade (Janssen) for the treatment of inflammatory diseases (Gameiro, 2016).

Table 2.3 *The best-selling therapeutic monoclonal antibodies in 2015 (Aggarwal, 2010; Gameiro, 2016).*

Product	Manufacturer	2010 Sales ($) B	Year Approved
Humira	AbbVie	14.0	2002
Rituxan	Roche	7.3	1997
Avastin	Roche	7.0	2006
Herceptin	Roche	6.8	1998
Remicade	Janssen	6.6	1998

As highlighted previously, therapeutic monoclonal antibodies are used diversely to treat chronic diseases including cancer and autoimmune diseases. The ability of this class of drug to treat such diseases increased the demand for this drug.

2.4.6 The significance of monoclonal antibodies

In recent years, the monoclonal antibody market has been the fastest growing within the biologics sector, enjoying high levels of market success (Rodrigues *et al.*, 2009). Monoclonal antibodies emerged as the best-selling biologics, with the sales of monoclonal antibodies representing 40% of the total biologics market (Stephenson, 2013; Aggarwal, 2010). In 2009, the total sales of monoclonal antibodies reached $75 Billion (Ecker *et al*., 2015). With 44 monoclonal antibodies currently on the market and many monoclonal antibodies in clinical trials, billions of dollars of revenues could be generated in the coming years, making monoclonal antibodies an attractive, and the single most important biologic to both manufacturers and investors (Liu, 2014; Ecker *et al*., 2015).

2.4.7 The monoclonal antibody production process

Monoclonal antibody production technologies have advanced significantly over the past decades. Most of the monoclonal antibodies in the market today are produced using recombinant DNA technology using mammalian cells expression systems (Birch and Racher, 2006). Mammalian cells expression system cultures are the predominant method for producing monoclonal antibodies due to advantages over other expression systems such as *E.coli* or yeast cells (Farid, 2007). The key advantage being that only mammalian cells can reliably perform all the post-translational modifications required for full antibody activity (Zhang, 2010). The current expression titre for monoclonal antibodies is 3-5 g/L although 10 g/L has been reported to be achieved using a Chinese Hamster Ovary (CHO) expression system (Kelley, 2007). The trend is toward higher titres.

The production capacities of monoclonal antibodies have doubled over these years (Kelley, 2007). In the 1990s, the largest volume of fed-batch bioreactor scale was 10,000 L. Presently, the production capacity of fed-batch monoclonal antibodies can go up to 200,000 L achieved with multiple bioreactors of 25,000 L (Kelley, 2009; Farid, 2007). The most common type of bioreactor used for batch/fed batch culture systems are stirred tank reactors though over recent years, there has been significant interest in the use of single use technologies in bio-manufacturing, especially single-use bioreactors (this will be further discussed in Section 2.4.8). Amongst the single-use bioreactors that are currently available on the market are XDR-Disposable Stirred Tank Bioreactor (GE Healthcare, Uppsala, Sweden), WAVE Bioreactor (GE Healthcare, Uppsala, Sweden) and BIOSTAT CultiBag STR (Sartorius Stedim,

Melsungen, Germany) (Eibl *et al*., 2010b). However, the single-use bioreactors have limited scales; the highest volume currently available is 2000 L (Eibl *et al*., 2010b).

The platform monoclonal antibody production process based on a mammalian cell expression system can be separated into three defined phases: upstream, downstream and formulation/fill/finish (Figure 2.3). The upstream phase consists of a cell culture stage where the cells expressing the product of interest are grown inside a fermenter typically operating in fed-batch mode. The downstream phase most often consists of harvest and clarification steps, a number of chromatography steps, two virus removal steps, an ultrafiltration/diafiltration step and finally a sterile filtration step. The final formulation/fill/finish phase consists of a number of steps to prepare the product for delivery whilst maintaining the product's stability, quality and efficacy (Petrides *et al*., 2011; Kovarcik; 2016).

During the cell culture stage, the cells will secrete the monoclonal antibody into the cell culture medium, therefore harvest and clarification steps are essential to remove the cells and cell debris. Centrifugation, tangential flow microfiltration, and depth filtration are the most common unit operations employed in the biopharmaceutical industry for harvest and clarification steps. Centrifugation is preferred over other harvesting technologies such as microfiltration due to its scalability for large volumes processing (typically 2-15,000 L/batch). Centrifugation acts as a harvesting step but cannot accomplish complete removal of cells and cell debris, which must be removed using a depth filter (Low *et al*., 2007).

Following this, a capture step follows. The Protein A chromatography serves as a primary capture step to capture the monoclonal antibody of interest (Shukla *et al*., 2007). Protein A chromatography has been shown to be highly selective for mAbs, resulting in >95% purity in a single step (Gagnon, 1995). The captured monoclonal antibody is then subjected to a number of purification steps.

The FDA requires two viral reduction steps in addition to adventitious reductions achieved on chromatography steps. Both viral inactivation and a viral filtration steps are necessary to meet the FDA's requirements (Shukla *et al*., 2007).

The cation-exchange chromatography (CEX) and anion-exchange chromatography (AEX) steps act as polishing steps to remove the impurities present in the product (Shukla *et al*., 2007; Kelley, 2007; Low *et al*., 2007). The CEX step clears host cell proteins and leached Protein A while the AEX flow through step removes DNA and achieves further reduction in host cell protein impurities (Shukla *et al*., 2007).

The ultrafiltration/diafiltration step is implemented in the process to concentrate and formulate the product (Kelley, 2007). Upon the completion of downstream purification, the product is buffer exchanged into the formulation buffer. And finally, the product undergoes a sterile filtration step to reduce the overall bio-burden of the product by removing bacteria and viruses (Van-Reis and Zydney, 2001; Shukla *et al*., 2007).

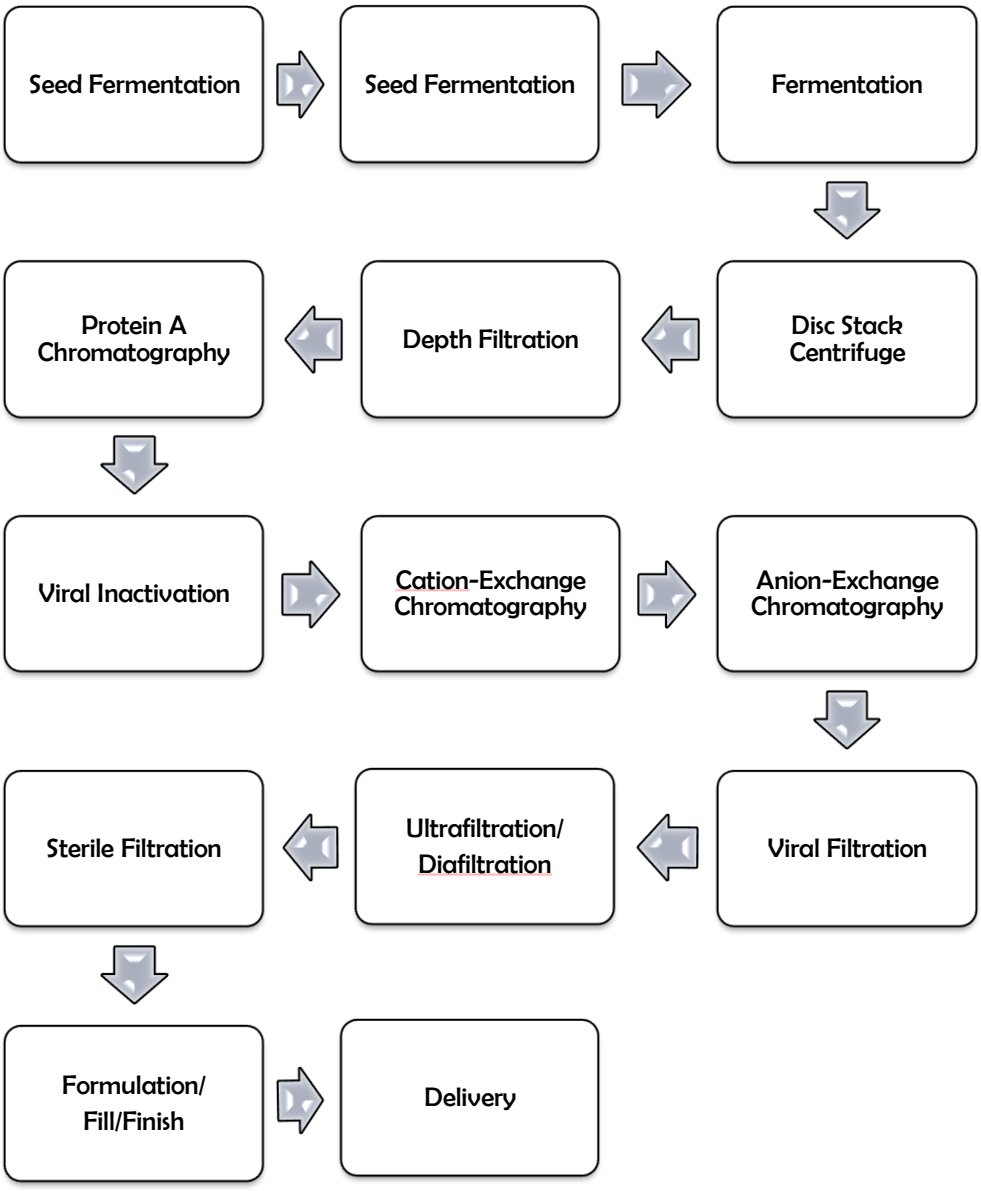


Figure 2.3 *A platform monoclonal antibody production process. Adapted from Shukla et al, 2007.*

The purified monoclonal antibody solution resulting from the downstream stage is referred to as the bulk drug substance. The bulk drug substance then undergoes the final formulation, fill and finish steps known as drug product manufacturing (Kelley, 2009). The formulation step involves taking the bulk drug substance at the desired concentration and dispensing it with the correct excipients that can ensure product quality and integrity during the subsequent fill/finish steps. The fill/finish steps

include filtration, filling, lyophilization, packaging, storage, and delivery to the customers (Kelley, 2009; Rathore and Rajan, 2008; Kovarcik, 2016).

2.4.8 Other expression systems to produce monoclonal antibodies

The growing and diverse need for the production of monoclonal antibodies as therapeutics has driven the development of a range of production systems (Andersen and Reilly, 2004). Though mammalians systems dominate, microbial cell expression systems such as *E. coli* are currently being considered to produce monoclonal antibodies because of the benefits they can provide in terms of lower cost of goods, faster drug development processes, production of protein in higher quantities, and the ability to grow at a fast rate when compared to mammalian cells. The latter results in shorter manufacturing times and faster delivery rate to patients (Verma *et al*., 1998). However, *E. coli* has been most commonly used for production of aglycosylated antibody fragments such as Fabs because of limits in protein folding, disulfide bonding and post-translational modifications (Humphreys, 2003; Nelson, 2010). Successful production of full-length monoclonal antibodies using *E.coli* expression systems has been reported, although these monoclonal antibodies were not glycosylated (Li *et al*., 2010).

The main advantage of fragment antibodies is that they can penetrate into tissues inaccessible to full length monoclonal antibodies. The manufacturing process may also be less costly and simpler due to lack of glycosylation, which permits the use of microbial expression systems. However, fragment antibodies rapidly degrade in humans and have short circulating half-lives (Nelson *et al*., 2010).

Figure 2.4 shows a typical fragment antibody manufacturing process using an *E.coli* expression system. Similar to full-length monoclonal antibodies production, the antibody fragment manufacturing processes can be separated into three defined phases: upstream, downstream and formulation/fill/finish. The upstream phase consists of a cell culture stage where the cells expressing the product of interest are grown inside a fermenter most often operating in fed-batch mode. The downstream phase usually consists of harvest, cell disruption and clarification steps, a number of chromatography steps, an ultrafiltration/diafiltration step, a formulation step and a sterile filtration step. The formulation/fill/finish phase is the final phase in manufacturing where the bulk drug substance is prepared for delivery without affecting its stability, quality and efficacy (Scanlan *et al.*, 2014; Kovarcik, 2016).

During the cell culture stage, the antibody fragment will be produced intracellularly in *E.coli*. Therefore, a cell disruption step is essential to break open the harvested cells in order to extract the product. Extraction systems such as homogenisers are commonly used to break down cells and extract antibody fragments (Humphreys, 2003; Farid, 2006).

Centrifugation, tangential flow microfiltration, and depth filtration are the most common unit operations employed in the biopharmaceutical industry for clarification step. Following this, Protein A chromatography typically serves as a primary capture step to capture the antibody fragment of interest. A subsequent polishing step is required to remove impurities such as cell debris, host cell proteins and toxins present in the product. Ion exchange and hydrophobic interaction chromatography are unit operations that are typically employed for the polishing step for production

of monoclonal antibodies using *E.coli* as the expression system (Shukla *et al*., 2007; Castano *et al*., 2014).

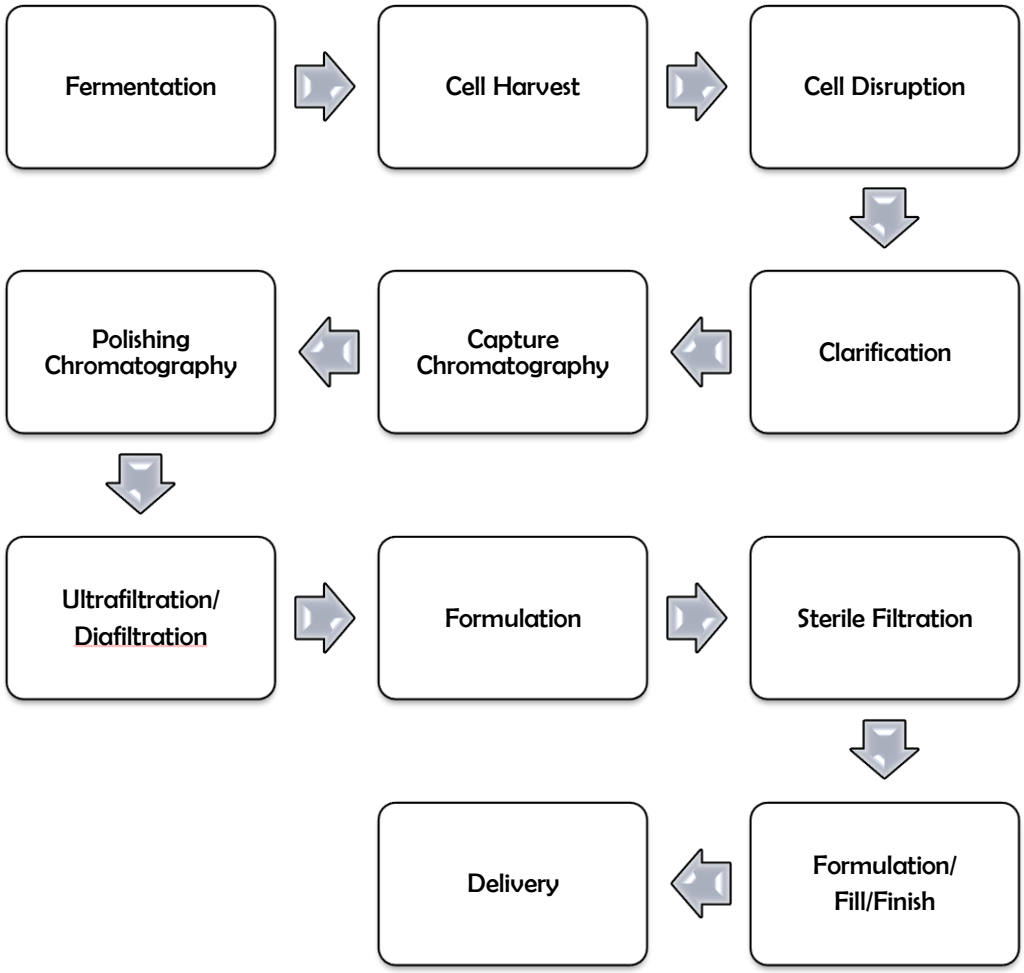


Figure 2.4 *A typical fragment antibody manufacturing process using E.coli expression system* (*Shukla et al, 2007).*

Following the polishing step, an ultrafiltration/diafiltration step is implemented in the process to concentrate and formulate the product (Kelley, 2007). Upon the completion of downstream purification, the product undergoes formulation and sterile filtration to reduce the overall bioburden. The primary consideration in formulation design for a fragment antibody is for stability. Antibody fragments have

a short circulating half-life which is a limitation for most therapeutic uses (Andersen and Reilly, 2004; Nelson, 2010). It is reported that the circulating half-life can be increased by up to 80% of that for IgG if site-specific attachment of a polyethylene glycol (PEG) moiety is introduced to a Fab' fragment. This is a process known as PEGylation. Other benefits of PEGylation include reduced antigenicity and immunogenicity of molecule, improved stability, and enhanced proteolytic resistance. PEGylation is however very costly (Zalipsky, 1995).

The third and final phase in fragment antibody manufacturing is the formulation, fill and finish steps (drug product manufacturing) where the bulk drug substance is subjected to several steps that include filtration, filling, lyophilization, packaging, storage, transport and delivery (Nesta *et al*., 2015; Kelley, 2009; Rathore and Rajan, 2008).

It is evident that *E. coli* as an expression system is likely to be used in the future as many antibody fragments derived from bacterial cell cultures are currently in clinical trials (Nelson, 2010; Birch and Racher, 2006). There are also two *E.coli* expressed fragment antibodies that have been approved: Lucentis (used mainly for the treatment of wet macular degeneration) and CIMZIA (used mainly for the treatment of rheumatoid arthritis). The extensive knowledge of *E.coli* bacterial genetics, its high growth rate and cost-effective production makes *E. coli* a promising host for antibody production, especially for full length monoclonal antibody production (Nelson and Reichert, 2009).

The biopharmaceutical industry not only focuses on different expression systems but also uses a range of manufacturing operations to achieve the exacting standards needed for monoclonal antibodies, run in either a traditional mode where equipment is cleaned in-between batches or in single-use mode where no such cleaning is required (Sinclair *et al*., 2008; Farid *et al*., 2005). These manufacturing alternatives will be described in detail in the following section.

2.4.9 Technologies used in monoclonal antibody manufacturing

Traditional batch processing still remains the predominant approach to manufacturing with items largely constructed of stainless steel. Therefore, they require assembly, and the subsequent clean-in-place (CIP), and steam-in-place (SIP) after each production batch (Sinclair, 2011). Although such traditional technology manufacturing operations are well established, their use is associated with (Shukla and Gottschalk, 2012; Farid, 2007):

- High levels of water consumption (a study carried out by Sinclair *et al* (2008) estimated that for a 1000 L operation scale, around 100,000 L of water is consumed per production batch for reagent preparations and CIP/SIP operations)
- Large capital investment (the capital investment for commercial antibody production facilities are reported to range from $40 M to $650 M)
- Increased manufacturing downtime.

These limitations have sparked interest in the wider adoption of manufacturing alternatives. One such alternative relies upon the deployment of single-use technologies, which employ disposable equipment. The earliest single-use

components adopted in the biopharmaceutical industry were basic filtration components, tubing, and connectors (Langer and Price, 2007). The industry is now increasingly employing single-use bioreactors, mixing devices, single-use membranes, single-use chromatography columns, sampling devices, and single-use probes (Langer and Price, 2007). Such single-use manufacturing process technologies can offer many benefits (Shukla and Gottschalk, 2012; Pierce and Shahbram, 2004; Rodrigues *et al*., 2009) including:

- A reduction in water consumption (a case study carried out at a biological plant operating at 2 x 2000 L operation scale determined that adopting a fully single-use manufacturing process could result in savings of more than one million litres of water)
- Reduction in the facility footprint
- Reduction in the high capital investment associated with stainless steel equipment (single-use facilities have the potential to reduce capital investment requirements by 33%-40%)
- Reduction in the frequency of process cross-contamination
- Process time reductions.

Process time reduction is an important factor for biologics as timely market penetration can be key to success (Farid *et al*., 2005). Although, single-use technologies can provide many benefits, there are several limitations associated with this type of manufacturing process (Sinclair *et al*., 2008; Eibl *et al*., 2010a; Shukla and Gottschalk, 2012) including:

- Limited production scale; highest volume of bioreactor is 2000 L

- Production of leachables and extractables by the single-use bags that could contaminate the product
- Potential adverse effects to the environment due to the increased solid waste levels inherent in operation with single-use components

However, it is not clear how the environmental impacts of single-use items contribute to mitigating the consequences of normal operation using extensive water-based cleaning. Such trade-offs promise a valuable motivation for the research conducted in this study.

2.4.10 Processing strategies in monoclonal antibody manufacturing

The two processing strategies employed in the biopharmaceutical industry are batch and continuous. Batch processing is the conventional processing approach currently used in the monoclonal antibody production (Li *et al*., 2005). In batch processing for monoclonal antibody production, the upstream equipment (bioreactor) is run in a fed-batch mode and the downstream equipment (harvest, clarification, purification, etc.) are run batch-wise (Sinclair, 2011). The fed-batch option (upstream) is preferred in the monoclonal antibody production because it is easy to operate, scalable, and has high volumetric productivity (Li *et al*., 2005). The disadvantages are higher production cost (capital and cost of goods) and longer manufacturing downtime (Sinclair, 2011).

Continuous processing is uncommon in monoclonal antibody production and only used for small scale production, for example in diagnostics production (Valdes *et al*., 2001; Gorter *et al.*, 1993). However, there are now therapeutic monoclonal

antibodies produced using continuous processing strategies. Remicade, a monoclonal antibody approved for the treatment of Crohn's disease and rheumatoid arthritis, is produced via continuous processing (Chu and Robinson, 2001). In continuous processing for monoclonal antibody production, the upstream equipment (bioreactor) is run in perfusion mode and the downstream equipment (harvest, clarification and purification) are run continuously (Sinclair, 2011). The advantages of continuous processing strategies are high productivities, constant nutrients exchange to the cells and removal of potentially problematic by-products of growth (Farid, 2007). However, the limitations of continuous processing include scalability issues and higher risk of potential contamination (Farid, 2007; Jain and Kumar, 2008).

2.4.11 Future trends in monoclonal antibody production

To produce large volumes of monoclonal antibodies, large-scale, efficient and economically favourable manufacturing processes are required. Thorough understanding of how the industry is using its available production capacity is important because inaccurate production planning for therapeutic monoclonal antibodies can have serious financial repercussions. In the past, monoclonal antibodies could only be extracted in tiny quantities, but today's technology allows five times the concentration of antibody produced by technologies just 5 years ago (Langer *et al*., 2009).

Mammalian cell culture has been the predominant method of production for marketed antibodies, but the growing demand for monoclonal antibodies and the financial constraints caused by the current global economic crisis are likely to create a new trend in antibody production (Langer *et al*., 2009). Hence, alternative systems

are being used, with the aims of lowering capital investments and cost of goods associated with large scale production of recombinant monoclonal antibodies (Verma *et al*., 1998). These alternatives include transgenic animal production system such as goat and chicken and plant based production system. Other alternatives are microbial expression systems such as bacterial and yeast expression systems (Farid, 2007; Ferrer-Miralles *et al*., 2009).

In transgenic chicken, monoclonal antibodies are produced in egg whites and this process takes approximately 8 months (Chadd *et al*., 2001). This method offers more advantages compared to transgenic goat as they have easy and inexpensive breeding cycle; 21 days to hatch and 5-6 months to mature (Farid, 2007). This expression system has the potential to supply huge quantities of material for clinical trials with a minimum cost (Morrow *et al*., 2000). In transgenic goats, antibody DNA fused to a milk- specific regulatory element is inserted into a single cell embryo by microinjection (Chadd *et al*., 2001). Transmission of the mammary gland-specific transgene is achieved using Mendelian genetics (Meade *et al*., 1999). The generation of monoclonal antibodies from transgenic goat for Phase I clinical trials takes up to 24 months (Chadd *et al*., 2001). At the moment, most of these transgenic antibodies are in pre-clinical trials.

Antibody expression in transgenic plants is now a well-established technology and it can address the capacity issues in antibody production (Hood *et al*., 2002). This method offers many advantages such as low cost of production, ease of scale-up or scale-down to meet market demand and a lower capital cost than mammalian cell culture (Hood *et al.*, 2002).

Although protein production using *Escherichia coli* (*E.coli*) bacteria is the first choice for microorganisms type production system, there are several limitations to this method of production (Farid, 2007). One is that the recombinant protein obtained from *E. coli* lack the post-translational modifications which are present in most eukaryotic proteins (Rosano and Ceccarelli, 2014). Another limitation is that the frequency with which the different codons appear in *E. coli* genes is different from those occurring in human genes, and this could lead to reduced yield of expected protein versions (Makrides., 1996).

Various bioreactors have been used for antibody productions such as stirred tanks, airlift, hollow fiber, and rotary cell devices, some of which may be operated as disposables (Jain and Kumar, 2008). Disposable bioreactors provide advantages over other bioreactors in aspects such as low initial investment cost, reduced lifetime operating cost, reduced validation requirements, simplified scale-up and low risk of cross-contamination (Jain and Kumar, 2008). The Wave bioreactor, introduced in the late 1990's, is the first disposable bioreactor available for the biopharmaceutical industry for drugs manufacturing (Scott, 2007).

While disposable technologies are widely employed in upstream processing, adoption in downstream operations has been slow (Challener, 2014). Only recently have manufacturers started implementing disposable technologies in their operations. The disposable options have advanced in areas such as tangential flow filtration (TFF), but are still progressing in areas such as chromatography (Allison and Richards, 2014). With increasing validation studies being carried out, there is a high

possibility for single use options to replace fully the traditional technology in biopharmaceutical manufacturing in the future (Challener, 2014).

2.5 Conclusion

This chapter has highlighted the need for EIIs to evaluate and reduce their environmental impacts in order to achieve sustainable development. This chapter also highlighted the need for sustainable manufacturing practices within the EIIs. A large number of EIIs have conducted environmental studies in attempt to reduce their environmental contributions, and these have been highlighted.

The biopharmaceutical industry is one of the EII. The industry's best-selling products, monoclonal antibodies, employ high levels of energy and water during production. The processes are run in either a traditional mode where equipment is cleaned in-between batches or in single-use mode where no such cleaning is required (Sinclair *et al.*, 2008; Farid *et al.*, 2005). Presently, a significant challenge faced by the biopharmaceutical industry is selecting between traditional and single-use manufacturing processes.

Historically, process costs were used by the industry to support decision-making (Farid *et al.*, 2005). Now, with increasing environmental legislation, policies and governmental initiatives associated with climate change have triggered a broader biopharmaceutical industry interest in the environmental contributions made by manufacturing. Therefore, there is a pressing need for the development of environmental framework that will allow the industry to evaluate the environmental impacts of manufacturing alternatives.

The environmental impacts of manufacturing alternatives can be assessed using a framework based on the LCA tool. The ability of the LCA tool to quantify the full environmental impacts of a process, product or activity, whilst avoiding shifting of the environmental issues from one stage to another or from one media to another make it highly desirable as an environmental tool in be used in the framework developed in this work (Curran, 2006; Mauter, 2009). The next chapter will describe the development of the framework in-detail as this provides the background for the studies conducted in this doctorate.

Chapter 3. Framework Development and A Case Study

3.1 Introduction

As indicated in the preceding chapters, the EIIs face pressure from governments worldwide to reduce their environmental impacts. This has sparked the biopharmaceutical industry's interest to focus on the potential of manufacturing alternatives to reduce environmental impacts. The process economics of monoclonal antibody manufacture have been studied extensively (Farid, 2007). Presently however, decision-making in the biopharmaceutical industry is strongly influenced by process economics and not by the environmental impacts of different manufacturing alternatives. This is likely to change as the industry becomes subject to increasingly stringent environmental legislation and policies.

Selecting an effective manufacturing process requires exploring the balance between striving for improved process economics and reduced environmental impacts. Unless these aspects are addressed explicitly, the industry risks making decisions that are not best-suited to achieve all goals.

In order to make significant steps toward reducing the environmental impacts of manufacture, the industry must be able to quantify the environmental impacts of manufacturing alternatives. Therefore, there is a pressing need for the development of a framework to perform such environmental assessments. To date, no such frameworks exist for the biopharmaceutical industry. The framework developed in this work represents the first attempt to provide a framework for the holistic environmental assessment in this industry. It is anticipated that this framework would

be used along-side other tools currently deployed in the industry to enhance the robustness and relevance of the decision-making process.

This chapter is divided into eight sections. Section 3.2 highlights the features of a proposed environmental framework and identifies the evaluation tool that will be used in the framework. A general introduction to life cycle assessment (LCA) is provided in Section 3.3. Section 3.4 discusses the application of the LCA tool in the biopharmaceutical industry to select environmentally favourable manufacturing and solid waste management alternatives. Section 3.4 also examines the environmental studies carried out in the biopharmaceutical industry, and highlights the importance of LCA studies for this industry. The real challenges of LCA analysis in the biopharmaceutical industry are discussed in this section. Section 3.5 presents the systematic approach used to develop the framework based on the LCA tool. In this section, the scope of the framework and the application of the LCA tool in the framework are discussed. Section 3.6 discusses the set-up of a hypothetical case study to demonstrate the functionality of the framework. In section 3.7, the environmental impacts reference is provided in order to have an understanding of the magnitude of the impacts produced by the biopharmaceutical processes. Finally, the summary of this chapter is provided in Section 3.8.

3.2 Key Aspects of an Environmental Assessment Framework for Biopharmaceutical Processes

As discussed earlier, there is a strong need for the development of an environmental framework to facilitate decision-making processes in the biopharmaceutical industry.

This section presents the characteristics that this framework should possess, and identifies the quantitative tools that can be applied in the proposed framework.

3.2.1 Features of an environmental framework

In order for the biopharmaceutical industry to evaluate and quantify the environmental impacts of a given manufacturing process, to make appropriate engineering interventions, and to move to a position where the manufacture of drugs is achieved in a more sustainable fashion, a decision-support framework for this industry should possess certain features. These features are:

- Ability to evaluate the environmental impacts of different manufacturing technologies such as traditional (consisting of stainless steel equipment) and single-use (consisting of disposable equipment);
- Ability to evaluate the environmental impacts of different manufacturing strategies such as batch (upstream equipment is run in fed-batch mode; downstream equipment is run batch-wise) and continuous (upstream equipment are run in perfusion mode, downstream equipment is run continuously);
- Capacity to assess the environmental impacts produced by different solid waste disposal options such as landfill, incineration with energy recovery and pyrolysis; and
- Capacity to identify the environmental "hot spots" (phases or stages of the process where environmental burdens are high) of a given manufacturing process.

3.2.2 Tools that can be used in the environmental framework

It is imperative to select appropriate tools that will assess the environmental issues within a single framework. However, selecting the appropriate tool is a challenging task as there are a number of tools available for environmental analysis studies Jeswani *et al*., 2010; Kissinger and Rees, 2010; Torío and Schmidt, 2010, Bebbington *et al*., 2007). These tools include:

- Risk assessment (RA)
- Material flow analysis (MFA)
- Environmental impact assessment (EIA)
- Energy/exergy analysis (EA)
- E-Factor (Environmental factor) tool
- Life cycle assessment (LCA)

Risk assessment (RA) is a tool typically used to evaluate the environmental, health and safety related risks posed by chemicals, harmful substances, and industrial plant (Jeswani *et al*., 2010). The RA tool focuses on a specific harmful endpoint resulting from product, process or event. Although RA is used as decision-support tools for policy and regulation, the results generated by the tool are prone to public distrust because of the complexity of the issues, and under- or over-estimation of risks due to multiple uncertainties (Jeswani *et al*., 2010).

Material flow analysis (MFA) is a tool, which accounts for material and energy inputs and waste outputs associated with an entire specified system (Fiksel, 2010; Cain *et al*., 2007). This tool combines a large amount of data on the materials required for a particular economic activity (Jeswani *et al*., 2010). However, it should

be noted that this tool is directed towards reducing the number of substances of study as much as possible to maintain transparency and manageability, which could lead to incomplete evaluation (Jeswani *et al*., 2010).

Environmental impact assessment (EIA) is a tool for evaluation of local environmental impacts, which generally takes into account time-related aspects, the specific local geographical situation and the existing pressure on the environment (Toro *et al*., 2013). However due to the lack of data, the uncertainty of the results can be an issue (Toro *et al*., 2013).

Energy/exergy analysis (EA) is a tool that measures both the quantity of energy (energy analysis) and the quality of energy – the maximum amount of work that can be theoretically obtained (exergy analysis). This tool can be employed to understand the effectiveness of resource utilisation; show where losses occur and where improvements can be made to improve energy efficiency (Kissinger and Rees, 2010). However, this tool only concentrates on the energy issues and may leave out other environmental issues (Jeswani *et al*., 2010).

The E-Factor is an environmental tool/metric developed to quantify the extent of waste produced (in kilograms) by a process per kilogram of a product (Jimenez-Gonzalez *et al*., 2011). The closer to zero the value of E-Factor (E-Factor ~0) is, the less waste generated and more sustainable/greener the process is considered to be (Tobiszewski *et al*., 2015). This tool/metric was initially developed for reducing waste, but it can be adapted to obtain more environmental information on the manufacturing processes such as process water consumption and consumables usage

(Jimenez-Gonzalez *et al*., 2011; Pollock *et al*., 2013). This tool has been widely used by many industries including the pharmaceutical and biopharmaceutical industries to guide in their green/lean manufacturing initiatives (Ho *et al.,* 2011). Although this metric is useful to measure the "greenness or sustainability" of a given manufacturing processes, this metric/tool cannot be deployed to quantify impact categories such as the cumulative energy demand and carbon emissions which are important measures to determine the environmental performance of industries (Tobiszewski *et al*., 2015).

And finally, life cycle assessment (LCA) tool is based upon a comprehensive analysis which estimates the cumulative environmental impacts of a process, product or activity, avoiding shifting of the environmental issues from one stage/phase to another (Curran, 2006).

Although other tools exist, LCA has been identified as the most appropriate tool to be applied in the framework to evaluate the environmental impacts of manufacturing and solid waste management alternatives. This is because LCA is the only tool that allows comprehensive assessment of the entire life cycle of products (from cradle-to-grave), processes or activities (Baumann and Tillman, 2004). The ability of an LCA tool to quantify the full environmental impact of a process, product or activity makes it highly desirable as an environmental assessment tool, enabling organisations to identify the environmental trade-offs associated with particular products or processes (Curran, 2006; Mauter, 2009). The detailed LCA description is provided in the following sections.

3.3 Life Cycle Assessment (LCA)

This section provides an overview of the LCA methodology. It provides a summary of various LCA approaches and types, and highlights the applications and limitations of LCA.

3.3.1 Brief history of LCA

The LCA tool first gained recognition in the late 1960s in the United States, the United Kingdom, Switzerland, and Sweden, and was initially developed by scientists to understand the impacts of energy consumption on fossil fuel depletion as an emerging issue (Udo De Haes and Heijungs, 2007; Svoboda, 1995). In 1969, the Coca Cola company employed the Mid-West Research Institute to carry out LCA studies to compare the resource consumption and the environmental impact of using different beverage containers (EEA, 1998; Svoboda, 1995). As a result, Coca Cola switched from glass to plastic bottles due to the lower predicted level of environmental impact exhibited by plastic bottles. Early LCA studies mainly focused on levels of energy consumption and waste production (Baumann and Tillman, 2004). Contradictory results were often obtained because different methodologies, data and terminology were used (Azapagic, 1999; Baumann and Tillman, 2004). The breakthrough in LCA occurred in the 1980s when the Society of Environmental Toxicology and Chemistry (SETAC) developed a code of practice for LCA (Frankl and Rubik, 1999). The SETAC code of practice established a general and systematic methodology by which to conduct LCA studies. In the 1990s, the International Organisation for Standardisation (ISO) developed a general framework for LCA. Today LCA tools are used to carry out environmental studies over a broad range of

areas such as product/process comparison, eco-labeling, product/process improvement, decision and policy support (EEA, 1998).

3.3.2 Introduction to LCA

The International Organisation for Standardisation (ISO) has classified LCA as a process of "compilation and evaluation of the inputs, outputs, and the potential environmental impacts of a product, process or activity throughout the entire period of its life cycle" (Guinee and Heijungs, 2005). The LCA tool takes into account the entire life cycle of a product or process including raw material extraction, processing, manufacture, packaging, transportation, distribution, use, re-use, maintenance, recycling, and final disposal (UNEP, 1996; Pennington *et al*., 2004). The tool is based upon a comprehensive analysis which estimates the cumulative environmental impacts of the process, product or activity, avoiding shifting of the environmental issues from one stage/phase to another (Curran, 2006).

3.3.3 Principles and general framework of the LCA methodology

The methodological framework developed by the International Organisation for Standardisation (ISO) as the basis from which to conduct LCA studies consists of four phases: goal definition and scope, life cycle inventory analysis (LCI), life cycle impacts assessment (LCIA) and interpretation, as illustrated in Figure 3.1 (Curran, 2006; Martins *et al*., 2010; Rebitzer *et al*., 2004).

The **goal definition and scope** phase of LCA includes creating a detailed description of the objectives of the study, defining the functional unit, defining the system boundaries and the intended use of the outcome of the LCA as well as the targeted

audience (Miettinen and Hamalainen, 1997). The functional unit is a crucial basis that enables alternative products, processes and services to be compared (Rebitzer *et al*., 2004).

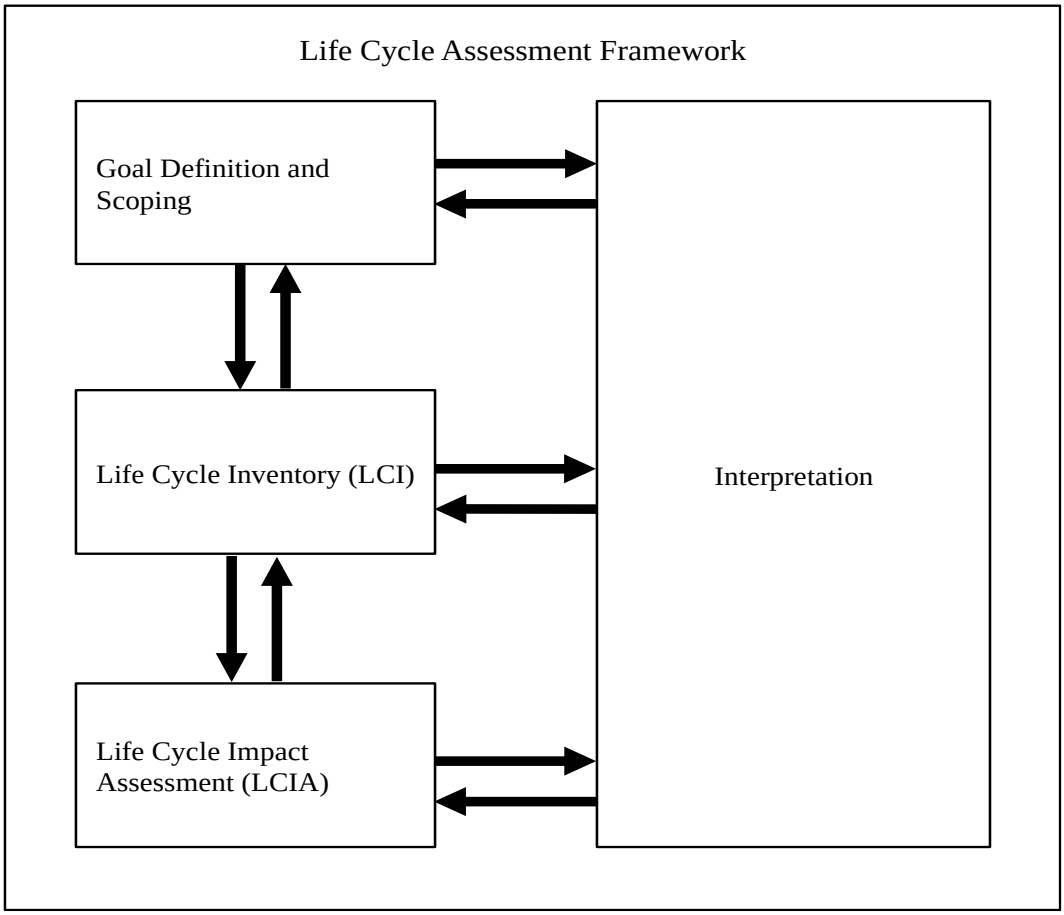


Figure 3.1 *The general framework of the LCA methodology according to the ISO standards. The general framework of the LCA methodology consists of four phases; the goal and scope definition phase, the life cycle inventory phase (LCI), the life cycle impact assessment phase (LCIA) and finally, the interpretation phase. The development of LCA is not linear; throughout the process it is necessary to return to previous steps and interpret the results found (Pennington et al., 2004; Raymond et al., 2010).*

The **LCI phase** involves the compilation of environmental burdens; the raw material requirements, air emissions, waste (solid and liquid), energy requirements, and waterborne effluents that are released throughout the life cycle of the system studied in the LCA (Svoboda, 1995).

The **LCIA phase** translates the environmental burdens quantified in the LCI analysis into impact categories (Pennington *et al*., 2004). There are two types of impact category: mid-point (problem-orientated) and end-point (damage-orientated category) (Jolliet *et al*., 2003). The mid-point impact category translates the LCI results into environmental themes such as climate change, ozone depletion and acidification potential, to name a few (Jolliet *et al*., 2003). It is located on the impact pathway at an intermediate position between the LCI results and the end-point category (Shaarai *et al*., 2010; Jolliet *et al*., 2003). The end-point impact categories allocate the mid-point impact categories to one or more damage categories; damage to human health, damage to natural resources, and damage to the ecosystem (Jolliet *et al*., 2003). Decision makers prefer the end-point impact categories to the mid-point impact categories because it can be easily interpreted and understood. However, it has been argued that the end-point impact categories increase the level of uncertainty than the mid-point impact categories (Pennington *et al*., 2004). The choice of impact categories (mid-point/end-point or both) depends purely on the objectives and the targeted audience of an LCA study.

And finally, **the interpretation phase** involves a complete review of all the three phases of LCA (De Benedetto and Klemes, 2009).

3.3.4 System boundaries

In an LCA study, the system boundary is defined during the goal and scope definition phase. It is important to define the system boundary approach in order to determine the unit processes that will be included in the study. Amongst the different LCA system boundary approaches that can be considered by an LCA practitioner

when carrying out LCA studies include:

- Cradle-to-grave
- Cradle-to-gate
- Gate-to-gate.

The **cradle-to-grave** LCA system boundary approach involves the evaluation of environmental impacts of a product/process from its raw material extraction and refining, through component manufacturing, distribution, use and disposal at end-of-life (includes the supply-chain, use and end-of-life phases) (Pietrzykowski *et al*., 2013). In the **cradle-to-gate** LCA system boundary approach, the evaluation of environmental impacts of a product/process from its raw material extraction and refining, through component manufacturing, distribution and use (includes the supply-chain and use phases) is made. The cradle-to-gate LCA system boundary approach does not account for the end-of-life phase of a product/process (Kara *et al*., 2010). Finally, the **gate-to-gate** LCA system boundary approach involves the evaluation of environmental impacts of a product/process during its use phase. The gate-to-gate LCA system boundary approach does not account for the supply-chain and end-of-life phases of a product/process (Puettmann and Wilson, 2005).

The different LCA system boundary approaches can be used for particular types of study. For example, the cradle-to-gate approach is often used by the chemical industry to provide a comparative assessment between two chemical processes to aid in process selection. The cradle-to-gate approach however does not provide a full assessment of the LCA impacts of a final product/process (Kara *et al*., 2010). It should be acknowledged that the outcome of an LCA study is particular to the

system boundary approach each adopts. The schematic representations of the LCA system boundary approaches are provided in Figure 3.2. This schematic diagram, based on a typical biopharmaceutical manufacturing process, illustrates the differences between each system boundary approach.

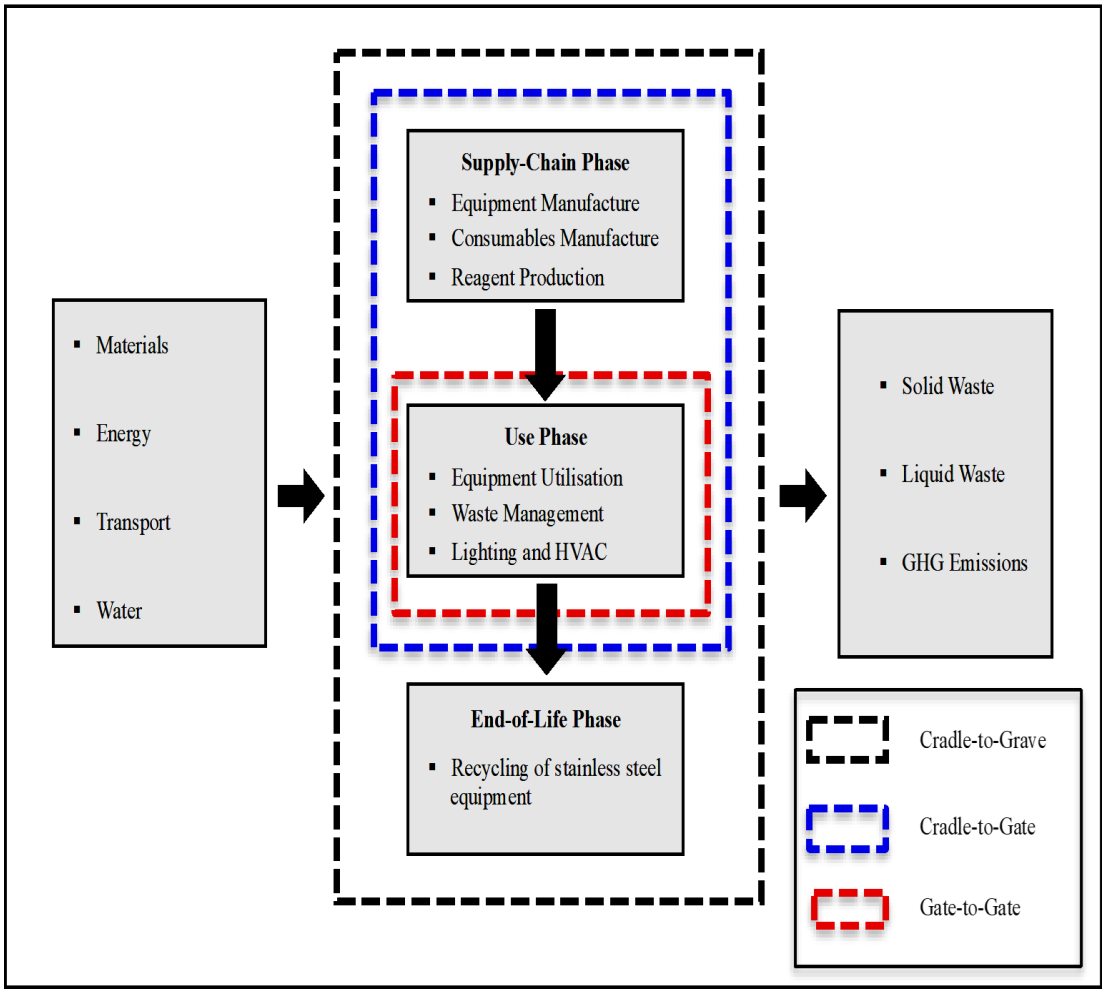


Figure 3.2 *LCA system boundary approaches. The LCA analysis of a monoclonal antibody manufacturing process using cradle-to-grave, cradle-to-gate or gate-to-gate system boundary approaches.*

3.3.5 Types of LCA

Consequential LCA (CLCA) and Attributional LCA (ALCA) are two different LCA types that aim to answer distinct questions (Finnveden, 2008). ALCA, also known as

retrospective LCA, is ideal for consumption-based accounting as it focuses on describing the environmentally relevant physical flows to and from a life cycle and its sub-systems, assuming a status-quo situation (Curran *et al*., 2005; Finnveden *et al*., 2009; Thomassen *et al*., 2008). CLCA, also known as prospective LCA, is commonly used to identify the cause and effect relationships arising from possible decisions, and the associated environmental burdens (Mathiesen *et al*., 2009).

The most significant difference between CLCA and ALCA is the way they treat co-production. In ALCA, co-products are either treated through allocation (allocates emissions to co-products based on either economic value, energy content, or value) or system expansion meanwhile in CLCA, the co-products are typically treated through system expansion (the product system is expanded to include the additional functions related to the co-products) (Suh *et al*., 2004; Schmidt, 2008; Brander *et al*., 2008). LCA with system expansion includes foreground and background systems.

The foreground system is a set of processes whose choice or method of operation is affected directly by decisions based on the study. The background system includes all other processes interacting with the foreground system, normally through materials and energy transfer (Clift *et al*., 2000). The emissions arising from the foreground activities are known as *Direct Burdens* and the emissions arising from the background activities are known as *Indirect Burdens* (Clift *et al*., 2000). Indirect burdens can be divided into upstream and downstream burdens (Clift *et al*., 2000; Bernstad and la Cour Jansen, 2012). Upstream burdens are those arising from the extraction and manufacturing processes, transportation and use of a given product prior to its final disposal. Upstream burdens associated with the provision of energy

– electricity, diesel or oil are included in most LCAs (Andersen *et al*., 2012; Manfredi *et al*., 2009). Downstream burdens, usually referred to as avoided burdens, associated with those economic activities which are displaced by materials, nutrients and energy recovered through waste treatment process (Clift *et al*., 2000). Most integrated waste management LCA studies include calculations of avoided burdens by displacing emissions associated with grid energy and virgin materials production by amounts of energy and materials recovered in a given system (Manfredi *et al*., 2009; Møller *et al*., 2009). Therefore, the total emissions are calculated as:

Total emissions = Direct emissions + Indirect Emissions – Avoided emissions

Another key difference between ALCA and CLCA is the type of data used. CLCA tends to use marginal data (marginal data represent those technologies that are affected by changes in demand) while ALCA ideally uses average data (data that are reflective of a point in time or current time) (Weidema *et al*., 1999; Schmidt, 2008; Brander *et al*., 2008).

The choice of the type of LCA must be appropriate so as to meet the objectives of a given study, and also to obtain accurate results (Brander *et al*., 2008). Although the biopharmaceutical industry has not been formally identified as an EII, this industry uses high levels of energy to manufacture their products. Given the lack of LCA studies applied to this industry at present, ALCA is the type of LCA that might be applied usefully initially in order to evaluate the environmental impacts of the biopharmaceutical industry's manufacturing processes. At present, CLCA would be difficult to apply to the biopharmaceutical industry due to the limited availability of

marginal data for biopharmaceutical processes. CLCA seeks to inform policy and decision makers on the broader impacts of policy changes, and on how these will affect the biopharmaceutical industry and the associated environmental footprint (Brander *et al*., 2008). Hence, the expectation is that as LCA studies are developed for this industry, an assessment of future changes that may impact the performance of this industry may be more easily tackled.

3.3.6 Applications and limitations of LCA analysis

LCA is generally accepted as a tool for environmental management (Azapagic, 1999). According to Jensen *et al* (1998), users of LCA can be categorised into four groups that include industry and commercial organisations, regulatory bodies and governments, NGOs, and finally consumers. LCA analysis is widely employed by organisations for process or product design, product or process improvements, product eco-labeling, product certification, company environmental policy, business strategy developments, policy and decision-making (Frankl and Rubik, 1999). LCA is preferred over other environmental assessment because it is either product or process orientated, integrative and quantitative (UNEP, 1996). Moreover, no other tools provide a comprehensive environmental analysis (Baunmann and Tillman, 2009).

A number of limitations have been identified with the use of LCA as an environmental assessment tool. Primarily it is seen as being time-consuming and data intensive to implement (Curran, 2006; Mauter, 2009). The lack of LCA data and of a standardised LCIA methodology can hinder industries from using this tool for their own environmental studies. However, with the advent of the UNEP/SETAC life

cycle initiative, a global UNEP/SETAC LCI database and a set of standardised LCIA methodologies have been developed (Udo de Haes *et al*., 2002). LCA remains the most efficient environmental assessment tool due to the "cradle to grave" approach, which extends beyond the usual boundaries of impact assessments for the environment (DEAT, 2004).

3.3.7 LCA databases and software packages

The LCI phase is the most time-consuming phase as a large amount of data is required, and often, insufficient data is available for the process, product or activity being studied. Several public national, industry, regional, and consultant LCA databases have been developed to overcome these issues by providing inventory data for the LCI phase (Finnveden *et al*., 2009). These databases include US NREL (NREL, US), DEFRA (UK), SPINE (CPM, Sweden), ELCD (European Commission), German PROBAS (Federal Environmental Agency, Germany), Japanese JEMAI (Japanese Environmental Management Association for Industry, Japan), Swiss Ecoinvent (Swiss Centre for Life Cycle Inventories, Switzerland), and Australian LCI (Australian Life Cycle Assessment Society, Australia) (Finnveden *et al*., 2009).

An LCA analysis can be carried out in by using a spreadsheet or by using a software package. The choice of using a proprietary spread sheet or software package depends entirely on the LCA practitioner. At present, there are about thirty LCA software packages available on the market. However, these packages do not incorporate inventory data for the biopharmaceutical industry. Such data are limited due to the paucity of environmental studies in the industry. Thus, there is a strong need for the

compilation of life cycle inventory data for this industry. Once this has been established, LCA analysis in the biopharmaceutical industry can be carried out using software packages, and this will offer faster and reliable ways for environmental studies.

In this doctoral project, the LCA analysis using the framework developed was carried out using Excel V. 2010 (Microsoft Corporation, Redmond, Washington, USA). The screenshots of this framework on Excel are provided in Appendix 4.7.

3.4 LCA in the Biopharmaceutical Industry

This section reviews and discusses the use of LCA in the biopharmaceutical industry, provides a critical review of the environmental studies conducted in the industry and highlights the importance of LCA analysis for the biopharmaceutical industry. The challenges of carrying out LCA analysis for the biopharmaceutical industry are also described in this section.

3.4.1 LCA for manufacturing process selection

Historically, traditional manufacturing processes employing stainless steel equipment have been the prevalent mode for the biopharmaceutical industry by which to manufacture proteins such as monoclonal antibodies and vaccines (Lim *et al*., 2007). However, more recently single-use manufacturing processes employing disposable equipment have been gaining increased attention due to their inherent benefits (Shukla and Gottschalk, 2012). Whilst, process economics and risk evaluation studies clearly highlight the advantages of full implementation of single use technologies in biopharmaceuticals production, the environmental impacts of the

traditional and single use manufacturing processes remain ambiguous, and despite some preliminary work, have not been the subject of significant and rigorous engineering analysis (Pietrzykowski *et al*., 2013; Newman, 2008; Mauter, 2009; Rawlings and Pora, 2009).

In principle, single-use manufacturing processes might be expected to lead to higher levels of environmental impact than would a traditional manufacturing process due to the production of consumables (plastics) with every manufacturing batch. Conversely, it might be expected that traditional manufacturing processes will produce higher levels of environmental impact due to the large amount of water consumed, the need for the fabrication of stainless steel equipment and the actual impact involved in the disposal of stainless steel equipment at the end of the plant life (5-15 years). Therefore, there is a pressing need for the development of an environmental framework based on the LCA tool that will allow the industry to identify in a strictly quantitative fashion, the actual environmental impacts of manufacturing alternatives. Such a capability would allow the industry to make decisions based on facts rather than depending on "rule of thumb" methods (Baumann and Tillman, 2004). This forms the basic driver and motivation for the thesis.

3.4.2 LCA for solid waste management selection

Waste, as defined by the Environmental Protection Act 1990, is any material, effluent or unwanted surplus substance or article that requires disposal because it is damaged, worn out, contaminated or spoiled. Waste that is regulated includes household, industrial and commercial waste. The Environmental Agency is

responsible for regulating waste in England (CIPS, 2007).

Industrial waste can be categorised into four groups, which include hazardous liquid waste, non-hazardous liquid waste, hazardous solid waste and non-hazardous solid waste. The disposal of hazardous liquid and solid waste is the main concern for the industries as improper disposal could cause adverse effects in the environment. An integrated solid waste management system must be in place in order for the industries to reduce the environmental impacts caused by waste disposal activities. An integrated solid waste management system comprises a range of activities such as reduction, recycling, segregation, treatment and finally disposal at the disposal site (Hamer, 2003). An integrated waste management system is an imperative for the industries as it can provide effective and standardised ways to manage waste. Such a waste management system places an emphasis on waste prevention and resource management (Clift *et al*., 2000).

The biopharmaceutical industry could potentially benefit by employing an integrated solid waste management system. The increasing level of implementation of single-use systems in manufacturing is forcing the industry to seek effective and sustainable ways in which to dispose of solid wastes (e.g. consumables such as single-use bags and single-use filters) (Rawlings and Pora, 2009). The disposal options that can be considered by the biopharmaceutical industry include landfill, incineration with/without energy recovery and pyrolysis (Rawlings and Pora, 2009). Landfill is a method where solid waste is dumped into or onto land (SEPA, 2012). Incineration is a method that involves burning solid waste, whilst incineration with energy recovery involves burning the solid waste to produce combined heat and energy (Environment

Agency, 2012). Pyrolysis is a method that involves thermal conversion of organic solid waste by heat in the absence of oxygen to produce combined heat and energy (Brownsort, 2009).

Each disposal method has benefits and limitations. Landfill remains a commonly available disposal method in many countries, but in the UK is rapidly being replaced due to a number of drawbacks. The drawbacks include tax introduced by the UK Environmental Agency on landfill disposal methods, noise produced by vehicles on the landfill sites, and odor produced by the waste dumped at the landfill sites (Rawlings and Pora, 2009). Incineration disposal methods are increasingly gaining popularity for reasons that include low operating costs, wide availability across the UK, and energy recovery possibilities. The major drawback of the incineration disposal method is the high initial investment costs for new incineration facilities (Rawlings and Pora, 2009). Pyrolysis is an alternative disposal method to incineration, which produces bio-char, bio-oil and synthetic gas by burning solid waste. The bio-oil and synthetic gas can be upgraded to produce fuel, electricity and heating. Even the bio-char produced from pyrolysis has economic value as it can be sold to the metallurgical and agricultural industries (Al-Salem *et al*., 2009; Parkes *et al*., 2015). Additionally, in the UK, the energy produced from pyrolysis is eligible for more Renewables Obligation Certificates (ROCs) than for that produced from incineration with energy recovery (Ofgem, 2014). ROCs are certificates issued by the Office of the Gas and Electricity Markets (Ofgem) to electricity generators in order to encourage generation of electricity from eligible renewable sources. Electricity generators can sell their ROCs to electricity suppliers, which will allow them to receive premiums. Electricity suppliers are required to present their ROCs in

order to meet the renewables obligation, and if they fail to do, electricity suppliers are required to pay a penalty (DECC, 2012; Smith and Watson, 2002). Although pyrolysis appears to be a sustainable solution, insufficient understanding of the reaction pathways is currently preventing many industries from employing this as a possible solid waste disposal option (Al-Salem *et al*., 2009).

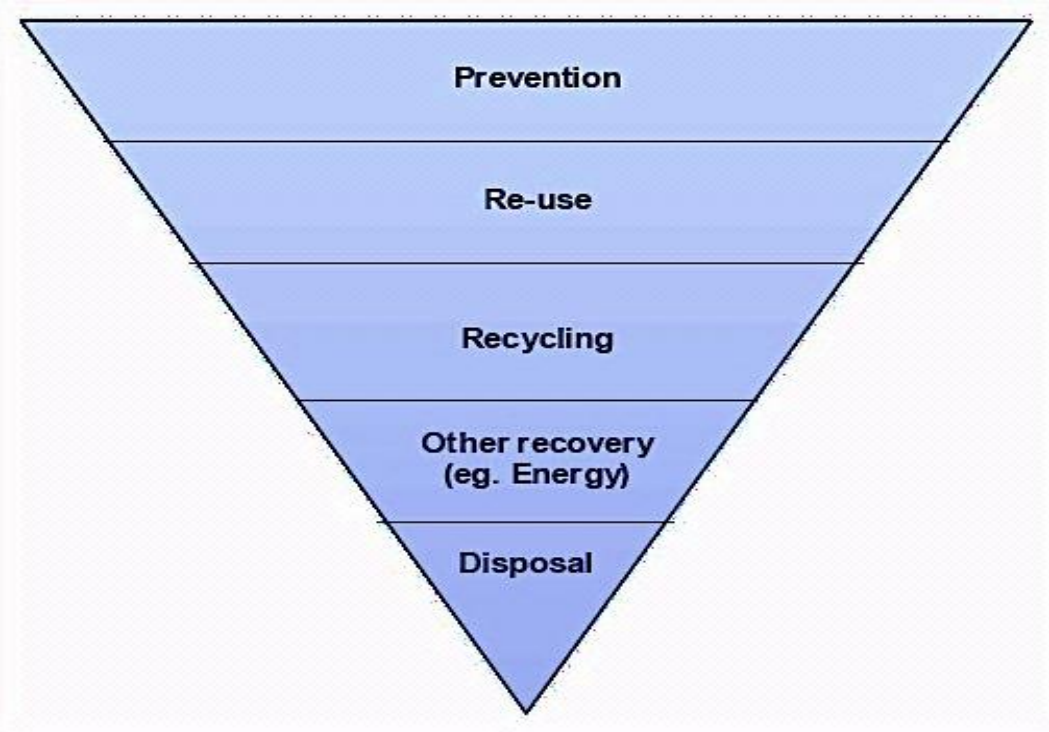


Figure 3.3 *The European waste hierarchy. Adapted from The Scottish Government, 2010. This diagram illustrates the European Waste Hierarchy developed by the European Commission.*

Presently, there is no one single ideal disposal option for the biopharmaceutical industry. The European Waste Directive's waste hierarchy states that landfill should be used as the option of last resort for any industrial waste disposal (Figure 3.3) (DEFRA, 2010). According to the hierarchy, any industry should aim for waste prevention, re-use, re-cycling, energy recovery disposal options, and finally landfill

(in that order). By employing LCA, the actual environmental impacts produced by each disposal option can be identified, and in principle an integrated waste disposal method can then be designed for the biopharmaceutical industry (Cherubini *et al*., 2009). In theory, this will allow the industry to plan manufacture and to manage their waste in a more sustainable manner.

3.4.3 Critical review of environmental studies in the biopharmaceutical industry

Most of the current environmental studies have been carried out in the energy and automotive industries. A broad analysis applied in the three different sectors (energy, automotive and biopharmaceutical) reveals that the majority of the LCA studies are in the energy sector (encompassing coal, nuclear, electricity, etc.); more than 500 studies exist (Whitaker *et al*., 2012; Warner and Heath, 2012).

A study carried out in 1996 estimated that there were more than 56 LCA studies available for the automotive industry (encompassing vehicle engines, vehicle materials and parts, and vehicle's end-of-life) (Ecobilan, 1996). By comparison, there are only 5 environmental studies (in which only 2 are LCA studies) available for the biopharmaceutical industry (Mauter, 2009; Pietrzykowski *et al*., 2013; Rawlings and Pora, 2009; Pollock *et al*., 2013; Sinclair *et al*., 2008). Table 3.1 highlights the environmental studies currently available for the biopharmaceutical industry, the methods applied, the outcomes and the limitations of these studies.

Table 3.1 *The environmental studies available for the biopharmaceutical industry.*

Environmental Study	Impact Categories Investigated	Outcomes	Limitations
Mauter, 2009: This study estimated the shifts in environmental impacts when a conventional stainless steel bioreactor is replaced with single-use bioreactors (LCA analysis)	Environmental impacts are expressed using mid-point impact categories (see the list of mid-point impact categories in Section 3.3.3)	Environmental shifts are observed when a traditional bioreactor is replaced with a single-use bioreactor. For climate change impact category, the stainless bioreactor contributes to higher environmental impact than the disposable option	The environmental analysis is not comprehensive; the study only looked at one unit operation (bioreactor). This is a product-orientated study rather than a process-orientated study
Rawlings and Pora, 2009: This study evaluated the energy demand of 3 stages (cleaning, sterilisation and materials production) of a monoclonal antibody manufacturing process using a traditional stainless steel system and a disposable system	Energy consumption (MJ)	The study identified that the manufacturing process employing the stainless system consumes more energy than the manufacturing process employing the disposable system in all the 3 stages investigated	The study did not use an established environmental analysis methodology such as LCA that would enable thorough (cradle-to-grave) analysis
Sinclair *et al*., 2008: This study evaluated the carbon, water and land footprint savings of a Mab process (3 × 2000 L) when traditional equipment facility is replaced with single-use facility	Water, land and carbon footprint	The study identified that around 87% of water, 38% of land and 30% of carbon savings can be achieved when single-use equipment are employed	The study did not use an established environmental analysis methodology such as LCA that would enable thorough (cradle-to-grave) analysis The study is not comprehensive, as it did not include the materials that are common to both operations (e.g. only considers the differences in consumables, did not account for disposables that are same in both facilities). The HVAC systems were not included in the study too

Pietrzykowski *et al*., 2013: This study compared the environmental impacts associated with the production of mAbs using either single-use or traditional technologies using the LCA methodology at 3 different production scales; 100L, 500 L and 2000 L. This study employed a cradle-to-grave LCA analysis	Environmental impacts are expressed using end-point impact categories: damage to ecosystems, damage to human health and damage to natural resources	The study identified that the traditional manufacturing process has greater impact across all the end-point damage categories than the single use alternative at 100 L, 500 L and 2000 L	The study did not quantify the environmental impacts produced by the processes, however only expressed the impacts in-terms of end-point impact (the actual impacts produced are unknown)
Pollock *et al*., 2013: The study compared the environmental performance (water consumption and consumables production) of different processing strategies for mAbs production: fed-batch, spin-filter perfusion, and an alternating tangential flow (ATF) perfusion strategies. The strategies were compared across a range of scales (100, 500 and 1000 kg/year) and titres (2, 5, and 10 g/L)	E-Factor (kg/kg)* scores for water and consumables production	The fed-batch processing strategy performed better in terms of E-Factor scores (has lower water and consumable usage profile) than the perfusion processing strategies	The E-factor metric only looked at water consumption of process and non-process related steps for mAbs processing. The aspects related to energy consumption and carbon emissions were not evaluated This study is not a comprehensive environmental analysis that reflects the actual impact associated with mAbs production. A tool such as the LCA would also allow the full environmental impacts produced by the different processing strategies evaluated. LCA also allows various impact categories to be evaluated, such as toxicological impacts, global warming potential and acidification potential

**E-Factor is defined as the amount in kilograms of organic solvents, reagents, and consumables used per kilogram of the product produced.*

It is clear from Table 3.1 that there are a number of methodologies/tool that can be used to carry out environmental studies in the biopharmaceutical industry. However, these tools/methodologies are not ideal for comprehensive environmental impact quantification. LCA is the only tool/methodology that allows a thorough and detailed evaluation (ranging from raw material extraction to equipment disposal) of environmental impacts produced during biologics manufacture. It is also a quantitative tool that can handle several environmental issues (e.g. GWP, cumulative energy demand and cumulative water consumption) at one time.

.

3.4.4 The importance of LCA studies in the biopharmaceutical industry

Presently, the main priorities of the biopharmaceutical industry include manufacturing drugs that are safe for the patients (drugs that do not cause harmful effects when administered into patients) and manufacturing drugs in the most efficient and cost-effective manner without sacrificing quality or efficacy, and to achieve all of this in the shortest period of time (Deloitte, 2014). Process time reduction is an important factor for biologics as timely market penetration can be key to success (Farid *et al*., 2005). Another main priority of this industry is to secure a reliable and timely supply of raw materials for manufacturing, as delays may cause the biopharmaceutical industry to have serious financial losses (Van Trieste, 2014).

At present, assessing the environmental performance of biopharmaceuticals manufacture is not one of the main priorities for the industry. This is because the industry is relatively new (25-30 years), and it has not been heavily subjected to environmental legislation/policies. Unlike energy-intensive industries such as the steel, petroleum and chemical, the biopharmaceutical industry is also not facing

serious sustainability concerns at present. Thus, not many relevant environmental studies (especially LCA studies) exist.

However, this is expected to change as the industry matures. As more biologics enter the pipeline, the industry will contribute higher levels of environmental impact, driving governmental agencies to subject the industry to increasing environmental legislation and policies in order to control and then reduce the environmental impacts. This will increase the industry's sustainability concerns and will create the need for more comprehensive and rigorous environmental studies of the industry. LCA is the most ideal tool that will allow the biopharmaceutical industry to quantify the environmental impacts of manufacturing processes without shifting the environmental burdens from one stage/phase to another. The comprehensiveness of this tool should make it highly desirable to be employed in the biopharmaceutical industry's decision-making processes.

The biopharmaceutical industry can also benefit significantly through deploying the LCA tool to evaluate the environmental impacts of manufacturing processes. By quantifying and understanding the environmental impacts of manufacturing processes, the industry can for example decide to adopt clean technology concepts to reduce the environmental impacts. Adopting clean technology concepts in the industry not only improves the health of the industry, but also contributes to revenue growth, cost reduction, process waste and pollution reduction, whilst increasing operational efficiency and profit (Fiksel, 2010). Adopting clean technology concepts should also help the industry to be recognised as "environmentally responsible"

organisation and will improve the industry's reputation amongst consumers and investors (Fiksel, 2010).

This analysis clearly shows that the industry should make LCA studies a main priority, and incorporate them into their decision-making processes when selecting manufacturing processes that are efficient, cost-effective, reliable and environmentally feasible. This will not only help the industry to meet increasingly stringent regulatory requirements, but also improve overall manufacturing performance. This in turn will allow the industry to deliver products that are efficient, reliable, affordable and safe without harm to the environment.

3.4.5 Challenges of LCA analysis in the biopharmaceutical industry

Though LCA has been identified as the most suitable tool for environmental analyses of biopharmaceutical manufacturing processes, a number of challenges exist in carrying out LCA studies and include (Rebitzer, 2004):

- Identifying the system boundary for a biopharmaceutical manufacturing process;
- obtaining the LCI data for a biopharmaceutical manufacturing process given the relative lack of experience with LCA in the biopharmaceutical industry;
- and verifying the LCI data.

3.4.5.1 Identifying the system boundary

Identifying the system boundary for a biopharmaceutical manufacturing process can be challenging as the current LCA methodologies and the standards set by the ISO create difficulties when defining system boundaries (Suh *et al*., 2004). The different

LCA system boundary approaches that can be considered for environmental analysis of biopharmaceutical processes include cradle-to-grave, cradle-to-gate and gate-to-gate (detail explanations of these system boundary approaches are provided in Section 3.3.4). The different LCA system boundary approaches relate to particular types of study. It should be acknowledged that failure to define properly the system boundaries could cause a study to be considered invalid (Matthews and Small, 2000). This is because important activities that may have significant impacts are not captured. An LCA practitioner should analyse the objectives of a given study carefully before drawing a system boundary.

3.4.5.2 Obtaining the LCI data

LCI data for biopharmaceutical manufacturing processes typically consist of data on equipment/consumables fabrication, media/buffer preparation, WFI/PW production, equipment utilisation, waste management activities and recycling of equipment at the end-of-life phase. Obtaining these data can be hard given the relative lack of experience with LCA in the industry. Unlike in other industries, only limited numbers of biopharmaceutical LCA studies exist (Mauter, 2009; Pietrzykowski *et al*., 2013). The life cycle inventory available in these studies is, however, specific to the system boundaries considered. For example, the LCA studies carried out by Mauter (2009) focused on a single unit operation (bioreactor/filtration system) rather than on a whole manufacturing process. Hence, LCI data for biopharmaceutical processes are very limited. There are no databases specific to the biopharmaceutical industry, thus, seeking data for studies on biopharmaceutical processes can be laborious. This challenge could be addressed to some extent by using data from similar industries such as the chemical and pharmaceutical industries. One example

might be on the impact of water and energy usage. Carrying out interviews with experts within the industry to gather information and data on the specific process steps involved in biopharmaceutical manufacturing processes might be a suitable way forward. Clearly, a major effort is required to establish a proper data collection and to build the relevant LCI data needed for LCA studies in the biopharmaceutical industry (Udo De Haes *et al*., 2002).

3.4.5.3 LCI data verification

Upon obtaining the LCI data, there is a need to verify and validate it. This represents a further challenge for the biopharmaceutical industry. One approach could be to compare the data with that from similar industries (e.g. chemical and pharmaceutical). This is possible because the manufacturing processes have some common features (Mata *et al*., 2012). Validation of the robustness of the LCI data can also be carried out by sensitivity and uncertainty analyses (Baumann and Tillman, 2004; Pietrzykowski *et al*., 2013). Sensitivity analyses evaluate how parameter choices affect the outcome of an LCA study (Pietrzykowski *et al*., 2013). Uncertainty analyses on the other hand evaluate the effect of imprecise data on the outcome of an LCA study (Baumann and Tillman, 2004; Pietrzykowski *et al*., 2013). Once conducted, the results of such analyses give further confidence in the prediction made and hence of the value of the model to real life decision-making.

It is clear that responding effectively to these challenges requires a broader understanding of both the biopharmaceutical processes and of the relevant life cycle assessment methodology. This research will demonstrate the efforts carried out to overcome these challenges in order to carry out environmental studies for a

biopharmaceutical manufacturing process. By addressing these challenges, it is hoped that robust and reliable environmental analyses of biopharmaceutical manufacturing processes can be carried out in the future.

This section highlighted the roles and importance of LCA in the biopharmaceutical industry to evaluate environmental impacts and the challenges of carrying out LCA in the biopharmaceutical industry. The next section will detail the systematic approach used to develop the environmental framework.

3.5 A Proposed Environmental Assessment Framework for Biopharmaceutical Processes

3.5.1 Scope of the framework

A systematic approach was used to develop an environmental assessment framework for biopharmaceutical processes. The following questions were considered before defining the scope of the framework:

- What are the objectives of the framework?
- What life cycle phases are included in the assessment (whether it is cradle-to-grave, cradle-to-gate or gate-to-gate)?
- Which environmental impacts can be evaluated using the framework?

The final design of the framework will depend strongly on the answers to the questions above and, therefore, these aspects must be carefully considered. Figure 3.4 shows the schematic representation of the proposed environmental framework developed in this work. This framework is seen as a complex system with different

life cycle phases (subsystems). Each of the life cycle phases consists of various activities. These activities require resources and results in environmental impacts.

The first stage of defining the scope of the framework was to identify the main life cycle phases considered by the overall system. The system includes three life cycle phases: supply-chain, use and end-of-life. The following step was to define the activities involved in these life cycle phases. The supply-chain phase activities included stainless steel equipment fabrication (fabrication process of stainless steel equipment used in manufacture such as vessels and columns), consumables fabrication (fabrication process of consumables used in manufacture such as filters and resins) and reagent production (preparation of different types of reagent used in manufacture such as media and buffer).

The activities in the use-phase included equipment utilisation (equipment operations in the facility to manufacture biologics), waste management (treatment and disposal of solid and liquid waste produced in the manufacture) and lighting & HVAC (lighting and HVAC requirements of the facility where manufacture takes place).

The end-of-life phase considered the disposal of fixed-in-place stainless steel equipment after a period of 15 years (this period was assumed fixed in this work). These life cycle activities are described in detail in Chapter 4, Section 4.3.
Having identified the scope of the framework, the next section will highlight the application of LCA in the proposed framework.

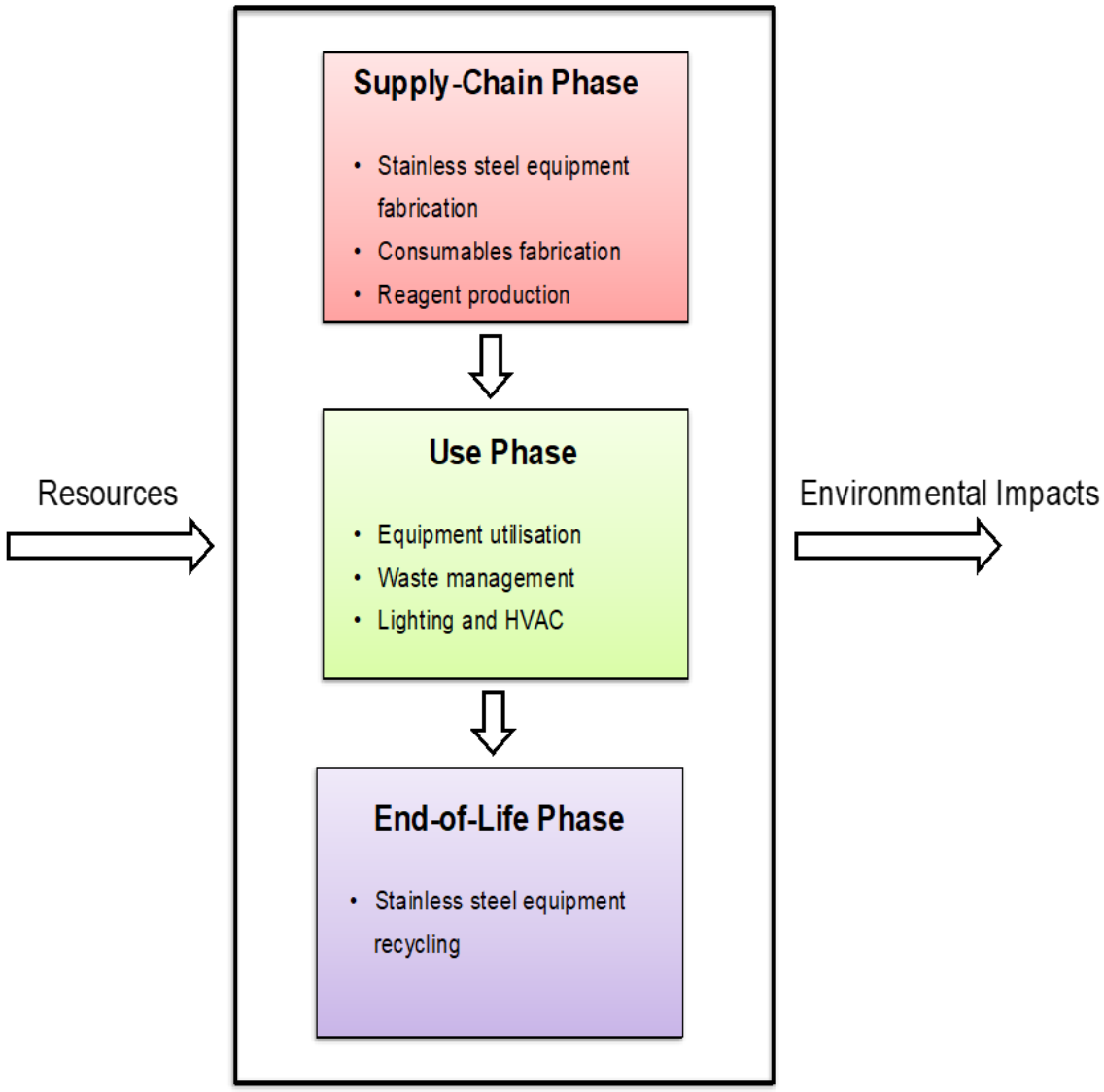


Figure 3.4 *A framework developed using a systematic approach for environmental assessment of biopharmaceutical manufacturing processes.*

3.5.2 Application of LCA in the proposed framework

The goal of this framework is to use it to determine the environmental impacts produced by the biopharmaceutical industry taking the manufacture of monoclonal antibodies as a test case. This will illustrate how such a capacity may allow the industry to make decisions in which the environmental impact is a factor. The assessment in this thesis looked at the production of monoclonal antibodies over a 20-batch campaign at 200 L operating scale. This is the basis for comparison in the

study. Attributional LCA with system expansion was used in the analysis as it provides a better method to treat co-product allocation (Finnvaden, 2008). The advantages of using system expansion are the ability of a system to reproduce the real world situation and to avoid difficult allocations as recommended in the ISO standards (ISO, 2006a). The overall LCA approach developed and applied in the framework is shown in Figure 3.5. The system boundary of the framework was selected to be cradle-to-grave (see Section 3.3.4). The phases and activities considered by the framework are provided in Figure 3.4 (Section 3.5.1).

The foreground system (highlighted in grey in Figure 3.5) includes the main activities that are being considered in the study (*direct burdens*). Foreground activities of this analysis include equipment utilisation, waste management, lighting and HVAC. In this analysis, only the use-phase activities were from the foreground system.

The background system included activities that are not directly involved in the study but however were included in the system boundary of the study (*indirect burdens*). These activities include equipment and consumables fabrication, reagent production and stainless steel equipment recycling. In this analysis, the activities involved in the supply-chain and end-of-life phases were from the background system. Another key aspect considered by the framework is transport. The transport involved in the foreground and background systems were included in this study.

The LCA methodology diagram highlights the manufacturing and solid waste management alternatives considered within the framework. This methodology

diagram also shows the scenarios that were developed in order to demonstrate the functionality of the framework in evaluating the manufacturing and solid waste management alternatives. The proposed framework was applied to a case study to evaluate these scenarios. A detailed description of this case study is provided in Section 3.6.

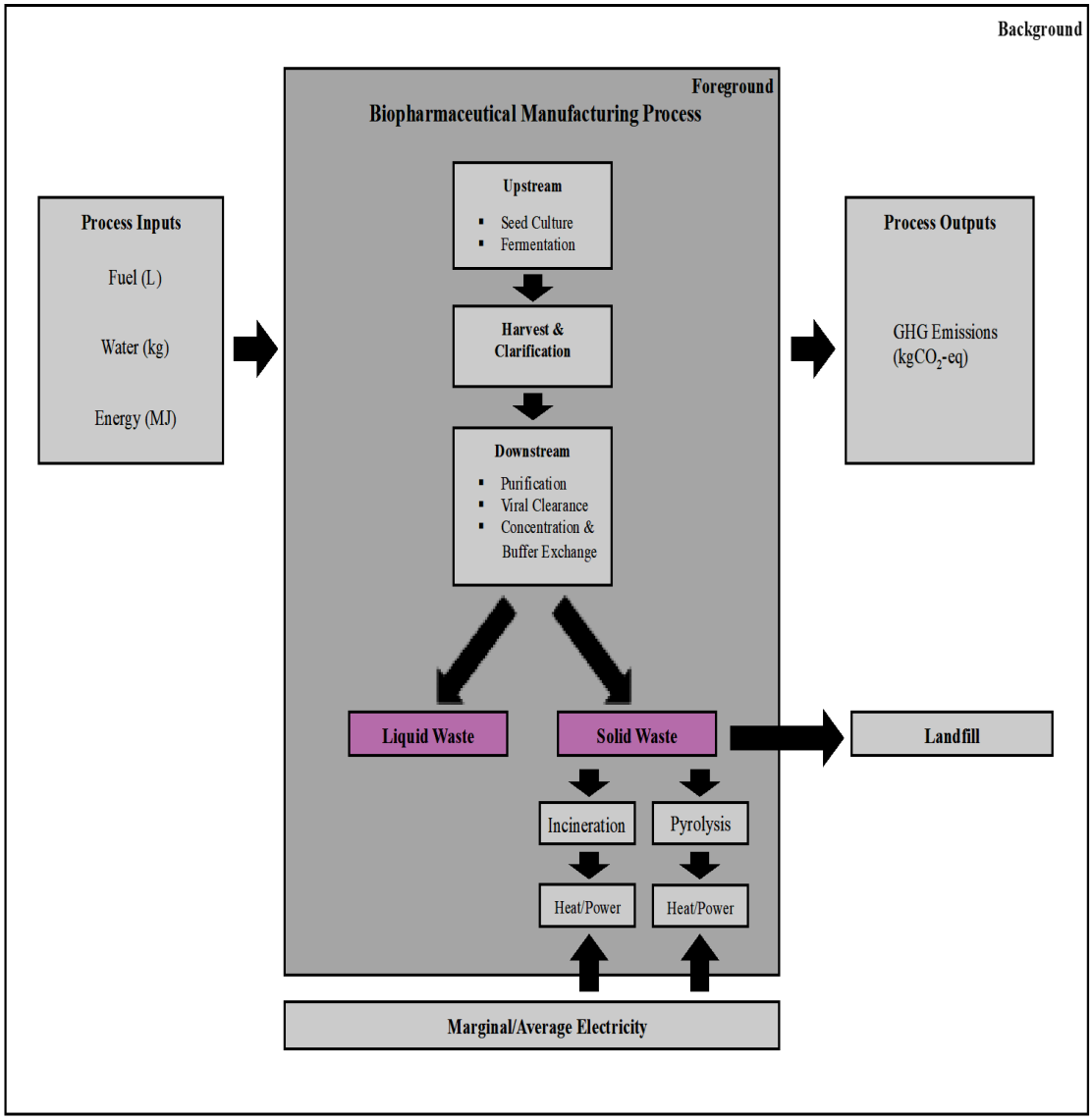


Figure 3.5 *LCA methodology diagram. The diagram shows the different solid waste management options available to the biopharmaceutical industry. The activities in the foreground and background systems and the process inputs and outputs of the system studied are shown.*

In this framework, the following environmental impacts were evaluated: resource use (cumulative energy and water consumption) and global warming potential (GWP) (see Table 3.2); the cumulative energy consumption is expressed in megajoule (MJ), the cumulative water consumption is expressed in kilogram (kg), and GWP is expressed in kilogram carbon dioxide equivalent ($kgCO_2$-eq). GWP was evaluated using the CML 2001 characterisation method (Guinee *et al*., 2002).

These environmental impacts in turn determine the types of life cycle inventory data collected in the analysis.

Table 3.2 *Environmental impacts considered by the framework.*

Resource use:	
• **Cumulative Energy Consumption**: Comprises energy consumption from all the activities involved in the supply-chain, use and end-of-life phases. The energy consumed for water generation is also considered • **Cumulative water consumption**: Comprises water consumption from all the activities involved in the supply-chain, use and end-of-life phases	• **Global Warming Potential (GWP)**: Comprises carbon emissions from all the activities involved in the supply-chain, use and end-of-life phases. The carbon emissions produced from electricity and water generation and transport were also included in the framework

As highlighted earlier, the Excel Software Package V. 2010 was used to carry out the LCA analyses. Each activity within the life cycle phase was modelled as a process flow sheet that analyses the levels of energy, water and carbon emissions of the system studied.

3.6 Case Study

3.6.1 Manufacturing processes and solid waste management methods considered in the case study

The functionality of the proposed framework in environmental assessments was demonstrated by application to a case study. The objectives of the framework were:

- To evaluate the environmental impacts of monoclonal antibody manufacturing alternatives, operated on a batch basis,
- To evaluate the environmental impacts of solid waste management methods for the biopharmaceutical industry.

The manufacturing alternatives considered in the framework were traditional and single-use technology processes. In this study, adoption of a fully single-use process was not pursued because for some of the unit operations, single-use equipment is not yet well-established (Guldager, 2009). Thus, a monoclonal antibody process employing traditional equipment, and a hybrid monoclonal antibody process employing a mix of traditional and single-use equipment were both established. The design of these process models was based upon discussions with industrial experts combined with extensive literature and analyses to guide process selection and

equipment sizing. These process models are described in-detail in Sections 3.6.1.1 and 3.6.1.2.

The system boundary of the framework was only set to include the primary processes (upstream and downstream manufacturing stages). The framework did not include drug product manufacturing stage (secondary processes), which involves the formulation/fill/finish steps, packaging and global delivery of the products to the customers/patients for the traditional and hybrid processes (see Chapter 2, Section 2.4.7). A drug product manufacturing stage typically involves thawing of the frozen product that has been stored at a validated storage temperature, preparation of the pH buffering agent, sterile filtration of the solution, and filling of the solutions into vials or syringes. The vials are often lyophilized (freeze dried) as they contain sensitive products (e.g. monoclonal antibody) (Kovarcik, 2016). The lyophilization processes is known to be energy-intensive as it is time consuming and extensive (involves four stages including pretreatment, freezing, primary drying and secondary drying) (Nireesha *et al.*, 2013). However, it is anticipated that the environmental impacts from the secondary processes/drug product manufacturing are significantly lower than the impacts produced from the bulk drug substance manufacturing (upstream and downstream stages). The drug product manufacturing stage handles material in smaller volumes (typically in mLs) compared to the bulk drug substance manufacturing, where large volumes of materials are handled (Petrides *et al.*, 2011). Thus, compared to the bulk drug substance stage where large vessels/equipment are typically employed, the drug product manufacturing stage employs smaller sized vessels/equipment. The smaller inventory used in the drug product manufacturing results in this stage contributing to substantially lower environmental impacts than

the bulk drug substance manufacturing stage.

Another activity that could contribute a significant environmental impact in the drug product manufacturing stage is the delivery of products to the customers/patients. Again, it is anticipated that the levels of impact from the distribution of monoclonal antibodies to the customers/patients will be significantly lower to the movement of bulk inputs (e.g. raw materials, equipment and consumables) to the upstream process. The industry can further reduce the levels of environmental impact from transport in the drug product manufacturing stage by deploying efficient transport modes, and locating the monoclonal antibody manufacturing sites strategically (e.g. establishing manufacturing facilities nearer to their main customers) (Petrides *et al*., 2011).

The solid waste management methods that were considered in this study include landfill, incineration and pyrolysis. As highlighted in Section 3.4.2, these are the methods that can be considered in this industry at present, and are described in-detail in the following.

3.6.1.1 Traditional process model

The traditional process model was assumed to consist solely of fixed-in-place stainless steel equipment, where cleaning-in-place (CIP) and sterilisation-in-place (SIP) are required between production batches. Each batch in the traditional process is produced by a series of operations that proceed from fermentation (cell culture) through to product recovery and finally purification, in a train of vessels. Following this, the bulk drug substance produced undergoes two more additional steps: the

formulation/fill/finish and packaging steps before the final product is sent for delivery (drug product manufacturing). As described in Sections 2.4.7 and 3.6.1, the drug product manufacturing stage (secondary processes) was not considered in this analysis.

The cell culture stage is associated with large-scale production of product targeting cells. This stage involved two batch seed fermentation operations to provide the material for a single fed-batch fermentation operation (Kelley, 2007). Stainless steel stirred tank bioreactors were employed in the traditional flow sheet. Harvest and clarification steps were employed immediately after the fermentation step to remove the production cells and to prepare a supernatant essentially cell-free from which to recover the protein of interest (Kelley, 2009). For the traditional process, disc-stack centrifuge (Alfa Laval, Lund, Sweden) and depth filtration (Pall Life Sciences, Lund, Sweden) unit operations were employed for cells removal.

The subsequent downstream stages consisted of a number of purification steps selected to reflect a commonly used flow sheet. Purification is typically achieved using a combination of column chromatography and membrane filtration steps. In this process model, affinity chromatography (GE Healthcare, Uppsala, Sweden), cation-exchange chromatography (GE Healthcare, Uppsala, Sweden), anion-exchange chromatography (GE Healthcare, Uppsala, Sweden), ultrafiltration/diafiltration (Pall Life Sciences, Lund, Sweden) and sterile filtration (Merck Millipore, Darmstadt, Germany) operations were employed (Shukla *et al*., 2007; Low *et al.*, 2007). In addition, viral clearance steps are required to remove viral contaminants derived from mammalian cells or contaminated biological

reagents (Low *et al*., 2007). In this process model, a combination of chemical inactivation and nanofiltration steps were employed.

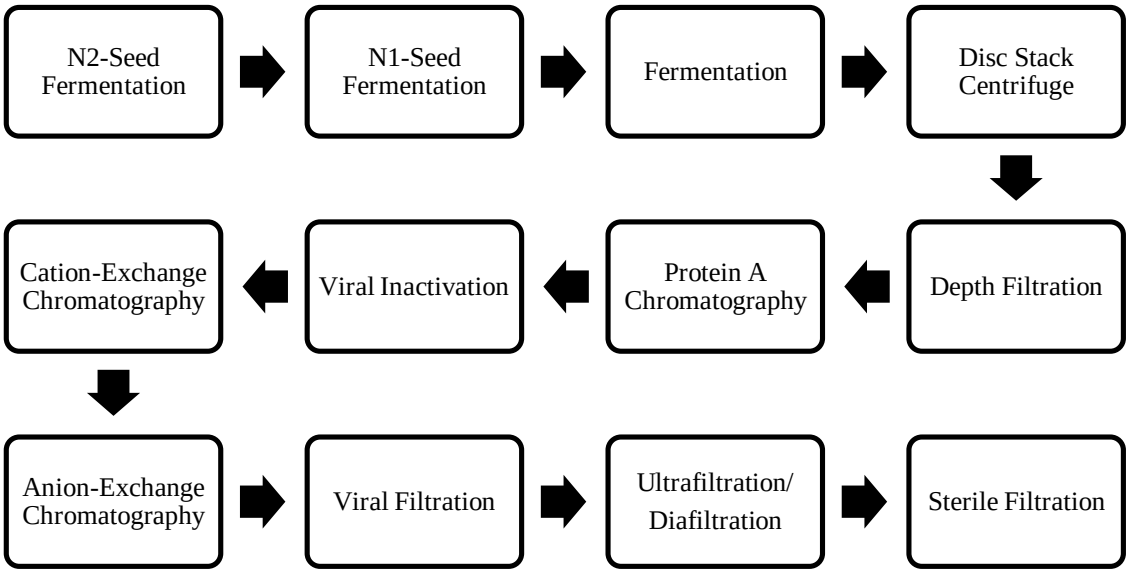


Figure 3.6 *Diagram represents a traditional monoclonal antibody manufacturing process employing entirely stainless steel equipment.*

Additional manufacturing operations are involved indirectly in the traditional process model. These include the preparation of intermediate materials such as media and buffer solutions. The vessels used to prepare and hold these solutions are made of stainless steel material. Further support equipment vessels such as product storage vessels, water-for-injection (WFI) still, clean steam generator, WFI and pure water (PW) storage and waste collection vessels are also made of stainless steel material in the traditional process model. Figure 3.6 shows the flow sheet for a traditional process model.

3.6.1.2 Hybrid process model

A hybrid process model was used to provide a comparison against the conventional process. As mentioned earlier, adoption of a fully single-use process was not pursued because for some of the unit operations, single-use equipment are not yet well-established. The hybrid process therefore consisted of a mix of fixed-in-place stainless steel and disposable equipment. The disposable equipment was assumed to be made of plastics, and that such equipment was disposed of at the end of each production batch.

In the hybrid process model, Single-use Wave Bioreactors (GE Healthcare, Uppsala, Sweden) were employed in the cell culture stage for the large-scale production of cells expressing the target product. For the harvest/clarification step that immediately follows the cell culture stage, a single-use tangential flow microfiltration (Pall Life Sciences, Lund, Sweden) and depth filtration (Pall Life Sciences, Lund, Sweden) were employed to provide a cell and cell debris-free process stream suitable for applications in packed bed purification streams (Johnston, 2010). The purification stages for the hybrid process model were assumed to be similar to the traditional process. However, the anion-exchange chromatography and viral filtration unit operations were replaced by single-use membrane adsorber chromatography (Sartorius Stedim, Gottingen, Germany) and viral filtration (Pall Life Sciences, Lund, Sweden). This decision was possible because the single-use membrane adsorber chromatography and viral filtration are widely employed in commercial bio-manufacturing processes and, therefore, the benefits are understood (Lim *et al.*, 2008).

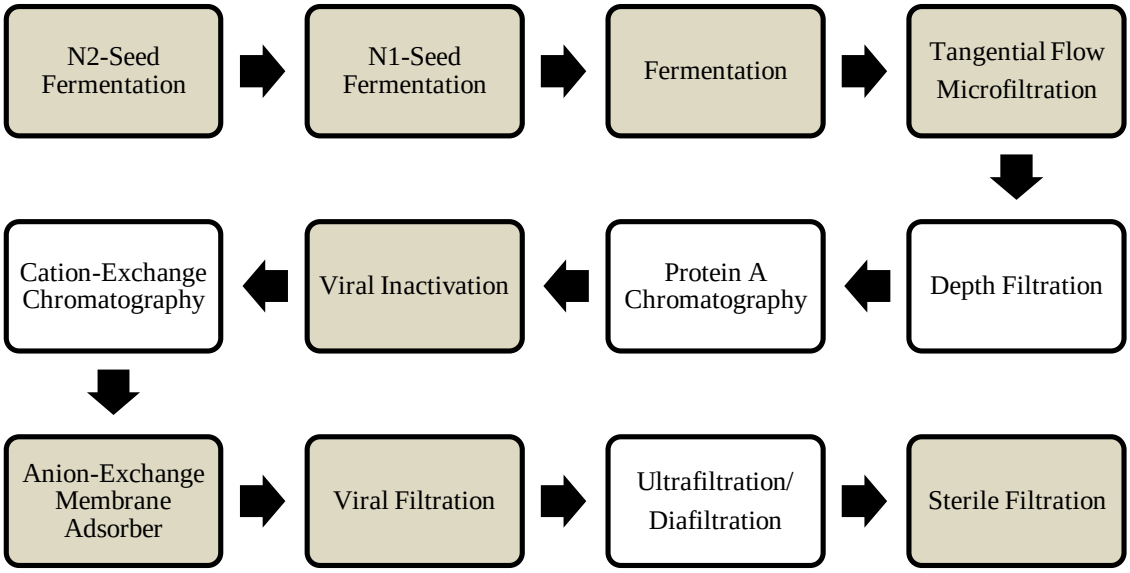


Figure 3.7 *Diagram represents a hybrid monoclonal antibody manufacturing process employing a mixture of stainless steel and single-use equipment. The grey boxes indicate single-use unit operations.*

As described in Sections 2.4.7 and 3.6.1, the formulation/fill/finish steps, packaging, and delivery of the final monoclonal antibody product (drug product manufacturing) for the hybrid manufacturing process were not considered in this analysis. Additional manufacturing equipment to prepare and hold media and buffer solutions were assumed to be single-use bags. The single-use bags were also used to collect the products. However, for waste collection, WFI and PW storage, stainless steel vessels were employed. The basis for this decision was; single-use bags were used when the volume of material collected is below 2000 L (single-use bags are available only up to this volume) and when the volume of material being collected was above 2000 L, stainless steel vessels were employed (Eibl *et al*., 2010a). Further support equipment such as WFI still and clean steam generator was included in the study. The support equipment in the hybrid process is much smaller in size compared to the support

equipment in traditional process due to the major reductions in the water use (Pietrzykowski *et al.*, 2012). Figure 3.7 shows the flow sheet for a hybrid process model.

3.6.1.3 Landfill

In this work, a conventional UK landfill with surface and basic sealing was assumed as a possible solid waste management method. The landfill model was obtained from the GaBi 6.0 software (the description of this software is provided in Section 3.6.4 and the landfill model is provided in Appendix 4.4). The model considered electricity and thermal energy and also fuels to be used on-site. The method also considered leachate treatment, sludge treatment and deposition. Leachates are defined as aqueous effluent generated from rainwater percolation through wastes, biochemical processes in waste's cells and the water content from the wastes itself (Renou *et al.*, 2008). Leachate treatment in this model included active carbon and flocculation/precipitation processing. The process for sealing materials was included for this method. The sealing contains gravel, sand, clay and polyethylene films. Gravel and sand are used as filter layers, PE film provided a waterproof sealing and clay as mineral coverage to the surface and basic sealing. All manufacturing processes of the sealing materials were also considered.

3.6.1.4 Incineration with energy recovery

In this work, a waste-to-energy (incineration) plant with typical technology used in Europe was assumed. The incineration model was obtained from the GaBi 6.0 software (the incineration model is provided in Appendix 4.4). This model consisted of an incineration line fitted with a grate and a steam generator. In the incineration

model, the steam produced from the incineration plant was mainly used internally as process steam and the balance used either to generate electricity or exported as heat to local industry or households. The average efficiency of the steam production is 82%. In this study, the incineration model considered all utilities used in the waste incineration plant, flue gas treatment, the NOx removal system, the treatment of air pollution control (APC) residues, the operation of the underground deposit and the landfill for bottom ash, and the credits for metals' recovery. The incineration model employed dry flue gas treatment (FGT) for flue gas treatment and Selective Non-Catalytic Reduction (SNCR) for NOx removal. The manufacturing processes of the materials required for these activities were also considered. Credits for electricity and heat were considered in this model (GaBi, 2014).

3.6.1.5 Pyrolysis

In this work, pyrolysis was used to investigate whether a sustainable waste disposal approach could be achieved. The model studied consisted of a fast pyrolyser. The pyrolysis model was obtained from the Royal Dalam study (Rabou *et al*., 2009; Lombardi *et al*., 2015). The pyrolysis model considered all utilities used in the pyrolysis plant, the flue gas treatment, the tar treatment and the syngas treatment. The flue gas treatment was achieved using a bag house filter. The tar was treated using an advanced scrubbing technology where mixtures of tar and water were avoided. The tar was then used to fuel the pyrolyser. The syngas was treated using a wet scrubbing system to remove water, chloride and ammonia from the gas. The heat and electricity for this model is usually generated from the clean syngas. The manufacturing processes of the materials required for flue and clean gas and tar

treatments were also considered in this model. Credits ("avoided" burdens) for electricity and heat were considered in this model.

3.6.2 Case study scenarios

In order to determine the environmental impacts of different manufacturing alternatives and solid waste management methods, six scenarios were developed. The scenarios were based on the processes described in Section 3.6.1. The list of these scenarios are provided below:

- Traditional process with landfill solid waste management method (Trad/Landfill)
- Traditional process with incineration solid waste management method (Trad/Incineration)
- Traditional process with pyrolysis solid waste management method (Trad/Pyrolysis)
- Hybrid process with landfill solid waste management method (Hybrid/Landfill)
- Hybrid process with incineration solid waste management method (Hybrid/Incineration)
- Hybrid process with pyrolysis solid waste management method (Hybrid/Pyrolysis)

The data on the energy and water consumption and carbon emissions of the scenarios are provided in Chapter 4, where the life cycle inventories of the activities evaluated by the framework are presented, and the methodology in obtaining these inventories are clearly described.

In this analysis, the traditional and hybrid processes were run on a batch campaign at a 200 L operational scale. This scale is typically employed in the clinical phase of biopharmaceutical manufacture, and does not represent production scale (Pietrzykowski *et al*., 2013). Presently, the operational scale of biopharmaceutical manufacture can go up to 200 000 L (Gronemeyer *et al*., 2014).

An operational scale of 200 L was selected in this case study because it was perceived that the data collection process would be easier for biopharmaceutical manufacturing processes at such a scale of operation. This is mainly due to easy availability of biopharmaceutical inventory data, especially primary data (e.g. easy access to manufacturing data from the UCL's Biochemical Engineering Pilot Plant facility). Also at 200 L operational scale, the traditional and hybrid processes can be compared (one of the objectives of the research was to compare the environmental impacts between the traditional and hybrid processes). Choosing a larger operational scale (above 2000 L) would not allow this objective to be met as currently, single-use have limitations in the operational scales (the maximum scale of single-use bioreactors is 2000 L).

Finally, it is also easier to understand and carry out environmental modelling on manufacturing processes with smaller operational scales as limited manufacturing data/information are currently available for biopharmaceutical manufacturing processes. However, as significant knowledge and understanding are gained in this area of research, environmental analysis for manufacturing processes with larger operational scales can be carried out. Hence, this should be a motivation for future work, as it will reflect the industry's true environmental contributions.

For the environmental analysis in the case study, a number of parameters were established for each of the processes. The main parameters that were considered include; (a) process titre, (b) downstream processing yield and (c) number of batches.

(a) Process titre

The process titre for traditional and hybrid processes were assumed to be 5 g/L. This assumption was made based on the average process titre obtained for monoclonal antibody processes using mammalian host cells. Presently, the average process titres for such processes are between 2-5 g/L (Gronemeyer *et al*., 2014).

(b) Downstream processing (DSP) yield

The DSP yield is a function of the individual step yields and the number of downstream processing steps (Farid, 2007). The average DSP yields for the traditional and hybrid processes were estimated to be 75%. The DSP yields for the processes were estimated by using the Equation 3.1. A process yield ranging between 85%-95% was assumed for each downstream processing step (Hill *et al*., 2010).

$$\frac{\textbf{Total process yield of all the DSP unit operation}}{\textbf{Number of DSP unit operations}}$$

(**Equation 3.1)**

(c) Number of batches

The number of batches for the traditional and hybrid processes were estimated based on factors that include cell culture time (including equipment preparing time for the next production batch) and short plant shut down period (Kelley, 2009). Based on a cell culture time of 12-14 days (mammalian cell culture) and a short plant shut down

period, an estimate of 20 batches per year was made. This is consistent with the range of batches of 20-24 that are currently employed in the biopharmaceutical industry for mammalian cell based monoclonal antibody manufacturing processes (Kelley, 2009).

As highlighted earlier, the system boundaries of these scenarios were set to be cradle-to-grave. Thus all the scenarios investigated had 3 life cycle phases; supply-chain, use and end-of-life. The activities involved in these phases have been highlighted in Figure 3.4. The detailed descriptions of these activities, and the life cycle inventory of these activities are provided in the following chapter (Chapter 4). The outcome of the case study is presented in chapter 5, where the environmental impacts of all the scenarios will be highlighted. The manufacturing process and the solid waste management method with the least environmental impact will be identified in this chapter.

Having evaluated the environmental impacts produced by the traditional and hybrid process models, “hot spot” analysis on the process that contributes to the highest level of environmental impacts was carried out. The “hot spot” analysis is typically carried out to identify the individual contribution of life cycle stages/activities to the overall environmental impacts. Through this analysis, the life cycle activities or stages that contribute to the highest environmental impacts can be determined. The “hot spot” analysis on the manufacturing process is presented in Chapter 5, Section 5.3.

3.7 Environmental Impacts Reference

In order to provide an understanding of the magnitude of the impacts produced by bioprocesses, the annual levels of consumption of a typical UK household was used as a basis for comparison in this study. The annual levels of consumption are provided in Table 3.3.

Table 3.3 *Annual levels of consumption of a typical UK household (CC Water, 2015; Carbon Independent, 2015; Palmer and Cooper, 2012).*

Energy Consumption (MJ) x 10^4	1.7
Water Consumption (kg) x 10^4	30
Carbon Emissions ($kgCO_2$-eq) x 10^4	0.2

The information provided above can be valuable as a basis by which to compare the impacts arising from a monoclonal antibody process. This will enable the readers to understand truly the magnitude of the impacts produced by monoclonal antibodies manufacture.

3.8 Conclusion

A novel environmental assessment framework to facilitate the decision-making process in the biopharmaceutical industry has been proposed. A systematic approach was employed to develop this framework, and is presented in this chapter. This framework allows the biopharmaceutical industry to evaluate the environmental impacts of a given manufacturing process. By understanding the environmental impacts of the processes, the decision-makers can make informed decisions when

selecting processes and solid waste management methods, ensuring that they are environmentally feasible.

The framework in this work was based on an LCA tool because LCA provides a thorough evaluation of the environmental impacts produced by processes. LCA is also preferred over other tools such as RA, EIA, EA and MFA (see Section 3.2.2) because it is process/product oriented, quantitative and can handle several environmental issues at one time (e.g. GWP, water usage, energy consumption, etc.).

To test the feasibility of the proposed framework, it was applied to a case study. In the case study, six scenarios focusing on traditional and hybrid processes with landfill, incineration and pyrolysis solid waste disposal options were evaluated. The life cycle inventory of the activities evaluated by the framework in this research is presented in Chapter 4. The inventory data were obtained from a number of sources including expert interviews and discussions. The methods used to obtain the life cycle inventory data of these activities are also highlighted in the following chapter.

Chapter 4. Life Cycle Inventory

4.1 Introduction

This chapter presents the life cycle inventory for the environmental studies investigated in the case study using the framework developed (described in Chapter 3, Section 3.5) in this research. The framework based on the LCA methodology was deployed in the case study to evaluate the environmental impacts of manufacturing alternatives used to manufacture monoclonal antibodies. The framework was also deployed in the case study to evaluate different solid waste management disposal options for the biopharmaceutical industry (see Section 3.6.2 for all the scenarios investigated in the case study).

The primary objectives of this chapter are to present the inventory data of biopharmaceutical manufacturing processes, and to describe the methodology employed to obtain these data. This chapter also provides a detailed description of the life cycle phases evaluated by the framework in the case study, and all the activities within the life cycle phases. In this research, the life cycle inventory data were collected from a number of sources, including expert/vendor interviews, software packages (e.g. LCA software) and literature surveys.

This chapter is divided into five sections. Section 4.2 presents the sources used in this research to obtain the life cycle inventory of all the phases/activities evaluated by the framework. In Section 4.3, the data collection methodology is described. Section 4.4 highlights the overall assumptions made in the case study. Finally, in section 4.5, the summary of the chapter is provided.

4.2 Data Sources

As highlighted previously in Chapter 3, any life cycle assessment study requires extensive data. It is imperative to obtain data from reliable and valid sources to make the analysis as accurate as possible. As described in the previous section, the sources used to obtain the life cycle inventory data for all the activities in the case study include BioSolve Process Enterprise Software (Biopharm Services, Amersham, UK), GaBi Product Sustainability Software (GaBi 6.0) (PE International, Leinfelden-Echterdingen, Germany), expert interviews and suitable literature surveys and analyses.

The compiled life cycle inventory data obtained were tested to ensure these data were sensible and robust. This was done through a series of discussions with industrial experts. In addition, sensitivity analyses (Chapter 6) were carried out to study the effect of certain parameters/data on the overall environmental impacts that were estimated.

4.2.1 BioSolve process enterprise

BioSolve Process Enterprise is a software package that has been developed to model biopharmaceutical manufacturing such as for vaccines, monoclonal antibodies and therapeutic protein manufacturing. The software is widely used by the biopharmaceutical industry in early process development phases to gain a better understanding of protein manufacturing processes and in order to develop efficient, flexible and cost-effective processes. The BioSolve software package was mainly used in this research to obtain important information such as the list of stainless steel equipment and consumables for the traditional and hybrid processes, the amount of

reagents used in the manufacturing processes, and the types and amount of waste produced by the traditional and hybrid processes (see Figures A1-A6 in Appendix 3.1 for the screenshots of the BioSolve software). This software was also used to obtain the equipment sizing and consumables use levels.

4.2.2 GaBi 6.0

GaBi 6.0 is based on a mass and energy balance approach that calculates life cycle inventories and associated life cycle impacts. It uses a process flow sheet type representation of the system being examined and has been developed with an object-orientated programming approach. A number of databases are implemented in the GaBi 6.0, and include PE International, Plastics Europe, Ecoinvent, together with national and regional databases (see Figures A7-A10 in Appendix 3.2 for the screenshots of the GaBi software). The life cycle inventory for the work carried out in this research was mainly obtained from the PE International database (will be described in detail in the following sections). This database was mainly used in this research to obtain environmental impacts data (energy and water consumptions and carbon emissions) of activities involved in consumables and stainless steel equipment fabrication and transport. The solid waste management models (Sections 3.6.1.3-3.6.1.5) and inventories were also obtained from this database.

4.2.3 Expert/vendor interviews and literature surveys

The life cycle inventory collection methodology employed in this research used a series of discussions with expert/vendor and literature surveys. The main expert/vendors involved in this research included:

- H. Ihre (GE Healthcare, Stockholm, Sweden): mainly for information on resin fabrication and column fabrication and the inventory data.
- A. Dey-Chowdhury (Pall Life Sciences, Portsmouth, UK): mainly for information on cross-flow and normal flow filter fabrication.
- S. Ragunathan (TWI, Cambridge, UK): mainly for information on stainless steel equipment fabrication.
- T. Sau Haung (Sun Seng Lee Engineering, Penang, Malaysia): mainly for information on stainless steel equipment fabrication and inventory data.
- A. Sinclair (BioPharm Services, Amersham, United Kingdom): information on how the biopharmaceutical industry manages liquid and solid waste. The information on the industry's requirement for lighting and HVAC, and the industry's end of life phase management were discussed.
- K. Gebauer (GE Healthcare, Uppsala, Sweden): information on single-use Wave bioreactor fabrication.
- G. Jagschies (GE Healthcare, Uppsala, Swdeden): information on single-use bioreactor and column fabrication.
- S.Hassan (UCL, UK): information on clinical trials of mAbs and the batches/scales typically employed in the clinical development phase.

The discussions with the industrial experts and vendors clearly helped in developing the life cycle phases for the framework, and also in identifying the key activities/processes in the life cycle phases that could be significant contributors to the overall environmental impacts. Without the help from these experts, it would have been a challenging task to establish the system boundary of the framework.

As described before in Section 3.5, a systematic approach was used to develop the framework. This framework is seen as a complex system with different life cycle phases (subsystems). Each of the life cycle phases consists of various activities. These activities require resources and results in environmental impacts. The next section presents the data collection methodologies employed in this research to obtain the inventory data for all the activities evaluated by the systematic framework developed in the research. In this section, the life cycle phases evaluated by the framework, and the activities involved within these phases will be described. This includes describing the fabrication process of supply chain equipment, the production of reagents needed for monoclonal antibody development, the waste management activities involved in the use phase and finally the disposal of equipment after a certain period in the end-of-life phase.

4.3 The Life Cycle Inventory Collection Methodology

As described previously (Section 3.6), a case study was set up to demonstrate the functionality of the framework in evaluating the environmental impacts of biopharmaceutical manufacturing processes. In the case study, the environmental impacts of traditional and hybrid manufacturing processes run at 200 L operational scale with 20 batches per annum were evaluated.

The environmental impact produced per annum was used as the basis to compare (functional unit employed in this study) the environmental contribution of different manufacturing and solid waste management alternatives. The cumulative energy and water demand and GHG emissions of all activities were adjusted according to this functional unit. Employing a functional unit allowed environmental impact

comparisons to be made with different manufacturing and solid waste management alternatives. The disadvantage of employing this functional unit is that for some activities the total/true impacts produced by an activity (e.g. environmental impacts of bioreactor fabrication) are not presented. For example, in equipment fabrication, a stainless steel bioreactor has a life span of 15 years. Thus, in order to adjust the impacts produced according to the functional impact selected, the impacts were divided with the equipment life span to obtain the impacts per year (this will be explained in-detail in the following sections).

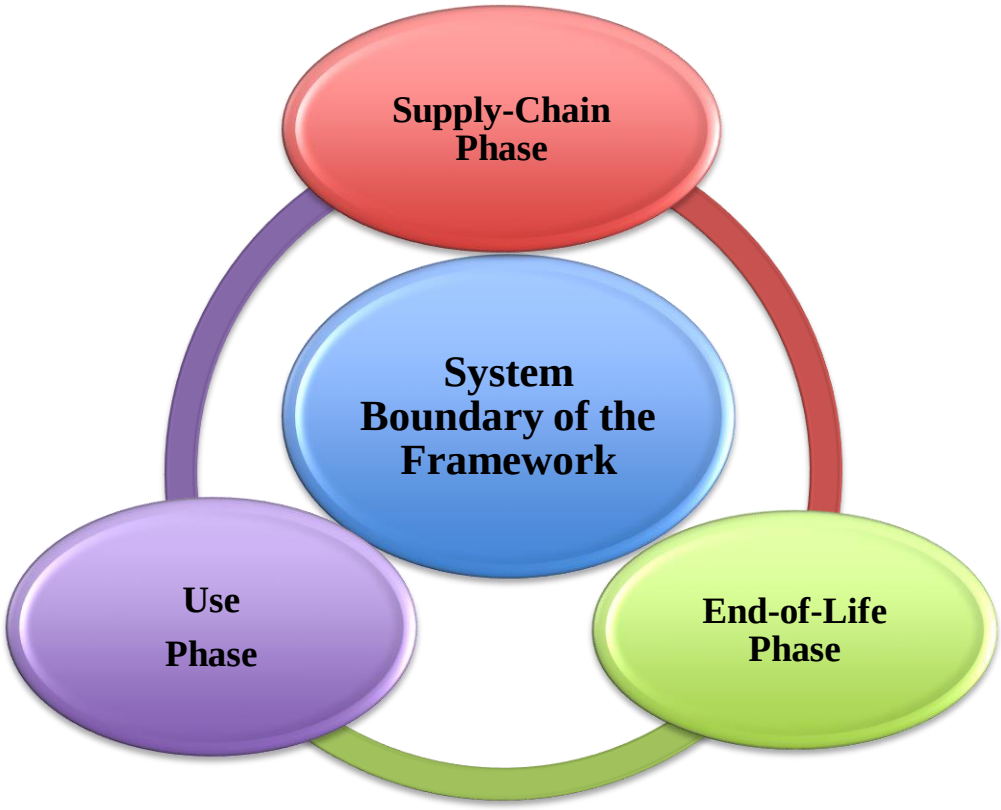


Figure 4.1 *The main life-cycle phases evaluated by the framework developed in this research (system boundary diagram).*

The framework based on LCA employed in the case study evaluates three main lifecycle phases: supply-chain, use and end-of-life (see Figure 4.1). The description

of the phases, the activities involved in these phases, and the life cycle inventory of all the activities evaluated by the framework are provided in the following sections. The methodology/approach used to obtain the data for the activities are also described.

4.3.1 Supply-chain phase

The supply-chain phase is one of the main phases evaluated by the framework (Figure 4.1). The supply-chain phase evaluates the background activities of the system studied. The environmental impacts produced in this phase contribute to indirect burdens. The three main activities of this phase include reagent production, stainless steel equipment and consumables fabrication. The activities evaluated in this phase and the inventory data are provided below.

4.3.1.1 Stainless steel equipment fabrication

This activity focused on the fabrication of all stainless steel equipment involved in manufacture. The fabrication of the support equipment such as waste collection tanks, clean steam generator and the WFI still for the traditional and hybrid processes were also included. The total stainless steel equipment required for the traditional and hybrid manufacturing facilities were obtained from the BioSolve software (see Tables A4 and A5 in Appendix 4.2 for the list of stainless steel equipment required by the traditional and hybrid manufacturing facilities for a 200 L production scale with 20 batches per annum).

The equipment sizes (e.g. vessel volume and chromatography column height and diameter) were obtained from the BioSolve software. It was assumed that these

fixed-in-place stainless steel equipment were used for a period of 15 years (Pietrykowski *et al.*, 2013). The information on the stainless steel equipment fabrication process was mainly obtained from Sun Sung Lee Engineering (Penang, Malaysia). The fabrication information was verified through discussions with experts from TWI (Cambridge, UK). Both TWI and Sun Seng Lee Engineering are established manufacturers of stainless steel equipment such as vessels, filter housings and skids.

The fabrication process (see Figure 4.2) included raw material extraction, processing and production of 316 L stainless steel plates at the processing site (transportation involved in the activities included), cutting and rolling of the stainless steel plates to form desired shapes, welding of the plates for the final assembly of the equipment and post-weld heat treatment (to increase the ductility of the stainless steel) within the stainless steel facility. The cleaning and final preparation of the equipment before delivery to the monoclonal antibody facility were also included in the fabrication process. Another aspect that was considered in the fabrication process was transportation. The transportation of the stainless steel plates from the raw material production facility to the equipment fabrication facility (supply chain facility), and from there to the monoclonal antibody facility were included.

Figure 4.2 also provides the inventory data (energy and water consumption and carbon emissions) of each activity in the fabrication process, obtained either from the GaBi 6.0 software database or through interviews with Sun Seng Lee Engineering, Malaysia (as indicated in the figure). These energy and water consumption levels and carbon emissions information are given per unit (e.g. per kilogram of the steel or per

metre of the steel), thus, these data were scaled according to the size of the equipment; multiplied with the size (e.g. weight of the tank) of the equipment to obtain the actual environmental impacts. For example, to obtain the environmental impacts of the 316 L stainless steel production activity for a vessel, the data were scaled to the weight of the vessel; the given inventory data were multiplied with the weight of the vessel. Meanwhile, to obtain the environmental impacts of the welding activity for a vessel, the data were scaled to the total welding length of the vessel; the given inventory data were multiplied with the welding length (height and circumference of the vessel). Thus, the fabrication impacts of equipment with different sizing can be evaluated, as long as the information such as weight and measurements (height and diameter) of the equipment are known.

To calculate the environmental impacts of transport, the energy (MJ/kg/km) and water (kg/kg/km) consumption and carbon emissions ($kgCO_2$-eq/kg/km) provided for transport were scaled according to the distance travelled and weight of the stainless steel equipment being transported. The environmental impacts (inventory data scaled to the size of the equipment) of each activity in the fabrication process of a stainless steel equipment are labelled as X_1-X_7 in Figure 4.2. The total environmental impacts (energy and water consumptions and carbon emissions) produced by a stainless steel equipment is the sum of the environmental impacts produced by each activity in the fabrication process (see Equation 4.1).

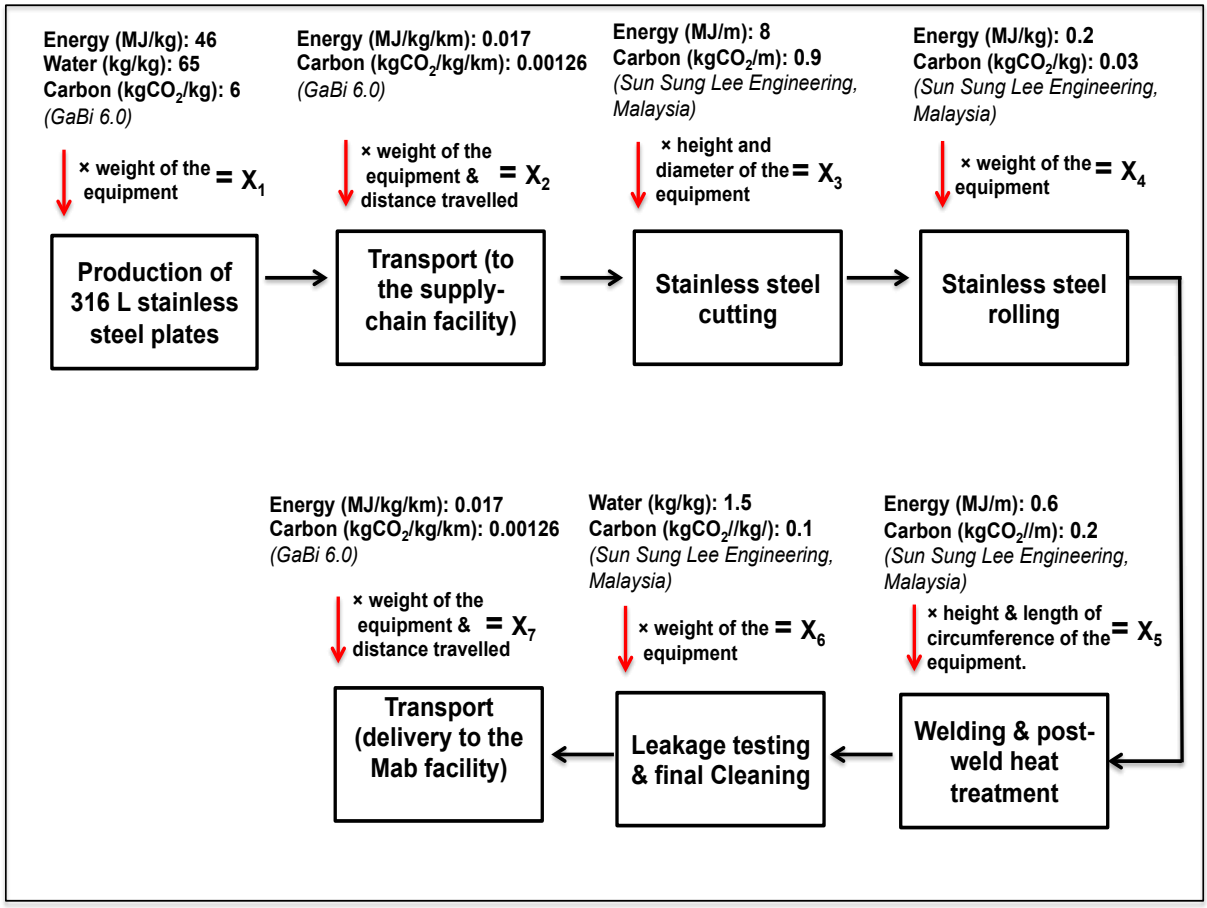


Figure 4.2 *The stainless steel equipment fabrication process and the life cycle inventory data.*

Table 4.1 is provided to illustrate the total energy and water consumptions and carbon emissions of stainless steel equipment fabrication. The purpose of this table is to highlight the environmental impacts produced by some of the stainless steel equipment employed in the case study. However, these impacts account for a period of 15 years (life span of the equipment was assumed to be 15 years). Since the aim of the case study is to evaluate the impacts per annum, the total environmental impacts of a stainless steel equipment was divided by 15 (life span of the stainless steel equipment) (Equation 4.2).

Table 4.1 *The energy and water consumptions and carbon emissions of stainless steel equipment fabrication.*

Stainless Equipment	**Energy (MJ)**	**Water (kg)**	**Carbon($kgCO_2$-eq)**
Vessel Volume: 20 L	105500	101400	26500
Vessel Volume: 2000 L	396000	380000	99300
Vessel Volume: 20000 L	1250000	1100000	320000
Chromatography Column (Column Height: 20 cm, Column Diameter: 44 cm)	15600	14900	4000
Filter Housing (Housing weight: 56 kg)	3900	3700	960

The total environmental impacts of a stainless steel equipment (for a period of 15 years) = $\sum (X_1 + X_2 + X_3 + X_4 + X_5 + X_6 + X_7)$

(Equation 4.1)

The total environmental impacts of a stainless steel equipment per annum

$$= \frac{\sum (X_1 + X_2 + X_3 + X_4 + X_5 + X_6 + X_7)}{15}$$

(Equation 4.2)

The total environmental impacts from the stainless steel equipment fabrication employed in the manufacturing process (traditional/hybrid) is the sum of the environmental impacts per annum produced by all the stainless steel equipment used in the manufacturing process.

Key assumptions for stainless steel equipment fabrication: A number of assumptions were made to simplify the environmental impacts evaluation of stainless steel

equipment. In this study, the stainless steel production facility was assumed to be located in China and the equipment fabrication facility was assumed to be located in Sweden. The raw material facility was assumed to be located in China as they are one of the largest producer of stainless steel in the world (Millbank, 2013). The equipment fabrication facility was assumed to be located in Sweden because a large number of biopharmaceutical processing equipment are fabricated/assembled there (Levine *et al*., 2012). The study also assumed that the fixed-in-place stainless steel equipment was used for a period of 15 years. Finally, it was assumed that all the stainless steel equipment to be delivered to the monoclonal antibody manufacturing facility were transported together from the supply-chain facility.

4.3.1.2 Consumables fabrication

This activity focused on the fabrication processes of all filters, single-use bags and resins involved in the monoclonal antibody manufacturing process (the types of single-use equipment employed are described in Chapter 3, Section 3.6.1.2). The total consumables required for the traditional and hybrid manufacturing processes per production batch were obtained from the BioSolve software (see Tables A6 and A7 in Appendix 4.2 for the list of consumables required by the traditional and hybrid manufacturing facilities for a 200 L production scale with 20 batches per annum). The consumables sizing (e.g. resin volume and filter weight) were also obtained from the BioSolve software. To simplify the analysis, the fabrication process of filters and single-use bags were assumed to be similar. In this study, it was assumed that the single-use bags were made of polyethylene (PE) and the filters were made of polypropylene (PP), as they are the most common materials used in the industry to manufacture single-use bags and filters (Eibl *et al*., 2010b).

Single-use bags/Filters fabrication: The fabrication process (see Figure 4.3) included extraction, processing and production of raw materials to form low-density polyethylene (or low-density polypropylene) granules at the processing site (transportation involved for these activities are included), extrusion and blown film extrusion of the granules (to form shapes), injection moulding (to make rigid components for the filters and single-use bags), final assembly of the filter components and gamma irradiation of the consumables (to ensure their sterility) at the facility (Eibl *et al.*, 2010b). The information on single-use bags/filters fabrication process was obtained from a literature survey (Eibl *et al.*, 2010b) and through discussions with industrial experts (G. Jagschies, GE Healthcare; A.Dey-Chowdhury, Pall Life Sciences). The activity also considered transportation. The transportation of the raw material from the processing site to the consumables facility and from the consumables facility to the monoclonal antibody facility were included.

Figure 4.3 provides the inventory data (energy and water consumptions and carbon emissions) of each activity in the single-use bag/filter fabrication process, obtained mainly from the GaBi 6.0 software database (indicated in the figure) (Rawlings and Pora, 2009; Sinclair, 2008, Mauter, 2009). These energy and water consumption and carbon emissions information are given per unit (per kilogram of the polyethylene/polypropylene), thus, these data were scaled according to the size of the single-use bag/filter; multiplied with the weight of the single-use bag/filter to obtain the actual environmental impacts. For example, to obtain the environmental impacts of the production of polyethylene/polypropylene for a single-use bag/filter, the given data were multiplied by the weight of the single-use bag/filter. Thus, the fabrication impacts of consumables with different sizing can be evaluated, as long as the weight

of the consumables is known.

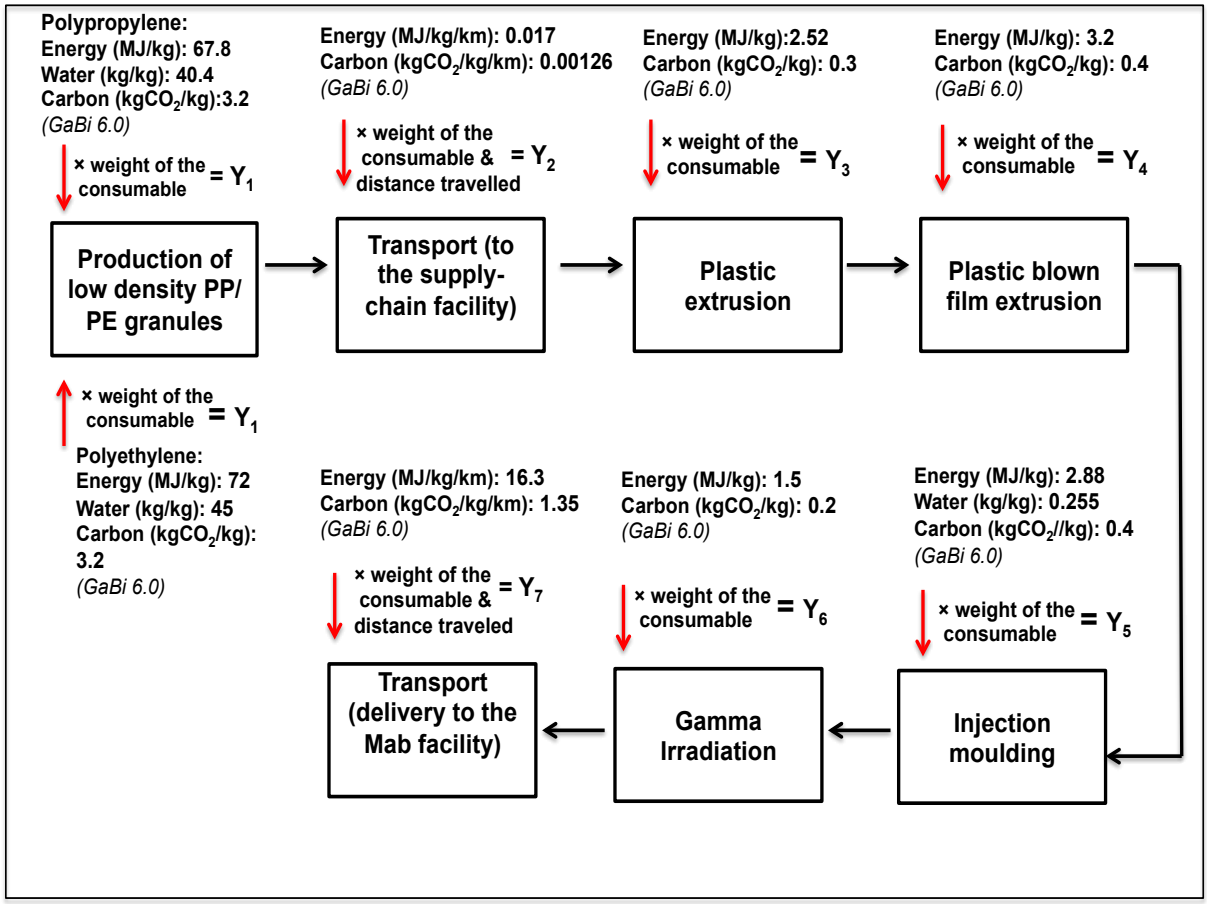


Figure 4.3 *The single-use bags/filter fabrication process and the life cycle inventory data.*

To calculate the environmental impacts of transport, the energy and water consumptions and carbon emissions provided for transport were scaled according to the distance travelled and weight of the single-use bag/filter being transported. The environmental impacts (inventory data scaled to the size of the equipment) of each activity in the fabrication process of a single-use bag/filter are labelled as Y_1-Y_7 in Figure 4.3. The total environmental impact (energy, water and carbon impacts) produced by a single-use bag/filter is the sum of the environmental impacts produced by each activity in the fabrication process (see Equation 4.3).

Tables 4.2 and 4.3 are provided to highlight the energy and water consumptions and carbon emissions of single-use bags and filter fabrications. The purpose of this table is to illustrate the environmental impacts of some of the single-use bags (10 L, 200 L, 500 L) and single-use filters employed in the case study. However, these environmental impacts are impacts produced per production batch. Since the aim of the case study was to evaluate the impacts per annum, the environmental impact of a single-use bag/filter was multiplied by 20 (the number of production batches were assumed to be 20) (Equation 4.4).

The total environmental impacts of a single-use bag/filter per production batch

$= \sum (Y_1+Y_2+Y_3+Y_4+Y_5+Y_6+Y_7)$

(Equation 4.3)

The total environmental impacts of a single-use bag/filter per annum

$= (\sum (Y_1+Y_2+Y_3+Y_4+Y_5+Y_6+Y_7) \times 20)$

(Equation 4.4)

Table 4.2 *The energy and water consumptions and carbon emissions of single-use bags fabrication.*

Bag Volume (L)	**Energy (MJ)**	**Water (kg)**	**Carbon ($kgCO_2$-eq)**
10	1365	86	35
200	2310	124	60
500	4180	273	126

Table 4.3 *The energy and water consumptions and carbon emissions of single-use filters/membrane adsorber fabrication.*

Filter Size- **Membrane area (m^2)/ weight (kg)**	**Energy (MJ)**	**Water (kg)**	**Carbon ($kgCO_2$-eq)**
Depth Filter: 0.2 m^2 /0.3 kg	220	13	8
Ultrafiltration/dialfiltration: 0.4 m^2 /1 kg	797	46	45
Anion-exchange membrane adsorber capsule: 30 inch	5100	416	161

Resins fabrication: A resin consists of a ligand and a support as beads or matrix that are typically covalently linked together so that the combination may be reused for a number of cycles (Tagliavia and Nicosia, 2012). The beads are typically manufactured using agarose harvested from algae through fermentation processes. The harvested algae are refined and purified to form agarose (powder form). The agarose powder is transported to the consumables facility where the beads were prepared using an emulsification process. The type of ligand to be used on the resin depends on the method of chromatography employed. In the research, two types of ligand were employed; recombinant protein (Protein A chromatography) and poly-methyl methacrylate (PMMA) ligand (ion-exchange chromatography). It was assumed that the ligands were manufactured at the consumables facility. The beads and the ligand were covalently coupled at the consumables facility before being transported to the manufacturing facility. Transportation of the beads and the ligand to the consumables facility, and the transportation of the resins from the consumables facility to the manufacturing facility were included in the analysis. The information on the resin fabrication process (Figure 4.4) was obtained from a discussion with an industry expert (H. Ihre, GE Healthcare). Figure 4.4 also provides the inventory data

(energy and water consumption and carbon emissions) of each activity in the resin fabrication process, obtained mainly from the literature survey and expert discussion (as indicated in the figure) (Rawlings and Pora, 2009; Ihre, 2011; Pandey *et al.*, 2008; Agargel, 2013). These energy and water consumptions and carbon emissions information are given per unit (per kilogram of the agarose/per kilogram of ligand), thus, these data were scaled according to the volume of the resin; multiplied with the weight of the resin to obtain the actual environmental impacts. For example, to obtain the environmental impacts of the production of agarose through fermentation to produce the beads for the resin, the data were scaled to the weight of the agarose; the given data were multiplied with the weight of the agarose. Thus, the fabrication impacts of resins with different volumes can be evaluated, as long as the weight of the resin is known.

To calculate the environmental impacts of transport, the energy and water consumptions and carbon emissions provided for transport were scaled according to the distance travelled and weight of the agarose/resin being transported. The environmental impacts (inventory data scaled to the size of the equipment) of each activity in the fabrication process of a resin are labelled as Z_1-Z_6 in Figure 4.4. The total environmental impacts (energy, water and carbon impacts) produced by a resin is the sum of the environmental impacts produced by each activity in the fabrication process (see Equation 4.5). Table 4.4 is provided to illustrate the total energy and water consumptions and carbon emissions associated with the manufacture of resins and highlights the environmental impacts produced by resins employed in the case study.

It was assumed in this case study that the Protein A resin can be used up to 200 times, and the cation-exchange and anion-exchange resins can be used up to 50 times. The assumptions were made based on the life span of the resins provided by the vendors (GE Healthcare). Since the resins can be used for a number of cycles, the environmental impacts of the resins per cycle should be calculated. This can be calculated by dividing the total impacts of a resin by the number of cycles they can be deployed in manufacturing (Equation 4.6). To obtain the environmental impacts of resin per annum, the impacts per cycle was multiplied by 20 (the number of production batches were assumed to be 20) and number of chromatography cycles (Equation 4.7).

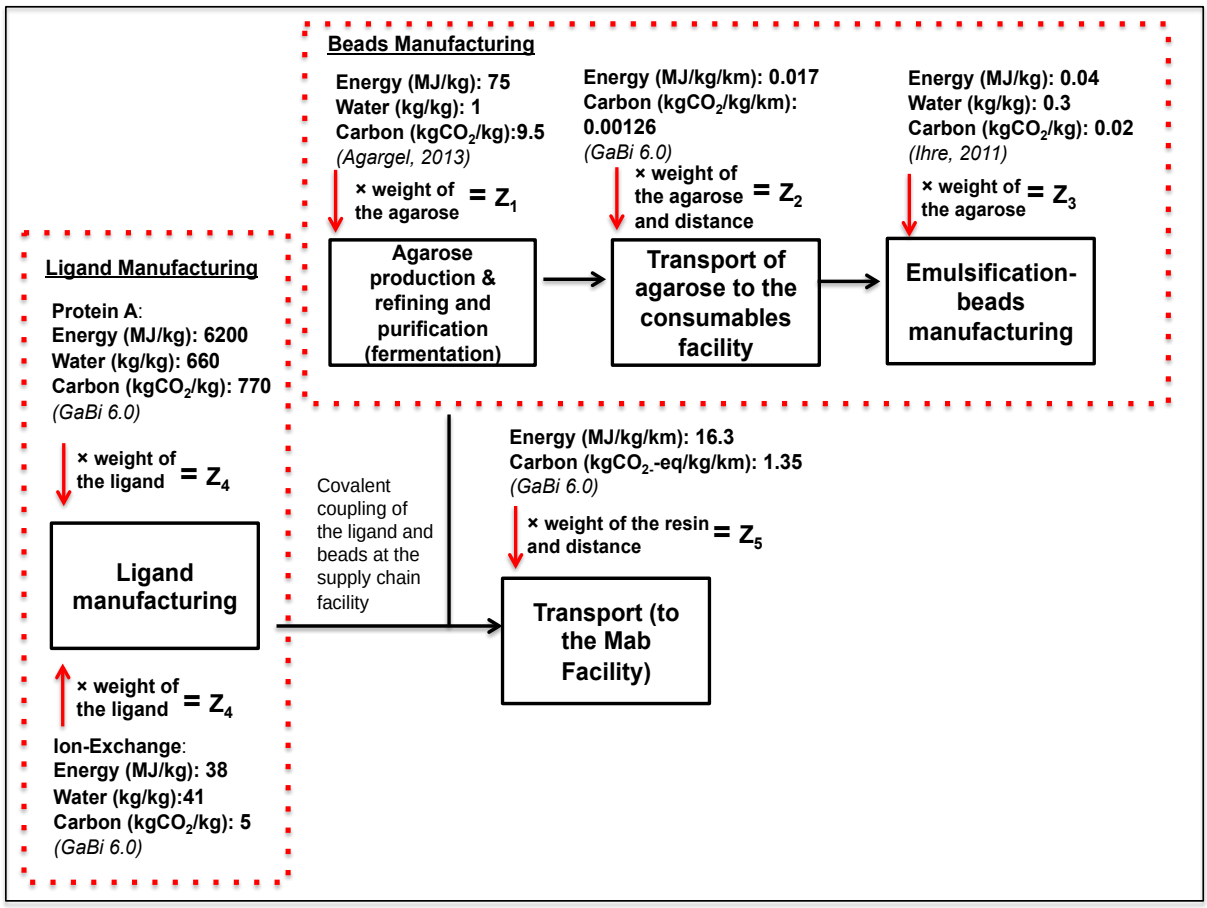


Figure 4.4 *The resin fabrication process and the life cycle inventory data.*

Table 4.4 *The energy and water consumptions and carbon emissions of resins manufacture.*

Resin Type	**Resin Volume (L)**	**Energy (MJ)**	**Water (kg)**	**Carbon ($kgCO_2$-eq)**
Protein A resin	26	13300	1100	2000
IEX resin	25	3500	115	460

The total environmental impacts of a resin = $\sum (Z_1+Z_2+Z_3+Z_4+Z_5+Z6)$

(Equation 4.5)

The environmental impacts of a resin per cycle $= \frac{\sum (Z_1+Z_2+Z_3+Z_4+Z_5+Z6)}{\text{Number of resin cycles}}$

(Equation 4.6)

The total environmental impacts of a resin per annum = the environmental impacts of a resin per cycle × 20 × number of chromatography cycles.

(Equation 4.7)

The total environmental impacts from consumable fabrication per annum for a manufacturing process (traditional/hybrid) is the sum of environmental impacts produced from all the single-use bags/filters and resins employed per annum.

Key assumptions for consumables fabrication:

All consumables (solid) waste produced by the processes were assumed to be disposed of at the end of a production batch except for protein A chromatography resins which were used for 200 cycles, ion-exchange chromatography resins which were used for 50 cycles (Low *et al.*, 2007; Hill *et al.*, 2010). The number of cycles for chromatography is assumed to be 2 cycles per batch for both Protein A and Ion

Exchange chromatography (Pietrzykowski *et al.*, 2013). The dynamic binding capacity (DBC) of the Protein A resin was assumed to be 30 g/L and the DBC of the ion-exchange chromatography resins were assumed to be 62 g/L. The DBC values were obtained from the literature (Low *et al.*, 2007). The DBC of resins impact the chromatography column sizing (Low *et al.*, 2007).

For the chromatography operations, it was assumed that five column (CV) of wash buffer, five CV of equilibration buffer and five CV of elution buffer were used, but only two CV of product was collected (Low *et al.*, 2007). The consumables (single-use components) used in manufacturing were assumed to arrive pre-sterilised (pre-sterilised using Cobalt-60 irradiation) to the facility (Sinclair *et al.*, 2008). In this case study, the packaging of consumables was not included.

In this study, the raw material processing facility was assumed to be located in Belgium and the consumables manufacturing facility was assumed to be located in Sweden. The raw material processing facility was assumed to be located in Belgium as it is the largest plastic producer in Europe (Plastics Europe, 2012). The consumables manufacturing facility was assumed to be located in Sweden as a large biopharmaceutical processes related consumables fabrication take place there (Sandstròm *et al.*, 2011). The study also assumed the consumables to be delivered to the monoclonal antibody manufacturing facility were transported together from the supply-chain facility.

4.3.1.3 Reagent production

The reagent preparation activities were all assumed to occur on-site (in the facility). The types of reagent that were employed included media, buffer, CIP agents (caustic and acid) and steam (Hill *et al*., 2010a). In this case study, media, buffer, and CIP agents were prepared using water-for- injection (WFI) and steam was prepared using pure water (PW) (Sinclair, 2008). The total environmental impacts for reagents preparation is the sum of environmental impacts produced from WFI and PW production, media, buffer and CIP agents mixing, and steam production. The media, buffer, CIP agents and steam requirements for the traditional and hybrid manufacturing processes at 200 L production scale were obtained from the BioSolve software (see Tables A8 and A9 in the Appendix 4.2 for the media, buffer, CIP agents and steam requirements of the traditional and hybrid processes run at 200 L production scale with 20 batches per annum). The environmental impact inventory data for WFI/PW production and reagent mixing were obtained from literature surveys (Sinclair *et al*., 2008; Rawlings and Pora, 2009; Blue Spring Corporation, 2016) (see Table 4.5). Using the inventory information, impacts produced from the reagents preparation for manufacturing processes at different production scales can be calculated, as long as the reagent requirements for the manufacturing processes are known.

Key assumptions for reagent production

To ensure process sterility, fixed-in-place stainless steel equipment for both the traditional and hybrid processes were assumed to undergo CIP and SIP operations between batches. The media, buffer and cleaning agents required in the manufacturing processes were assumed to be made using WFI generated on-site. The

steam required for SIP was made using PW generated on-site. The energy consumed for production of WFI and PW were determined to be almost similar, thus, these values were assumed to be the same in this analysis (Pietrykowski *et al.*, 2013). The energy consumption and carbon emissions of water production (that is delivered to the facility) are also included.

Table 4.5 *The inventory data for reagents production.*

Activity	Environmental Impacts
WFI/PW production/L	Energy (MJ/L): 0.7 (Blue Spring Corporation, 2016) Carbon emissions ($kgCO_2$-eq/L): 0.1 (Blue Spring Corporation, 2016)
Reagent (media, buffer, acid and caustic) mixing/L	Energy (MJ/L): 0.9 (Rawlings and Pora, 2009) Carbon emissions ($kgCO_2$-eq/L): 0.2 (Rawlings and Pora, 2009)
Steam production/L	Energy (MJ/L): 1.2 (Sinclair *et al.*, 2008) Carbon emissions ($kgCO_2$-eq/L): 0.4 (Sinclair *et al.*, 2008)

4.3.2 Use Phase

As highlighted in Figure 4.1, the use phase is one of the three main phases evaluated by the framework (part of the system boundary). The use-phase evaluates the foreground activities within the system boundary. The environmental impacts produced in this phase contribute to direct burdens and include equipment utilisation, waste management and finally lighting and HVAC. The descriptions of these activities are provided below.

4.3.2.1 Equipment utilisation

This activity considered the impacts arising from equipment operations to produce monoclonal antibodies. Tables 4.6 and 4.7 show the energy consumption and carbon emissions of all the unit operations employed in the traditional and hybrid manufacturing processes. The calculations to obtain these energy and carbon values are shown in Table 4.8. Table 4.8 shows the inventory data and energy (KWh) calculation methods for different unit operations in the traditional and hybrid processes. The inventory data were mainly obtained literature surveys/vendor reports (indicated in Table 4.8). The equipment sizing data and the cycle time (processing time) of each unit operation in the traditional and hybrid processes (also shown in Tables 4.6 and 4.7) were obtained from the BioSolve software.

The energy in KWh can be converted to MJ by multiplying with a conversion factor of 3.6 (DEFRA, 2015). The carbon emissions of each unit operation can be determined by multiplying energy in KWh with GHG conversion factor of 0.4455 (DEFRA, 2015).

Key assumptions in equipment utilisation

In this case study, it was assumed that the energy consumption of the traditional dead-end filters were similar to the energy consumption of the single-use dead-end filters. The traditional cross flow filters energy consumption were also assumed to be similar to the energy consumption of single-use cross flow filters. These assumptions were made because the energy difference values between the traditional (dead-end and cross flow) and single-use (dead-end and cross flow) filters are insignificant (Rawlings and Pora, 2008). The chromatography cycles were assumed to be 2 per

production batch (BioSolve software). In equipment utilisation, the impacts produced by the support equipment such as the WFI still and PW skids were also considered (information obtained from the BioSolve software).

Table 4.6 *The energy consumption and carbon emissions of all the unit operations in the traditional process.*

Unit Operation	**Equipment Size**	**Cycle Time (h)**	**Energy (MJ)**	**Carbon ($kgCO_2$-eq)**
Seed Fermentation	Bioreactor Volume: 10 L	58	105	13
Seed Fermentation	Bioreactor Volume: 20 L	59	200	25
Fermentation	Bioreactor Volume: 10 L	210	7500	930
Disc Stack Centrifuge	Flowrate: 200 L/h	5	5	186
Depth Filtration	Membrane area: 0.4 m^2	8	2	0.3
Protein A Chromatography	Column Height: 44 cm and Diameter: 20 cm. Ligand volume: 26 L	9	26	3
Viral Inactivation	Vessel volume: 100 L	3	9	1
Cation-Exchange Chromatography	Column Height: 44 cm and Diameter: 20 cm. Ligand volume: 25 L	8	23	2.8
Anion-Exchange Chromatography	Column Height: 44 cm and Diameter: 20 cm. Ligand volume: 23 L	8	23	2.8
Viral Filtration	Membrane area: 0.2 m^2	5	3	0.4
Ultrafiltration /Diafiltration	Membrane area: 0.4 m^2	11	7	1
Sterile Filtration	Membrane area: 0.1 m^2	7	0.4	0.1

Table 4.7 *The energy consumption and carbon emissions of all the unit operations in the hybrid process.*

Unit Operation	**Equipment Size**	**Cycle Time (h)**	**Energy (MJ)**	**Carbon ($kgCO_2$-eq)**
Seed Fermentation-Wave Bioreactor	Bioreactor Volume: 10 L	58	125	16
Seed Fermentation-Wave Bioreactor	Bioreactor Volume: 20 L	59	255	32
Fermentation-Wave Bioreactor	Bioreactor Volume: 200 L	210	9000	1113
TFF-Microfiltration (Disposable)	Membrane Area: $1m^2$	38	50	6
Depth Filtration	Membrane area: 0.4 m^2	10	2	0.2
Protein A Chromatography	Column Height: 44 cm and Diameter: 20 cm. Ligand volume: 26 L	9	26	3
Viral Inactivation (Disposable)	Bag Volume: 100 L	3	9	1
Cation-Exchange Chromatography	Column Height: 44 cm and Diameter: 20 cm. Ligand volume: 25 L	8	23	2.8
Anion-Exchange Membrane Adsorber	30 inch capsule	9	23	2.8
Viral Filtration (Disposable)	Membrane area: 0.2	5	0.3	0.03
Ultrafiltration /Diafiltration	Membrane area: 0.4	11	6	0.8
Sterile Filtration	Membrane area: 0.1	7	0.1	0.06

Table 4.8 *Inventory data and environmental impacts calculation methods for unit operations in the traditional and hybrid processes.*

Equipment	**Inventory Data**
Bioreactor	Based on vendor data, 100 L vessel consumes 5 KW of energy To obtain the energy (KWh) of a 200 L vessel=5 KW× $(\frac{200\ \text{L}}{100\ \text{L}})$ × cycle time (h) (Mauter, 2009)
Disc Stack Centrifuge	Based on vendor data, a disc stack centrifuge consumes around 2.5 KW of energy per m^3 of load volume entering the centrifuge (Milledge and Haven, 2011). The volume handled by the centrifuge can be obtained from the volume balance (see Appendix 4.1) To obtain the energy (KWh) of a disc stack centrifuge= 2.5 KW× $(\frac{\text{Load Volume (L)}}{1000})$ x cycle time (h)
Dead-end Filtration	Based on the vendor data, a dead-end filtration consumes 0.2 KW per m^3 of load volume entering the filter (Chew *et al*., 2014) To obtain the energy (KWh) of a dead-end filter= 0.2 KW × $(\frac{\text{Load Volume (L)}}{1000})$ m^3 × cycle time (h)
Cross-Flow Filtration	Based on the vendor data, a cross flow filtration consumes 2 KW per m^3 of load volume (L) entering the filter (Chew *et al*., 2014) To obtain the energy (KWh) of a cross-flow filter= 2 KW × $(\frac{\text{Load Volume (L)}}{1000})$ m^3 × cycle time (h)
Chromatography	Based on the vendor data, chromatography operation consumes around 0.8 KW of energy (GE Healthcare, 2016a) To obtain the power consumption (KWh) of a chromatography unit operation= 0.8 KW × cycle time (h) × number of chromatography cycles For chromatography unit operation, the cycle time included the time for the equilibration, loading, washing, elution and cleaning steps. Information on cycle time and number of chromatography cycles can be obtained from the BioSolve software
Single-Use Bioreactors (Wave Bioreactors)	Based on the vendor data, a 10 L bioreactor consumes 0.6 KW of energy, a 20 L bioreactor consumes 1.2 KW of energy and a 200 L bioreactor consumes 12 KW of energy. With every 10 L of volume increase in bioreactor, the energy consumption goes up by 0.6 KW (GE Healthcare, 2016b) To obtain the energy (KWh) of a single-use bioreactor= energy (KW) × cycle time (h)

4.3.2.2 Waste management

In this case study, it was assumed that the liquid and solid wastes produced by the manufacturing processes were hazardous (containing, and therefore must be treated before being disposed of (Sinclair *et al.*, 2008). The waste management activity considered the treatment of the liquid and solid waste produced during manufacture. The information on waste management was obtained through discussion with A.Sinclair (BioPharm Services, Amersham, UK). The liquid waste was treated using the "thermal inactivation" method where the liquid waste was subjected to a temperature of 90^0C for 2 hours (Moran *et al*., 2005). The treated liquid waste was then discharged to sewer (Moran *et al.*, 2005). The solid waste produced was treated on-site by autoclave in the facility (Sinclair *et al*., 2008). The treated solid waste was transported to the disposal site (landfill, incineration or pyrolysis). The transportation of the treated solid waste from the monoclonal antibody facility to the disposal site was also evaluated. In this study, the disposal site was assumed to be local to the monoclonal antibody manufacturing facility. Figure 4.5 shows the solid and liquid waste management activities of the traditional and hybrid manufacturing processes. The amount of liquid and solid waste produced per annum for the traditional and hybrid processes at 200 L production scale were obtained from the BioSolve software.

Figure 4.5 also provides the environmental impacts inventory data (energy and water consumption and carbon emissions) of each activity in the waste management activities obtained either from the GaBi software database or through literature surveys (Rawlings and Pora, 2009). These energy and water consumptions and carbon emissions information are given per unit (e.g. per L of liquid waste and per

kg of solid waste), thus, these data were scaled according to the amount of waste produced; multiplied with the volume of liquid waste produced or weight of solid waste produced to obtain the actual environmental impacts. For example, to obtain the environmental impacts of disposing waste in landfill, the landfill inventory data were multiplied with the weight of the waste sent to the landfill.

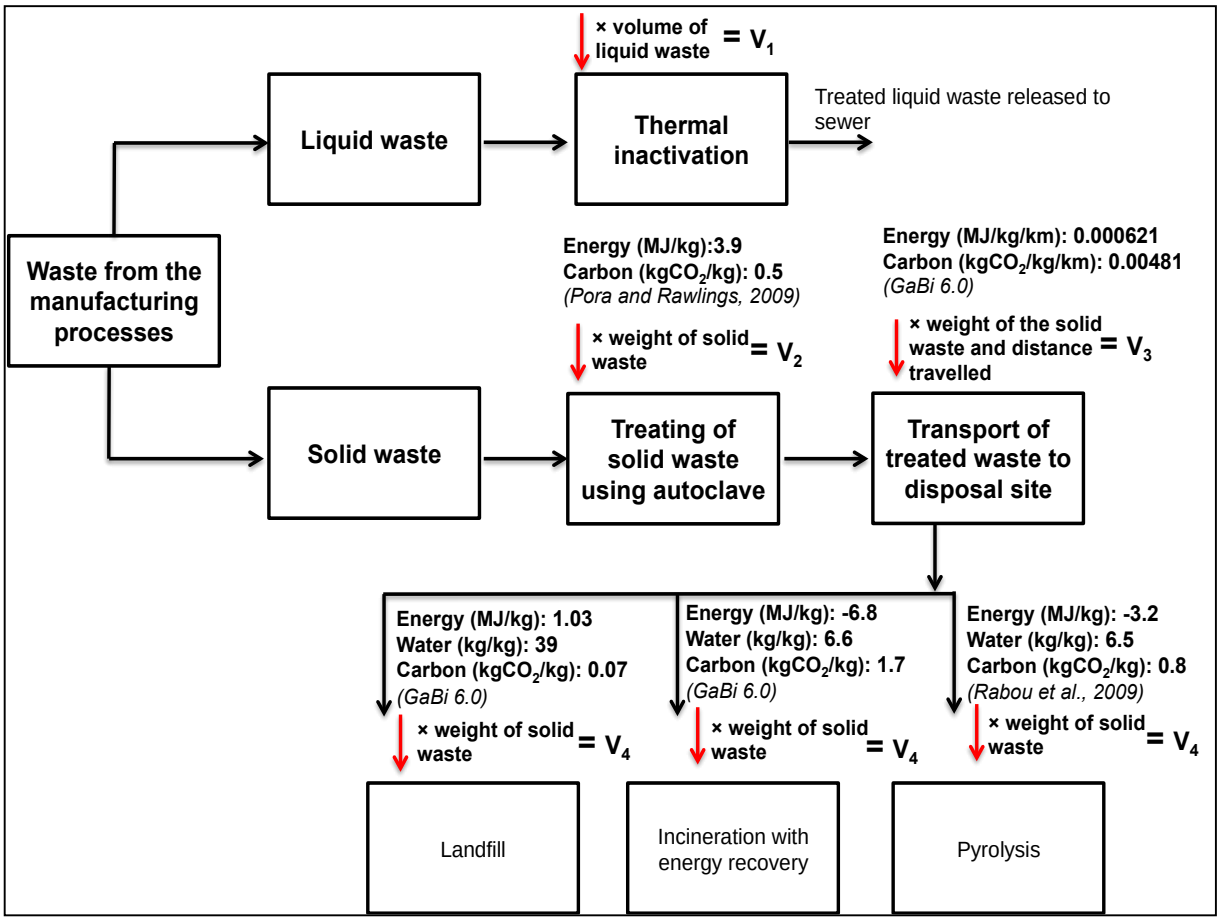


Figure 4.5 *The waste management process to treat the solid and liquid wastes from the manufacturing processes.*

The environmental impacts (inventory data scaled to the size of the equipment) of each activity in the solid waste management are labelled as V_1-V_4 in Figure 4.5. The total solid waste management environmental impact (energy, water and carbon impacts) is the sum of the environmental impacts produced by each solid waste

management activity (see Equation 4.8). These environmental impacts are impacts produced per production batch. Since the aim of the case study was to evaluate the impacts per annum, the total environmental impacts were multiplied by 20 (the number of production batches were assumed to be 20) (Equation 4.9). Tables 4.9 and 4.10 show the liquid and solid waste produced per annum by the traditional and hybrid manufacturing process. These tables also provide the environmental impacts of solid and liquid waste treatment and disposal activities of the traditional and hybrid processes.

The environmental impacts of solid waste management per production batch

$= \sum (V_1+V_2+V_3+V_4)$

(Equation 4.8)

The environmental impacts of solid waste management per annum

$= \sum(V_1+V_2+V_3+V_4) \times 20$

(Equation 4.9)

Table 4.9 *Environmental impacts of solid and liquid waste treatment and disposal activities per annum for the traditional process.*

Type of Waste	**Amount of Waste**	**Energy (MJ)**	**Water (kg)**	**Carbon Emissions ($kgCO_2$-eq)**
Liquid	23000 L	60000	-	7500
Solid (using landfill disposal method)	25 kg	150	640	22
Solid (using incineration disposal method)	25 kg	90	107	44
Solid (using pyrolysis disposal method)	25 kg	110	105	29

Table 4.10 *Environmental impacts of solid and liquid waste treatment and disposal activities per annum for the hybrid process.*

Type of Waste	Amount of Waste	Energy (MJ)	Water (kg)	Carbon ($kgCO_2$-eq)
Liquid	3000 L	8450	-	1044
Solid (using landfill disposal method)	133 kg	302	5170	42
Solid (using incineration disposal method)	133 kg	-49	875	259
Solid (using pyrolysis disposal method)	133 kg	111	861	134

4.3.2.3 Lighting and HVAC

The final activity considered in the use-phase was the impact produced from lighting and HVAC requirements of the facility. In this research, the impact of this activity was obtained from a bench marking study of cleanroom energy in biopharmaceutical facilities (Boyd, 2005). According to Boyd (2005), a 7000 m^2 biopharmaceutical facility (including laboratory and office space) consumes around 540, 000 MJ of energy for lighting and HVAC per month. Thus, the annual energy requirements for lighting and HVAC for a facility of this size is around 6.5 million MJ. Based on this information, it can be estimated that the facility's energy requirements per square metre is 930 MJ. It is important to understand that some spaces in the facility (e.g. clean room) may have higher energy requirements for lighting and HVAC, however, in order to simplify the analysis, it was assumed that the energy requirements for lighting and HVAC of all the areas in the facility are similar (Pietrykowski *et al.*, 2013; Cochet *et al.*, 2013). This information was used to estimate the lighting and HVAC energy requirements of the facility employed in the case study (Boyd, 2005). Estimating the lighting and HVAC energy requirements of a monoclonal antibody

manufacturing facility can be challenging. Thus, a number of reliable and valid assumptions were used to estimate the energy requirements. In this case study, it was assumed that the footprint of the facility is 2200 m^2 (24, 000 ft^2). This footprint includes the laboratory and office space. The assumption was based on the footprint of a monoclonal antibody facility producing monoclonal antibodies for clinical supply (production scale below 2000 L) (Cochet *et al*., 2013). Since the production scale employed in this case study (200 L) is a production scale typically employed for producing materials for clinical studies, the footprint of the facility in this case study was assumed to be similar to the footprint of the monoclonal antibody facility producing monoclonal antibodies for clinical supply (Cochet *et al*., 2013).

Using this footprint, the lighting and HVAC energy requirements for the facility was estimated. It was determined earlier that the monoclonal antibody facility consumes around 930 MJ of energy per square foot. By multiplying the energy requirements per square foot with the total facility footprint, it was determined that a 2200 m^2 facility will consume around 2 million MJ of energy annually for lighting and HVAC (Cochet *et al*., 2013). The carbon emissions resulting from the lighting and HVAC energy consumption is estimated to be around 0.3 million $kgCO_2$-eq (1 KWh of energy generates 0.4455 $kgCO_2$-eq of carbon).

4.3.3 End-of-Life Phase

The final life cycle phase evaluated by the framework is the end-of-life phase (see Figure 4.1). This phase evaluates the background activities of the study. The impacts produced in this phase contribute to indirect burdens. This phase includes the disposal of fixed-in-place stainless steel equipment after a period of 15 years (this

period was assumed fixed in all scenarios). The information on the activities involved in the end-of-life phase were obtained from expert discussion (A. Sinclair, BioPharm Services) and literature survey (Rawlings and Pora, 2009; Pietrykowski *et al*., 2013). The majority of the stainless steel equipment (~ 90%) was assumed to be recycled and the rest (~10%) were sent as waste to a landfill disposal site (Pietrykowski *et al*., 2013). The recycling process mainly involved equipment dismantling and scrap processing (where the steel is recovered). The transport of the dismantled equipment to the recycling facility, the transport of recovered steel to a stainless steel facility and the transport of waste to a landfill disposal site were each evaluated. In this study, the recycling facility and the landfill site were assumed to be located locally to the monoclonal antibody manufacturing facility. The reprocessed steel was transported to a stainless steel re-processing facility in China as they are one of the largest producer of stainless steel in the world (Millbank, 2013).

Figure 4.6 illustrates the activities involved in the end-of-life phase for the disposal of stainless steel equipment after a period of 15 years. Figure 4.6 also provides the environmental impacts inventory data (energy and water consumption and carbon emissions) of each activity in the end of life disposal obtained either from the GaBi software database or through literature surveys (Noorgate, 2004; Eurofer, 2012). The environmental impacts inventory data are given per unit (e.g. per kg of stainless steel equipment), thus, these data were multiplied with the weight of the stainless steel equipment to obtain the actual environmental impacts data. For example, to obtain the environmental impacts produced during the equipment dismantling and sorting process, the environmental impact inventory data was multiplied with the weight of the stainless steel equipment.

The environmental impacts (inventory data scaled to the size of the equipment) of each activity in the end-of-life phase are labelled as U_1-U_5 in Figure 4.6. The total environmental impact (energy, water and carbon impacts) produced in the end-of-life phase is the sum of the environmental impacts produced by each activity involved in this phase (see Equation 4.10). However, these impacts account for a period of 15 years (life span of the equipment was assumed to be 15 years). Since the aim of the case study was to evaluate the impacts per annum, the total environmental impact produced in the end-of-life phase was divided by 15 (life span of the stainless steel equipment) (Equation 4.11).

The total environmental impacts of the end-of-life phase = $\sum (U_1 + U_2 + U_3 + U_4 + U_5)$

(Equation 4.10)

The environmental impacts of the end-of-life phase per annum

$$= \frac{\sum (U_1 + U_2 + U_3 + U_4 + U_5)}{15}$$

(Equation 4.11)

Finally, the environmental impacts of the traditional and hybrid manufacturing processes per annum is the sum of the impacts per annum from the supply-chain, use and end-of-life phases. As stated previously, in this analysis, the environmental impacts from all the life cycles were adjusted to have a same basis (per annum) in order for a fair comparison.

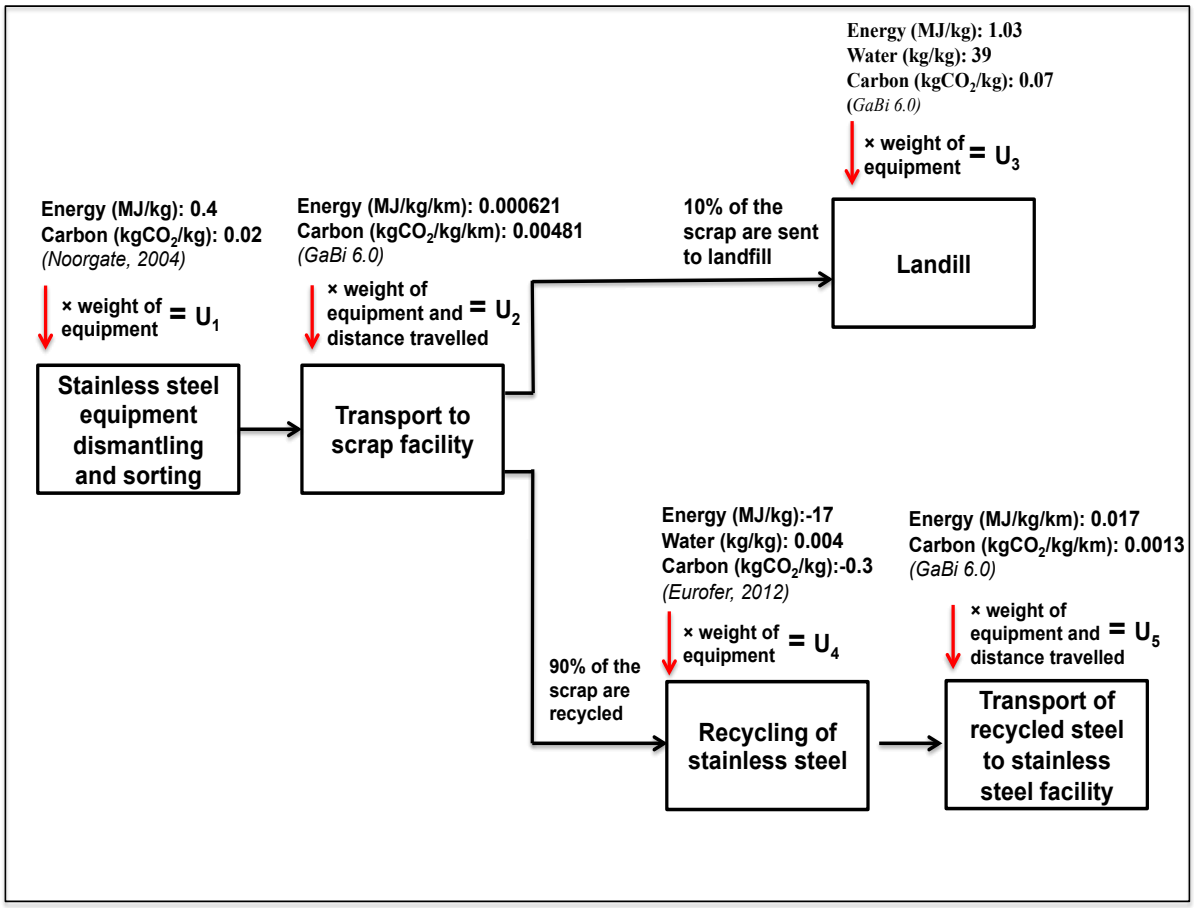


Figure 4.6 *The activities involved in the end of life phase activities and the life cycle inventory data.*

4.4 Overall Assumptions of the Case Study

The assumptions made in this research play a pivotal role. It is important to understand that changes in the assumptions can affect the outcome of the findings. Thus, assumptions made should be reliable, robust and within acceptable ranges (e.g. process titre selected must within the range currently employed for Mab manufacturing processes). The overall assumptions made in this case study were based on reliable resources and some of the assumptions were critically assessed later in sensitivity analysis studies (Chapter 6) to evaluate their robustness. In this section, the other assumptions (not listed above) that were made in this study were identified.

Mass and Volume Balances of the Processes

A number of assumptions were made (especially on the process parameters) in this research to carry out the mass and volume balances for the traditional and hybrid processes. Mass and volume balances are necessary to obtain the relevant information needed for the environmental analyses (see Appendix 4.1, Tables A2 and A3 for mass and volume balance of the manufacturing processes). The BioSolve software and reliable literature sources were used as a reference. For the fermentation unit operation, the operation yield was assumed to be 100%. The operation yield for the disc stack centrifuge was assumed to be 98%, and the centrate percentage of this unit operation was assumed to be 85% (Shukla *et al*., 2007; Rawlings and Pora, 2009). The operation yield for the depth filtration, viral filtration, ultrafiltration/diafiltration unit operations were assumed to be 95%, 98%, 98% and 98%, and their flux (flow rates) were assumed to be 200 LMH, 100 LMH, 40 LMH and 300 LMH (Pall, 2016; Pietrzykowski *et al*., 2013; Shukla *et al*., 2007). The ultrafiltration/diafiltration concentration factor was assumed to be 10.

For the chromatography unit operations, a number of parameters such as the operation yield, dynamic binding capacity (DBC), cycle times, and buffer column volumes (CV) were assumed. The operation yield for the protein A chromatography, anion-exchange chromatography and cation-exchange chromatography operations were assumed to be 97%, 89% and 98%. The DBC of the protein A resin was assumed to be 30 g/L and the DBC of the ion-exchange chromatography resins were assumed to be 62 g/L. The DBC values were obtained from literature (Low *et al*., 2007). The DBC of resins impact the chromatography column sizing (Low *et al*., 2007). The number of cycles for chromatography is assumed to be 2 cycles per batch

for both Protein A and Ion Exchange chromatography (Pietrzykowski *et al*., 2013). For these chromatography operations, 5 CV of wash buffer was used, 5 CV of equilibration buffer was used, and 5 CV of elution buffer was used, but only 2 CV were collected (Low *et al*., 2007; Ghose *et al*., 2014). Finally, the membrane adsorber chromatography unit operation was assumed to have an operation yield of 95% (Rawlings and Pora, 2009).

Transport

A large number of assumptions were made for the transportation involved in each phase of the life cycle. The assumptions were mainly on the distance travelled and the mode of transport. The transport distances and mode of transport for different activities were based upon discussion with industrial experts. Although the transport distances vary for different activities, the distances assumed were held constant across the scenarios to enable fair comparisons. The modes of transport involved in the analysis are transport by land (truck), air (plane) and sea (ship). The distances for the transportation involved in the life cycle phases are given in Table 4.11.

Energy

Electricity usage was assumed to be from an average UK grid mix. The average UK grid mix consists of a mixture of fossil fuels (~ 64 %), nuclear (~22 %) and renewables (~14 %) (Ecotricity, 2015).

Table 4.11 *Overall transport involved in the life cycle phases and the distances assumed.*

Transport Activities and Types of Transport Involved in the Life Cycle Phases	**Distance (Km)**	**Country-Country**
Supply-Chain Phase: Equipment Fabrication		
Transport from stainless steel production facility to equipment fabrication facility (cargo ship)	6500	China-Sweden
Transport from equipment fabrication facility to monoclonal antibody facility (cargo ship)	2500	Sweden-UK
Supply-Chain Phase: Consumables Fabrication		
Transport from PP/PE processing facility to consumables fabrication facility (from Belgium to Sweden) (cargo ship)	1700	Belgium-Sweden
Transport from consumables fabrication facility to monoclonal antibody facility (from Sweden to the UK) (cargo plane)	2500	Sweden-UK
Use-Phase		
Transport of treated solid waste to disposal site (landfill/incineration/pyrolysis) (truck)	50	Locally
End-of-Life Phase		
Transport from monoclonal antibody facility to stainless steel scrap processing facility (truck)	70	Locally
Transport from stainless steel scrap processing facility to the landfill site (steel waste transport) (truck)	50	Locally
Transport from stainless steel scrap processing facility to stainless steel production facility (cargo ship)	7700	UK-China

4.5 Conclusion

This chapter presents the life cycle inventory data of all the activities evaluated by the framework developed in this research. The collection methodology employed to obtain these inventory data are also presented in this chapter. In this research, the life cycle inventory was predominantly obtained from expert/vendor interviews, life cycle databases and literature surveys. The expert/vendors used in this research possess extensive knowledge of biopharmaceutical processes and their environmental contributions. The inventory data for the case study was also obtained from GaBi 6.0, which is an established LCA software package containing databases with verified LCI data. Using the data from established life cycle software packages will increase the robustness and quality of the study.

The compiled life cycle inventory data obtained were tested to ensure these data were sensible and robust. This was done through cross checking the data with industrial experts (Section 4.1), and also by carrying out sensitivity analyses. Sensitivity analyses help to study the effect of certain parameters/data on the overall environmental impacts produced.

As described in Section 4.1, the main objective of Chapter 4 was to present the life cycle inventory data of biopharmaceutical manufacturing processes. Since only limited life cycle inventory data on biopharmaceutical manufacturing processes is available, it is anticipated that this chapter will be useful for future environmental studies to be carried out in this area/industry. This chapter can be used as a guideline for the life cycle inventory data compilation.

The next chapter (Chapter 5) will present the environmental impacts of the six scenarios evaluated in the case study. The manufacturing process and the solid waste management with the lowest environmental impact will be identified. The "hot-spot" analysis carried out for the manufacturing process with the highest environmental impact will also be presented. This information is useful if the industry has a goal to improvise their manufacturing processes to become more environmentally sustainable.

Chapter 5. Results and Discussion of the Case Study

5.1 Introduction

This chapter aims to demonstrate the functionality of the framework developed in this doctorate through application to a relevant case study (description of this case study is provided in Chapter 3, Section 3.6), which uses the framework to evaluate and compare the environmental impacts of manufacturing alternatives and different solid waste treatment strategies applied to a biomanufacturing problem. The questions that will be addressed in this chapter are:

1. Which manufacturing process should be considered the 'best option' in terms of the lowest environmental impacts associated with monoclonal antibodies production?
2. Which stage within the traditional process train contributes to the highest levels of environmental impact?
3. Which solid waste management approach should the industry consider to employ to manage their waste?

Section 5.2 presents the outcome of the case study described in Chapter 3, Section 3.6. Section 5.3 presents the environmental hot spots study to determine the stages and activities within the life cycle that contributes to the most environmental impacts. Section 5.4 presents the solid waste management study carried out in this thesis to determine the most appropriate solid waste management method for the biopharmaceutical industry. The decision-making considerations for selecting the ideal solid waste management method are highlighted in Section 5.5. An overall

assessment of this chapter is provided in Section 5.6. Finally, a summary is provided in Section 5.7.

5.2 Environmental Impacts of the Scenarios

This section presents the outcome of an analysis to evaluate and compare the environmental impacts of traditional and hybrid monoclonal antibody processes with different solid waste management methods; landfill, incineration and pyrolysis. As described in the project methodology chapter, six scenarios were developed (see Section 3.6). Figures 5.1-5.3 show the overall levels of energy and water consumption, and carbon emissions of all the six scenarios. Figures 5.4-5.6 show the energy, water and carbon emissions breakdown according to the phases involved in the study for all the scenarios examined.

5.2.1 Energy consumption

Figure 5.1 shows the overall energy consumed by the different scenarios. This energy is the sum of all the energy consumed by the activities involved in the supply-chain, use and end-of-life phases. The supply-chain phase includes fabrication, construction and production of all process equipment, consumables and reagent required to support a 20-batch monoclonal antibody production campaign. The use phase includes all impacts that occur during monoclonal antibody production and waste treatment. The end of life phase includes the disposal of fixed-in-place stainless steel equipment after a period of 15 years (this period was assumed fixed in all analyses). The energy recovered ("avoided" energy) in steel recycling and solid waste management (incineration and pyrolysis) has been accounted for, and subtracted from the overall energy consumption.

It is evident from Figure 5.1 that the traditional process consumes significantly more energy than a hybrid process. The traditional process consumes around 571 x 10^4 MJ of energy and the hybrid process consumes around 564 x 10^4 MJ of energy annually. The traditional process consumes around 7 x 10^4 MJ more energy than a hybrid process (comparing traditional process/landfill with hybrid process/landfill). From this data, an estimate of the annual energy consumption of the processes per kg of monoclonal antibodies showed that the traditional process consumes around 21 x 10^4 MJ of energy, and the hybrid process consumes around 19 x 10^4 MJ of energy to produce one kg of monoclonal antibodies. The findings obtained agree with the outcome of an earlier study (Pietrykowski *et al*., 2013) where the single-use alternative was shown to consume considerably less energy than the traditional process mainly due to the reduction or elimination of CIP and SIP operations in the single-use process.

When the energy consumption of the traditional and hybrid processes is compared with the average household energy consumption in the UK, it was determined that the energy consumption of the traditional process per annum is equivalent to the average energy used by 335 houses in the UK per year, and the energy consumption of the hybrid process is equivalent to the average energy used by 332 houses in the UK per year (see Chapter 4, Section 4.5). This reference illustrates the magnitude of energy consumed per annum by the traditional and hybrid processes run at 200 L operation scale to produce monoclonal antibodies.

From Figure 5.1, it can be deduced that the choice of solid waste management method for a particular process does not significantly influence the overall levels of

energy consumption. This is probably because of the small operation scale (200 L) assumed in the case study. As the scale of operation increases, the differences are likely to become more significant. This will be investigated further (see Section 5.4).

Figure 5.2 shows the energy breakdown categorised by life-cycle stages/phases. From this figure it is evident that the substantial majority of the life cycle energy consumption occurs during the use phase. The use phase contributes to 69% of the life cycle energy for a traditional process, and 49% for a hybrid process (when landfill solid waste management is employed in each case).

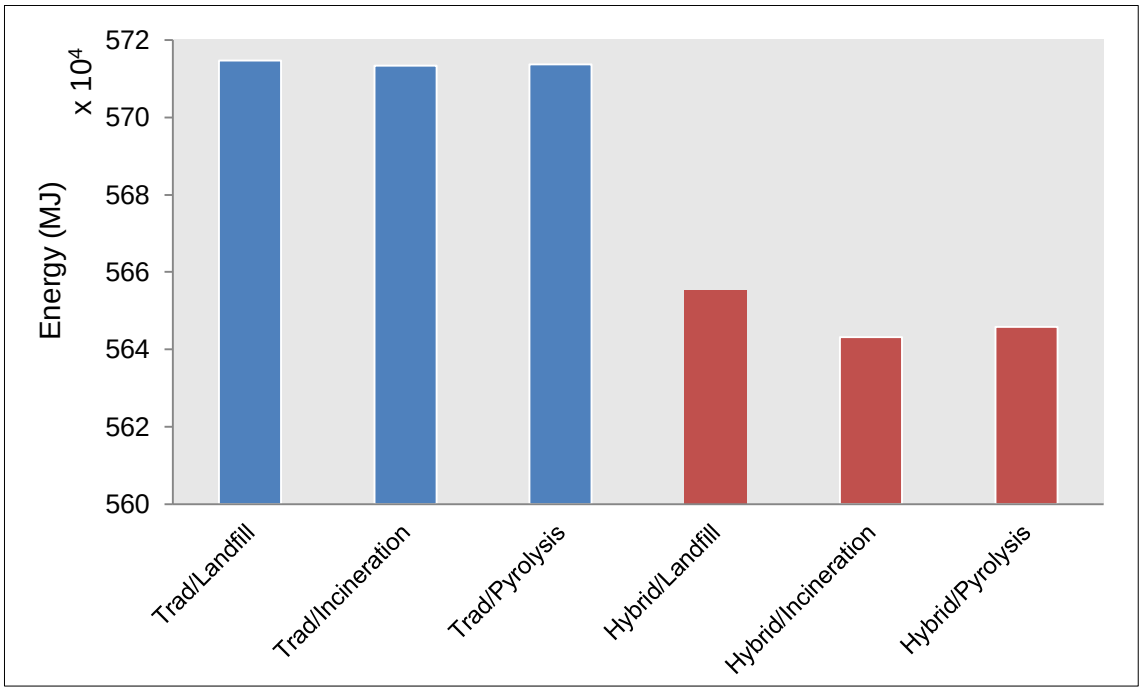


Figure 5.1 *Overall energy consumption of the scenarios. This figure shows the energy consumed by the traditional and hybrid manufacturing processes with different solid waste management methods.*

The most substantial levels of energy consumption in the use-phase are related to HVAC and lighting, and liquid waste treatment activities. The lighting and HVAC requirements itself contribute to 85% of the total use phase energy consumption.

Another observation that can be made from Figure 5.2 is that the use phase energy requirements are much higher in traditional processes compared to hybrid processes. The traditional process consumes 20% more energy in the use phase compared to a hybrid process. This can be attributed to the treatment of liquid waste, which is produced in higher quantities in traditional manufacture due to the increased requirements for CIP and SIP operations.

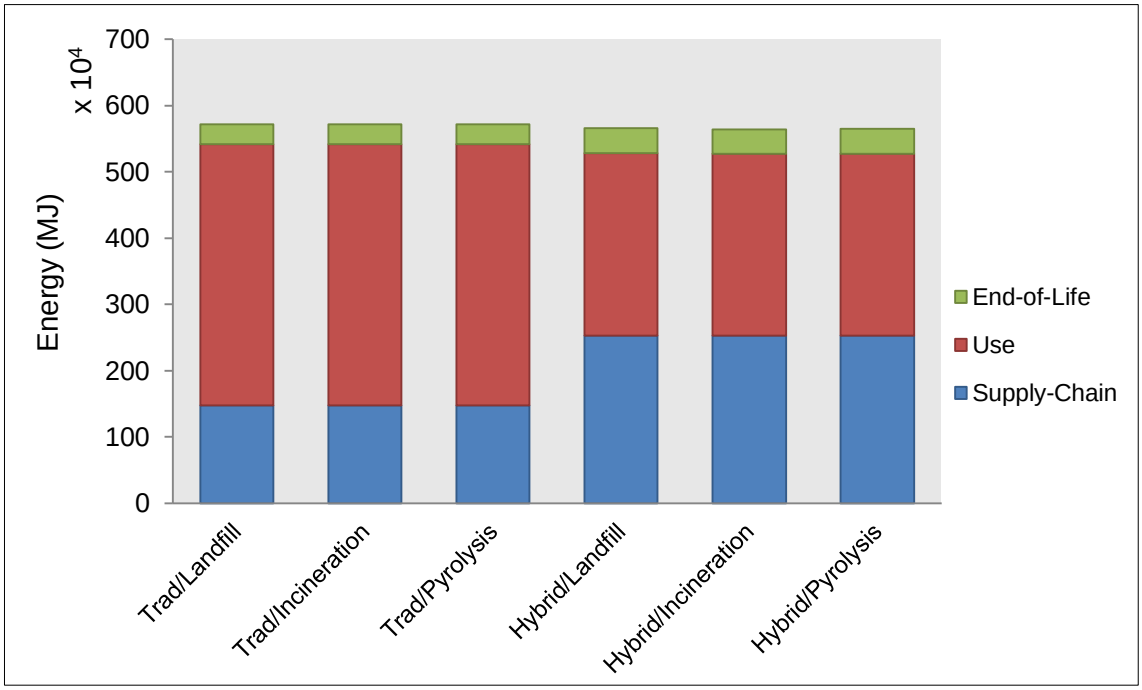


Figure 5.2 *Energy consumption breakdown of the scenarios. This figure shows the energy consumption in the supply-chain, use and end-of-life phases for the traditional and hybrid manufacturing processes with different solid waste management methods.*

Levels of supply-chain energy consumption are higher for the hybrid process option when compared to a traditional process due to the increased manufacturing required to produce the consumables used in the hybrid approach for a 20-batch monoclonal antibody manufacturing campaign. The supply-chain phase contributes to 26% of the life cycle energy for a traditional process, and 45% for a hybrid process. The hybrid

process consumes 19% more energy in the supply-chain phase compared to a traditional process.

In the traditional process (when a strategy of landfill solid waste treatment is employed), the consumables fabrication activity contributes to 47% of the total supply chain phase energy consumption, the equipment fabrication activity contributes to 32% of the total supply chain phase energy consumption, and the reagent production activity contributes to 21% of the total supply chain phase energy consumption. Meanwhile in the hybrid process (when a strategy of landfill solid waste treatment is employed), the consumables fabrication activity contributes to 89% of the total supply chain phase energy consumption, the equipment fabrication activity contributes to 9% of the total supply chain phase energy consumption and the reagent production activity contributes to 2% of the total supply chain phase energy consumption. It is clear that the consumables fabrication is the activity that consumes most energy in the supply chain phase for both the process models.

It is clear from Figure 5.2 that end-of-life impacts represent only a very small fraction of the total energy consumption for all the scenarios. Energy consumption from the end-of-life phase represents 5% of overall energy consumption for a traditional process, and 6% for a hybrid process (using landfill solid waste treatment in both cases). The transportation activities (transportation of stainless steel from the monoclonal antibody facility to the scrap processing facility, and the transportation of recycled steel back to the stainless steel manufacturing facility) consume the most energy in the end of life phase. Figure 5.2 also shows the end-of-life energy requirements are slightly lower than for a traditional process compared to a hybrid

process due mainly to the increased proportion of recycling of stainless steel equipment. This can be attributed to a higher level of energy that was recovered from increased stainless steel recycling in the traditional process.

The energy consumption values for all the activities evaluated by the framework for the traditional and hybrid processes are provided in Appendix 4.2.

5.2.2 Water consumption

Figure 5.3 shows the overall levels of water consumed by the different scenarios. This water is the sum of all the water consumed by the activities involved in the supply-chain, use and end-of-life phases. The activities involved in these phases have been described in the previous chapter (Chapter 4, Section 4.3).

It is evident from Figure 5.3 that the traditional process consumes significantly more water than a hybrid process. The traditional process consumes around 90 x 10^4 kg of water, and the hybrid process consumes around 50 x 10^4 kg of water. The hybrid process consumes 43% less water than a traditional process (comparing traditional process/landfill with hybrid process/landfill). Substantial levels of water savings are achieved for the hybrid processes due to the reduction of CIP and SIP operations between production batches.

The annual water consumption of the processes expressed per kg of monoclonal antibodies showed that the traditional process consumes around 3 x 10^4 kg of water, and the hybrid process consumes around 2 x 10^4 kg of water to produce one kg of monoclonal antibodies. So a very significant difference is observed.

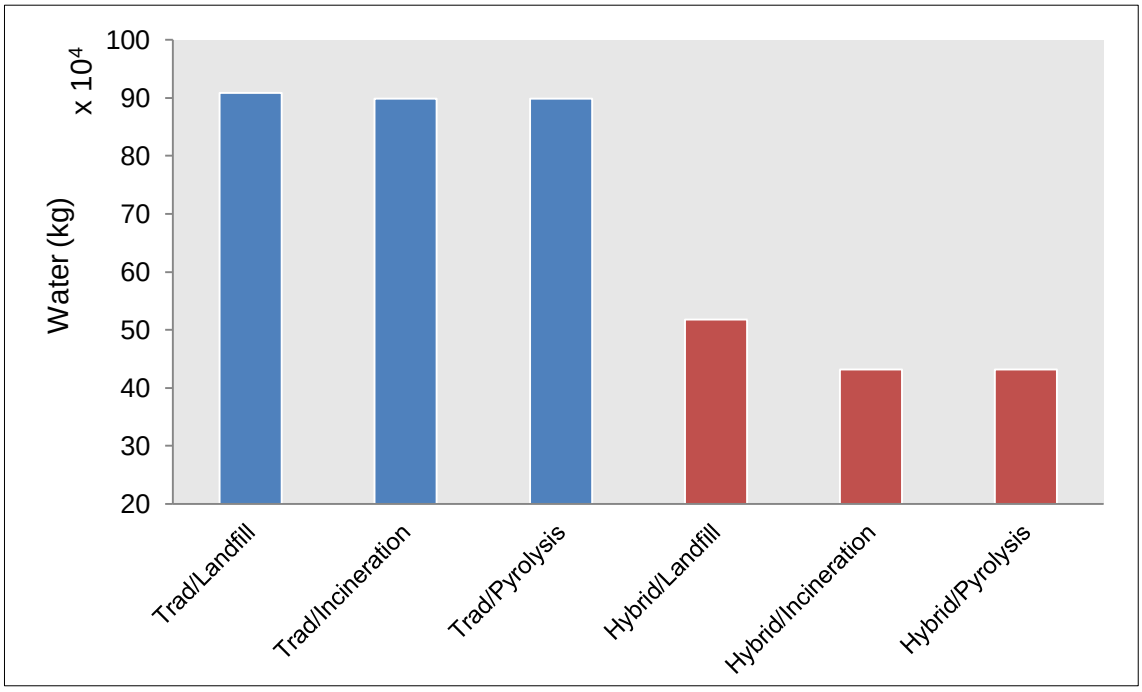


Figure 5.3 *Overall water consumption of the scenarios. This figure shows the water consumed by the traditional and hybrid manufacturing processes with different solid waste management methods.*

When the water consumption of the traditional and hybrid processes is compared with the average household water consumption in the UK, it was determined that the water consumption per annum of the traditional process is equivalent to the average water used by 3 houses in the UK per year, and the water consumption of the hybrid process is equivalent to the average water used by 2 houses in the UK per year (see Chapter 4, Section 4.5). This reference illustrates the true magnitude of water consumed per annum by the traditional and hybrid processes run at 200 L operation scale to produce monoclonal antibodies, which are rather small.

From Figure 5.3, it can be seen that disposal via landfill requires slightly higher levels of water compared to incineration and pyrolysis waste disposal methods. The landfill method consumes high levels of water for the preparation of materials for leachate and sludge treatment and deposition (see Section 3.6.1.3). However, it is

clear that the choice of solid waste management method for a particular process does not significantly influence the overall levels of water consumption. This is most likely a reflection of the small operation scale (200 L) considered in this case study. As the operation scale increases, the differences may become more significant and will be investigated further (see Section 5.4).

Figure 5.4 shows the water breakdown categorised by life-cycle stages/phases. From this figure it is evident that the substantial majority of the life cycle water consumption occurs during the supply-chain phase. The supply-chain phase contributes to 95% of the life cycle water for a traditional process, and 78% for a hybrid process (when landfill solid waste management is employed in each case).

The most substantial levels of water consumption in the supply-chain phase are related to reagent production and stainless steel equipment fabrication activities. In the traditional process, the reagent production activity contributes to 51% of the total supply chain phase water consumption, the equipment fabrication activity contributes to 47% of the total supply chain phase water consumption, and the consumables fabrication activity contributes to 3% of the total supply chain phase water consumption. Meanwhile in the hybrid process, the equipment fabrication activity contributes to 55% of the total supply chain phase water consumption, the reagent production activity contributes to 30% of the total supply chain phase water consumption, and the consumables fabrication activity consumes to 15% of the total supply chain phase water consumables. It is evident that reagent production and stainless steel equipment fabrication are the most water intensive activities in the supply chain phase.

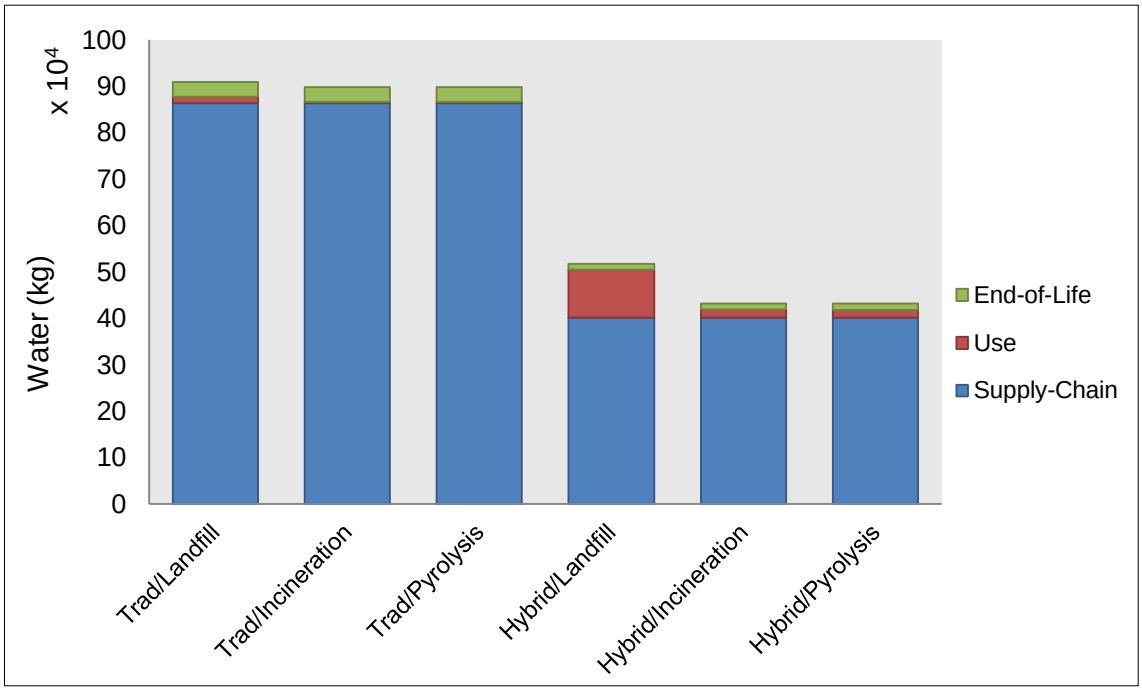


Figure 5.4 *Water consumption breakdown of the scenarios. This figure shows the water consumption in the supply-chain, use and end-of-life phases for the traditional and hybrid manufacturing processes with different solid waste management methods.*

Another observation that can be made from Figure 5.4 is that the supply-chain water requirements are much higher in traditional processes compared to hybrid processes. This is due to increased requirements for CIP and SIP operations in the traditional processes. The traditional process exhibits 17% more water in the supply-chain phase compared to a hybrid process.

Levels of use-phase water consumption are higher for the hybrid process option when compared with a traditional process due to the water consumed for the disposal of increased waste produced in the hybrid process. The water consumption in the use phase takes place during the solid waste treatment stage. The use-phase contributes to 1% of the life cycle water for a traditional process and 20% for a hybrid process (when a strategy of landfill solid waste treatment is employed). Figure 5.3 shows that the landfill waste disposal method consumes more water in the use-phase than the

other waste disposal methods. As mentioned earlier, the water in landfill disposal method is used in leachate and sludge treatment and deposition.

Water consumption from the end-of-life phase represents 4% of overall water consumption for a traditional process, and 2% for a hybrid process (using landfill solid waste treatment in both cases). The water consumption in the end of life phase mainly takes place during the stainless steel equipment recycling. Figure 5.4 shows the end-of-life water requirements are slightly lower than for a hybrid process compared to a traditional process due mainly to the increased proportion of recycling of stainless steel equipment. This can be attributed to a higher level of water that was consumed for increased stainless steel recycling in the traditional process.

The water consumption values for all the activities evaluated by the framework for the traditional and hybrid processes are provided in Appendix 4.3.

5.2.3 Carbon emissions

Figure 5.5 shows the overall levels of carbon emissions for the different scenarios. This is the sum of all the GHGs generated by all the activities involved in the supply-chain, use and end-of-life phases. The activities involved in these phases have been described in the previous chapter (Chapter 4, Section 4.3). The GHGs recovered ("avoided" GHGs) in steel recycling and solid waste management (incineration and pyrolysis) has been accounted for, and subtracted from the overall GHGs emitted.

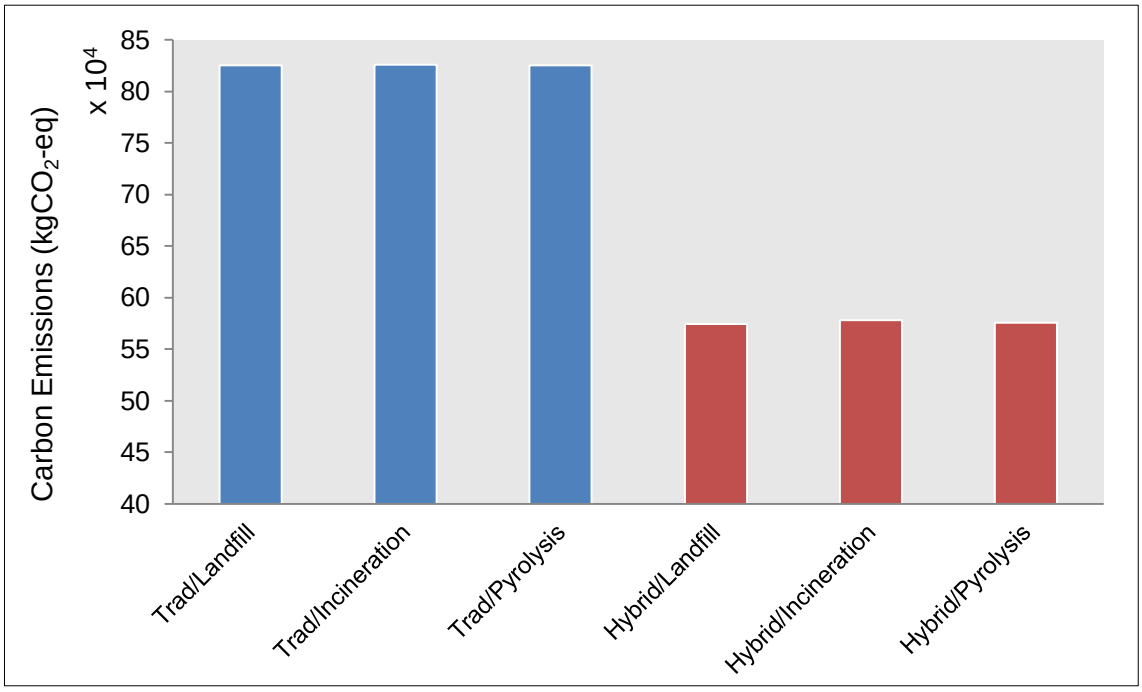


Figure 5.5 *Overall carbon emissions of the scenarios. This figure shows the carbon emitted by the traditional and hybrid manufacturing processes with different solid waste management methods.*

It is evident from Figure 5.5 that the traditional process generates significantly more carbon than a hybrid process. The traditional process generates around 82 x 10^4 $kgCO_2$-eq of carbon and the hybrid process generates around 60 x 10^4 $kgCO_2$-eq of carbon (comparing traditional process/landfill with hybrid process/landfill). The traditional process generates 30% more carbon than a hybrid process. The annual carbon emissions of the processes per kg of monoclonal antibodies showed that the traditional process generates around 3 x 10^4 $kgCO_2$-eq of carbon, and the hybrid process generates around 2 x 10^4 $kgCO_2$-eq of carbon to produce one kg of monoclonal antibodies. Again a significant difference dependent upon the mode of production adopted.

From Figure 5.5, it can be deduced that the choice of solid waste management method for a particular process does not significantly influence the overall carbon emissions. This is most likely a reflection of the small operation scale (200 L) considered in this case study. Again, as has been noted before it is likely that as the operation scale increases, so the differences may become more significant. This will be investigated in Section 5.4.

Figure 5.6 shows the carbon emissions breakdown categorised by life-cycle stages/phases. From this figure, it is evident that the substantial majority of the life cycle carbon emissions occurs during the use phase. The use phase generates 58% of the life cycle carbon for a traditional process, and 59% for a hybrid process (when landfill solid waste management is employed in each case).

The carbon emissions in the use phase are mainly from the lighting and HVAC activity. In the traditional process, the lighting and HVAC activity alone contributes to 90% of the total use phase carbon emissions, and in the hybrid process, the lighting and HVAC activity contributes to 87% of the total use phase carbon emissions. Another observation that can be made from Figure 5.6 is that the use phase carbon emissions are much higher in traditional processes compared to hybrid processes. The traditional process generates 29% more carbon in the use phase compared to a hybrid process. This can be attributed to the treatment of liquid waste which is produced in higher quantities in traditional manufacture due to the increased requirements for CIP and SIP operations.

Levels of supply-chain carbon emissions are higher for the traditional process option when compared to those of a hybrid process due to the increased production of reagents and increased manufacturing required to produce the equipment used in traditional process approach for a 20-batch monoclonal antibody manufacturing campaign. The equipment manufacturing is the key activity in the supply chain phase that leads to the high levels of carbon emissions in the traditional process. In the traditional process, the equipment fabrication activity contributes to 52% of the total supply chain phase carbon emissions, the consumables fabrication activity contributes to 37% of the total supply chain phase carbon emissions, and the reagent production activity contributes to 11% of the total supply chain phase carbon emissions. Meanwhile, consumables fabrication is the key activity in the supply chain phase that leads to the high levels of carbon emissions in the hybrid process. In the hybrid process, the consumables fabrication activity contributes to 59% of the total supply chain phase carbon emissions, the equipment fabrication activity contributes 39% of the total supply chain phase carbon emissions, and the reagent production activity contributes to 2% of the total supply chain phase carbon emissions.

Another observation that can be made from Figure 5.6 is that the traditional process generates more carbon in the supply chain phase than the hybrid process. The supply-chain phase generates 34% of the life cycle carbon emissions for a traditional process and 18% for a hybrid process (when a strategy of landfill solid waste treatment is employed). The traditional process generates 15% more carbon in the supply-chain phase compared to a hybrid process.

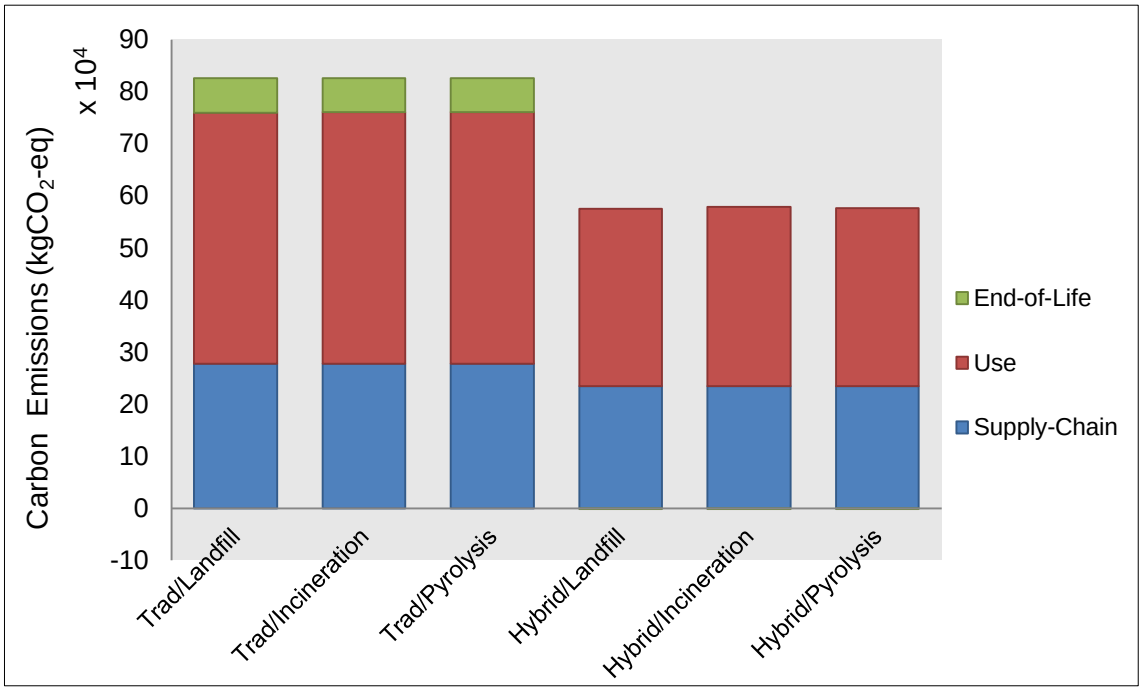


Figure 5.6 *Carbon emissions breakdown of the scenarios. This figure shows the carbon emissions in the supply-chain, use and end-of-life phases for the traditional and hybrid manufacturing processes with different solid waste management methods.*

It is clear from Figure 5.6 that end-of-life impacts represent only a very small fraction of the total carbon generated for the traditional processes. The carbon emissions from the end-of-life phase represents 8% of overall carbon generated from a traditional process. The carbon emissions in the traditional processes are produced from equipment recycling activities. Meanwhile from the same figure, it can be seen that carbon savings were achieved in the end-of-life phase for the hybrid processes. In the hybrid processes, the overall carbon generated from the stainless equipment recycling activities (equipment transport, steel processing and steel waste disposal) is less than the carbon savings achieved through energy recovery from stainless steel equipment recycling. However, for the traditional processes, the overall carbon generated from the stainless steel equipment recycling activities is more than the carbon savings achieved through energy recovery from stainless steel equipment recycling. This can be attributed to the increased levels of stainless steel equipment

recycling in the traditional processes. The carbon emission values for all the activities evaluated by the framework for the traditional and hybrid processes run at 200 L operation scale are provided in Appendix 4.2.

Having evaluated and compared the environmental impacts of the scenarios, the next step was to carry out a hot spot analysis of the traditional process in order to identify the unit operations contributing the highest levels of environmental impact and also the life-cycle phase within the unit operations with the highest levels of impact.

5.3 Environmental Hot Spot Analysis

This section seeks to identify accurately and transparently the underlying environmental burdens associated with monoclonal antibody manufacturing processes. This section focuses on identifying the key unit operation that appear to create the most significant contribution to environmental impact, followed by a detailed evaluation of that unit operation (see Section 3.6.1) for the description of this analysis). Since the traditional process has been identified as creating the most environmental impact, this process was adopted for analysis.

A traditional process employing landfill solid waste treatment strategy was utilised in this analysis to identify the key unit operation in the process train that contributes the highest level of environmental impact. The unit operations in the process trains were categorised into 7 stages that include upstream, harvest and clarification, capture, polishing, viral removal, concentration/buffer exchange/sterile filtration and finally support equipment (see Section 3.6.1.1 for the traditional process flow sheet). This procedure was employed in order to simplify the analysis.

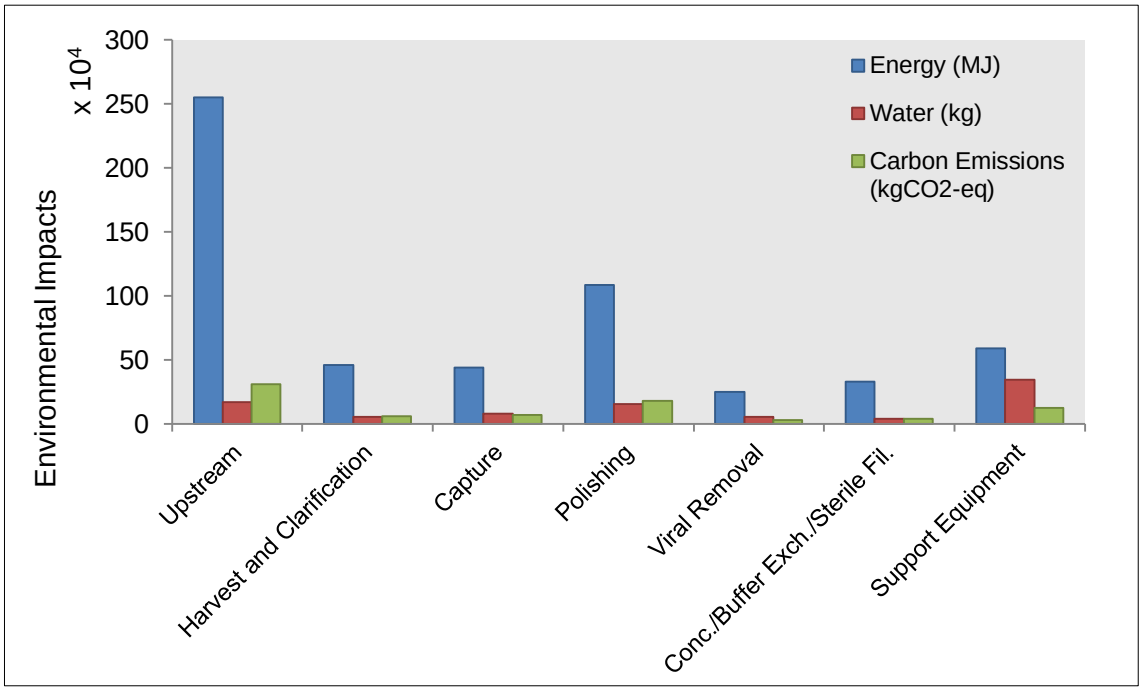


Figure 5.7 *Overall environmental impacts of the unit operations employed in the traditional monoclonal antibody manufacturing process.*

Figure 5.7 illustrates the contributions to the overall environmental impact of the unit operations in the traditional process train. The upstream stage contributes to the highest energy consumption and carbon emissions. Meanwhile, the support equipment stage contributes most to water consumption. The next largest contributions to the overall energy consumption and carbon emissions come from the polishing stage, and to the overall levels of water consumption from the upstream stage.

From the hot spot analysis, the upstream, polishing and support equipment stages were identified as the highest contributors to the overall environmental impact. The following sections will discuss these hot spot stages further.

5.3.1 Upstream stage

In Figure 5.7, the upstream stage was shown to be a significant contributor to energy usage and carbon emissions. The upstream stage consists of two seed fermenters (10 L and 20 L) and one 200 L scale bioreactor. This stage also considers vessels for media/buffer preparation, product hold and waste collection (see Appendix 4.2 for the list of equipment in the traditional process).

Figure 5.8 shows the individual contributions of each phase to the overall environmental impacts in the upstream stage. The largest contributions to energy usage and carbon emissions in the upstream stage come from the use phase, and the largest contribution to water consumption comes from the supply chain phase. The end of life phase creates the lowest environmental impact contribution in the upstream stage. Having described this, the Figures 5.9a-5.9c will explain this in detail.

Figures 5.9a shows the contributions of activities within the supply-chain phase to the overall upstream stage environmental impacts. As stated above, the supply chain phase creates the largest contribution to water consumption; significant levels of water are consumed for reagent production activities. The traditional bioreactors are dominated by the need for CIP and SIP operations between batches. At the 200 L scale, the volume of water associated with this contribution is 20500 L/batch. All of this water represents a large energy load contribution in the supply chain phase.

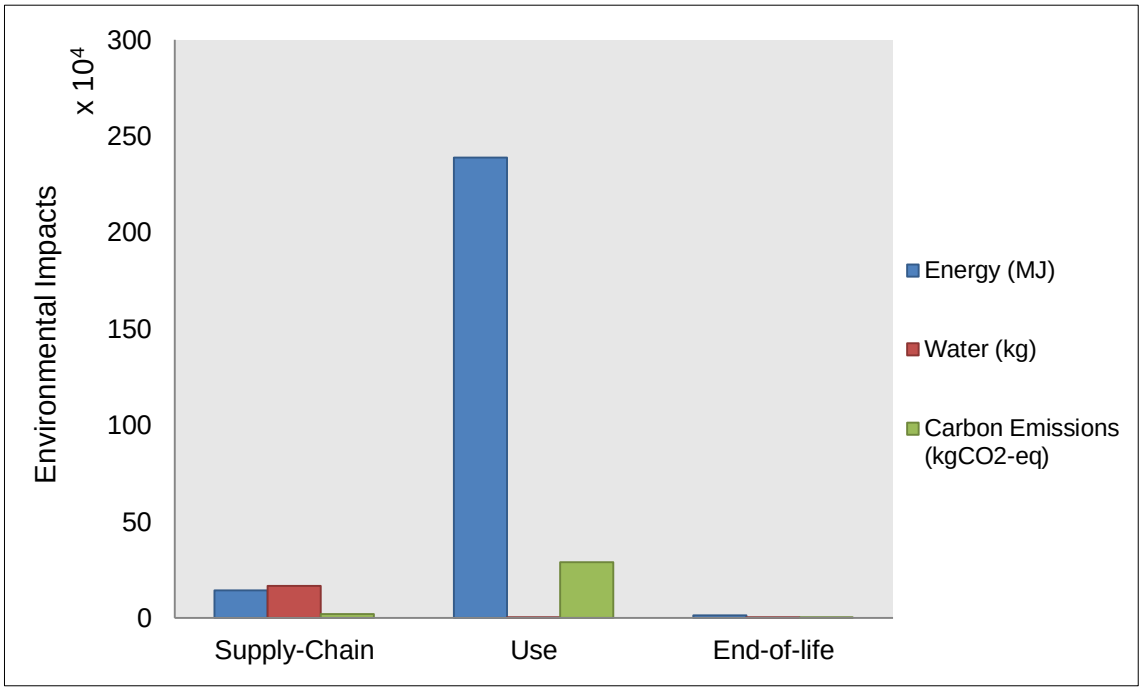


Figure 5.8 *Contributions of each of the life cycle phases to the overall upstream stage environmental impacts.*

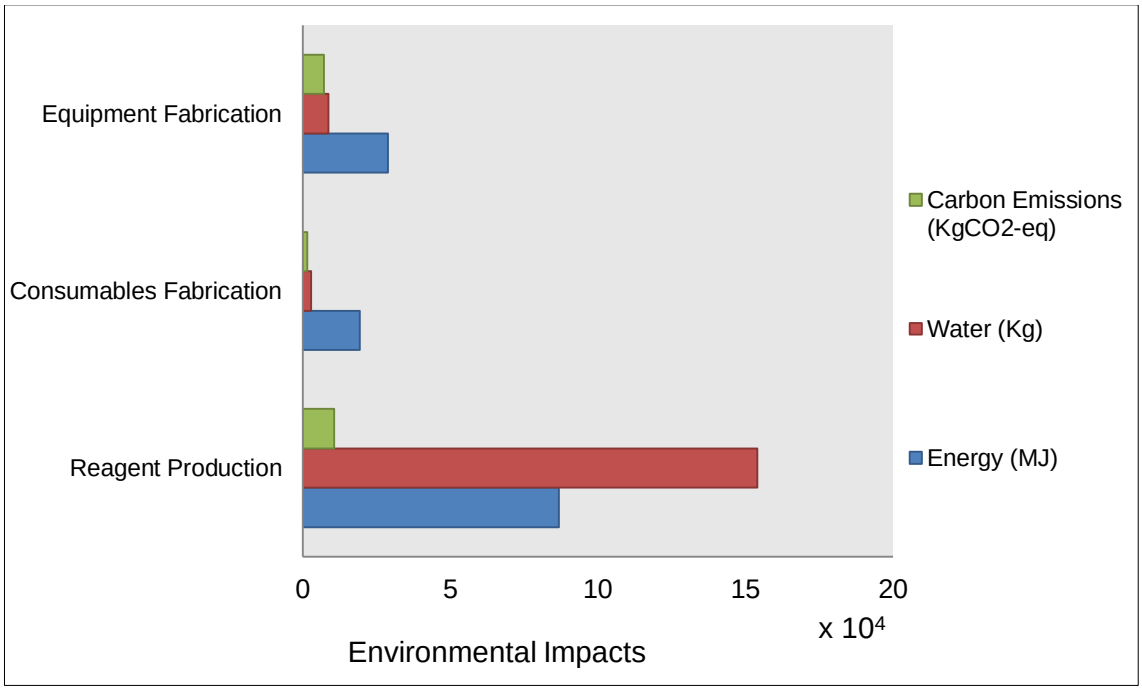


Figure 5.9a *Contribution of activities in the supply-chain phase to the overall upstream stage environmental impacts.*

Figure 5.9b can be used to examine the contributions of the activities from the use phase to the overall upstream stage environmental impacts. The substantial levels of energy consumption and carbon emissions in the use phase for the upstream stage come predominantly from lighting and HVAC requirements, followed by liquid waste management.

The lighting and HVAC energy requirements alone contribute to more than half of the total upstream energy requirements. A bench marking study of cleanroom energy in biopharmaceutical facilities showed that HVAC energy use comprises around 60% of the total energy (Ho *et al*., 2011). It is evident that in the upstream stage, the lighting and HVAC requirements represent the main cause for the high energy and carbon impacts of the use-phase. This is followed by the liquid waste management activity.

Figure 5.9c shows the activities involved in the end of life phase for the upstream stage. The end of life phase creates the lowest environmental impact contribution of all the other life cycle phases. In this particular case study, the recovered stainless steel was assumed to be transported to a raw material facility in China (see Table 3.2 under Section 3.6.3). This is a long distance transport and such transports are associated with huge environmental impacts.

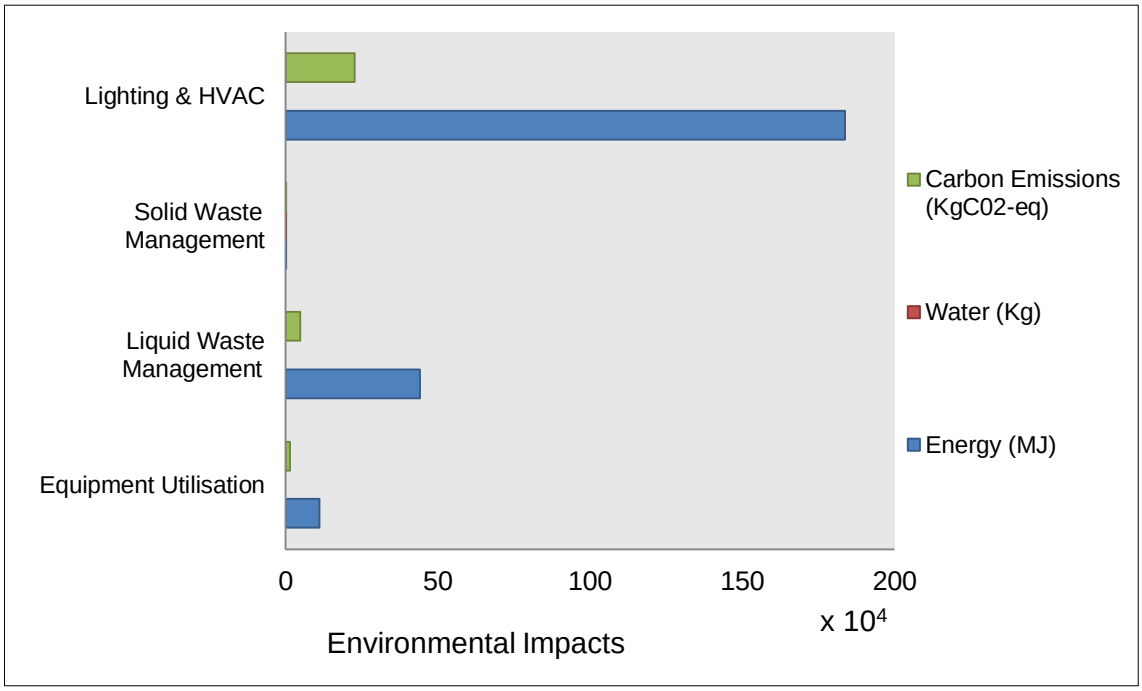


Figure 5.9b *Contribution of activities in the use phase to the overall upstream stage environmental impacts.*

It is evident that use of traditional equipment leads to high environmental burdens. Potentially, therefore introducing single-use alternatives at this stage may result in useful reductions and hence, environmental benefits. For example, replacing the traditional bioreactors with single-use WAVE bioreactors (GE Healthcare, Wisconsin, America), and replacing the vessels with single use bags in the upstream stage results in a significant decrease in both energy and water consumption and carbon emission levels. Conversion of the upstream stage to employ single use bioreactors is estimated to require 70% less energy, 71% less water, while 69% lower carbon emissions would be generated compared to a traditional process.

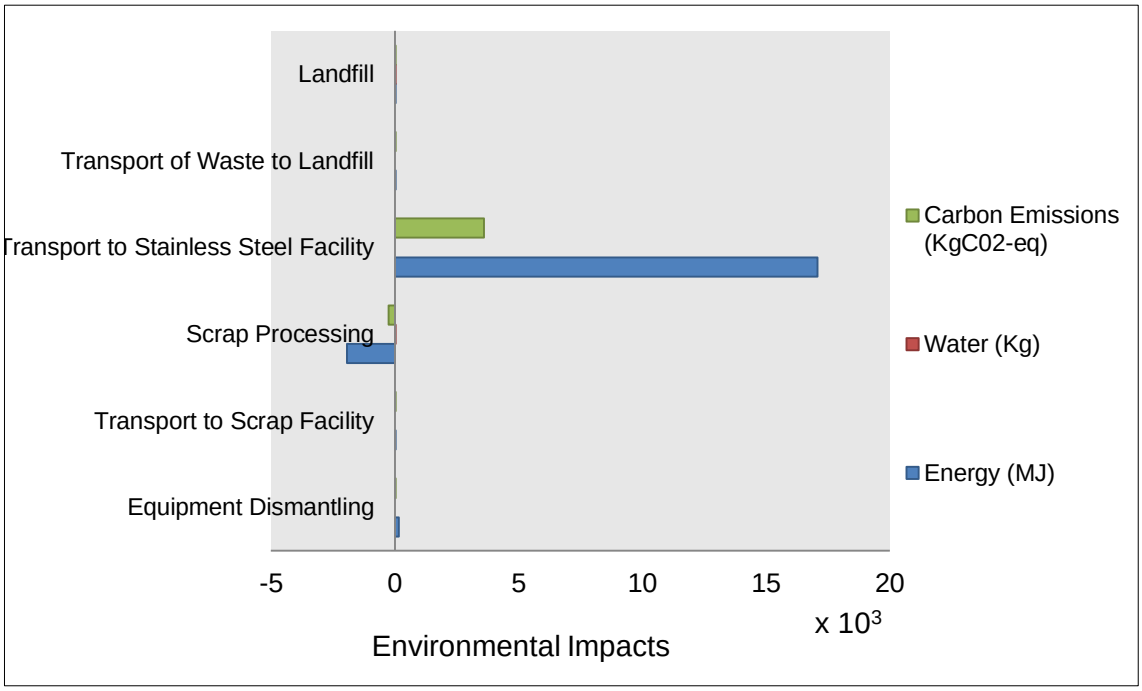


Figure 5.9c *Contribution of activities in the end-of-life phase to the overall upstream stage environmental impacts.*

5.3.2 Polishing stage

Following the upstream stage, the next most significant contributor to energy usage and carbon emissions is the polishing stage. The polishing stage consists of a bind and elute cation-exchage chromatography and a flow-through anion exchange chromatography. This stage also considers vessels for media/buffer preparation, product hold and waste collection.

Figure 5.10 shows the individual contributions of phases in the polishing stage. The supply chain phase forms the greatest environmental impact, followed by the use phase. The end of life phase provides the lowest levels of contribution to the environmental impact within the polishing stage. Having described this, the Figures 5.11a-5.11c will explain this in detail.

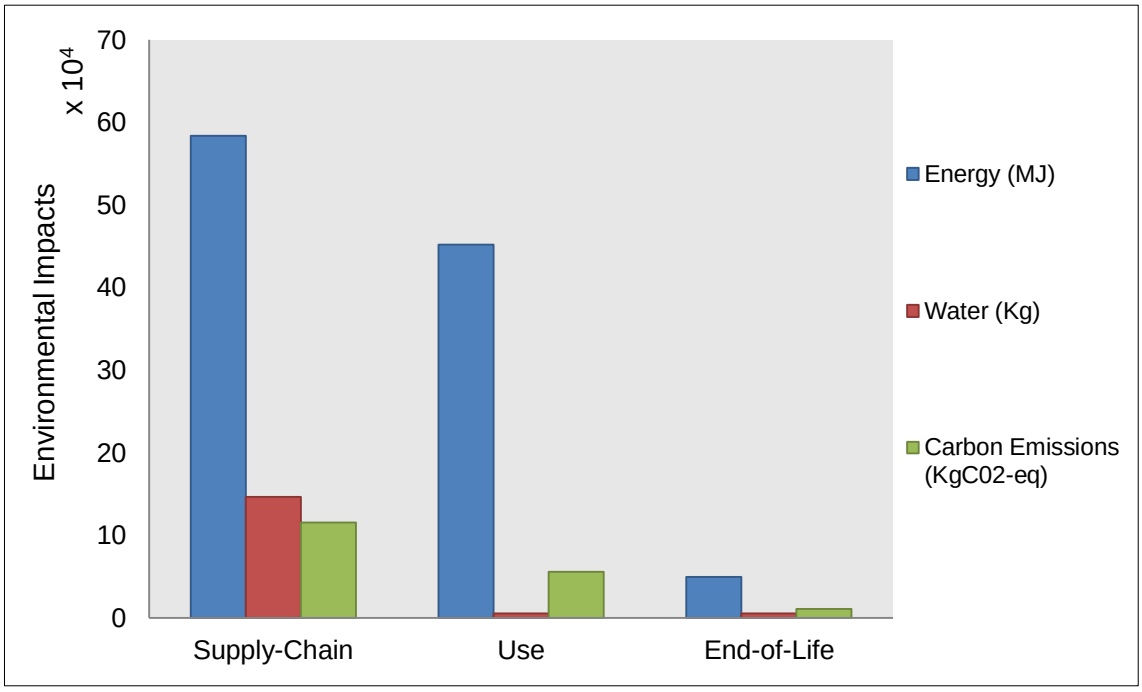


Figure 5.10 *Contributions of each of the life cycle phases to the overall polishing stage environmental impacts.*

Figure 5.11a can be used to examine the contributions of each activity involved in the supply chain phase to the overall environmental impact of the polishing stage. Unlike in the case of the upstream stage, the substantial energy and carbon impacts of the supply chain phase for the polishing stage are on consumables fabrication. The fabrication of consumables required for the polishing stage contributes to around 2 x 10^4 MJ of energy per production batch. The manufacture of resins contributes significantly to the high energy and carbon impact (resin manufacturing process is given in Section 4.3.1).

The high levels of water consumption in the supply chain phase for the polishing stage can be associated with reagent production activities (preparing buffers for chromatography unit operations). The polishing stage consumes around 0.5 x 10^4 kg

of water per production batch. High levels of reagent/buffer are required in the polishing stage to run the ion-exchange unit operations.

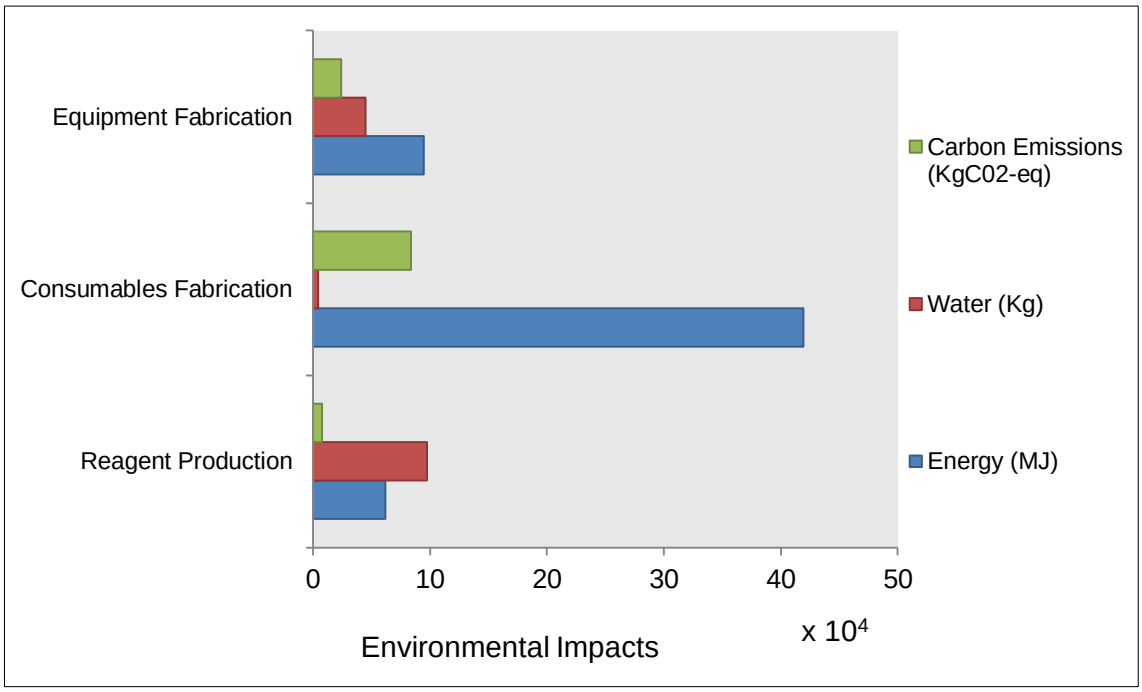


Figure 5.11a *Contribution of activities in the supply-chain phase to the overall polishing stage environmental impacts.*

Figure 5.11b illustrates the environmental contributions of activities in the use phase for the polishing stage. The highest environmental contributions in the polishing stage come from the liquid waste management activity. The liquid waste management activity contributes to 1.4 x 10^4 MJ of energy per production batch. The liquid waste management activity has the highest energy consumption because the polishing stage consists of a large number of vessels required for media/buffer preparation. These vessels are subjected to CIP and SIP operations, therefore high levels of liquid waste are produced. The lighting and HVAC activities also contribute to a high environmental impact in the use phase. Other activities in the use phase have much lower levels of environmental impact.

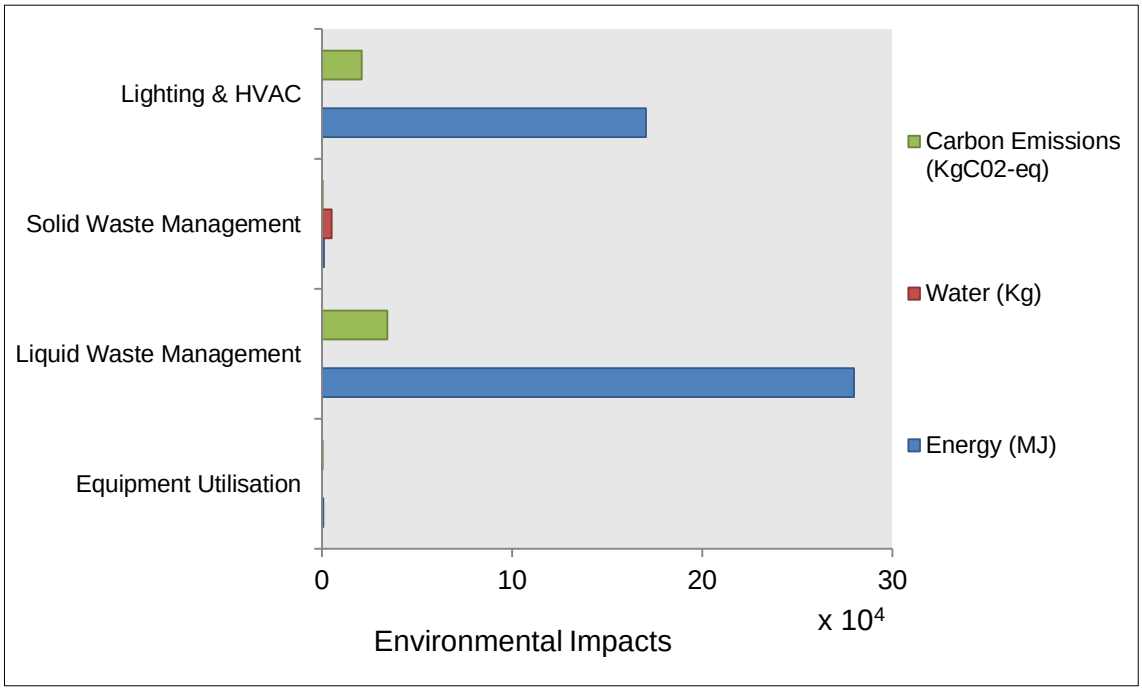


Figure 5.11b *Contribution of activities in the use phase to the overall polishing stage environmental impacts.*

As illustrated in Figure 5.10, the end of life phase creates the lowest levels of environmental impact. Figure 5.11c illustrates the environmental contributions of activities in the end of life phase for the polishing stage. As mentioned before, the impact in the end of life phase can be mainly associated with the transport of steel.

The choice of unit operation makes a big difference to the estimated environmental impacts. So for example, when the traditional anion-exchange chromatography step was replaced with an anion-exchange membrane adsorber, 69% less energy, 53% less water were consumed, while 67% less carbon emissions were generated. Replacing the stainless steel support with single use bags resulted in 63% less energy consumption, 43% less water consumption and 60% less carbon emissions. These savings were mainly achieved due to the lower requirements for CIP/SIP operations

and stainless steel equipment fabrication in the single-use alternatives. This clearly indicates the benefits of incorporating single use technologies in manufacturing.

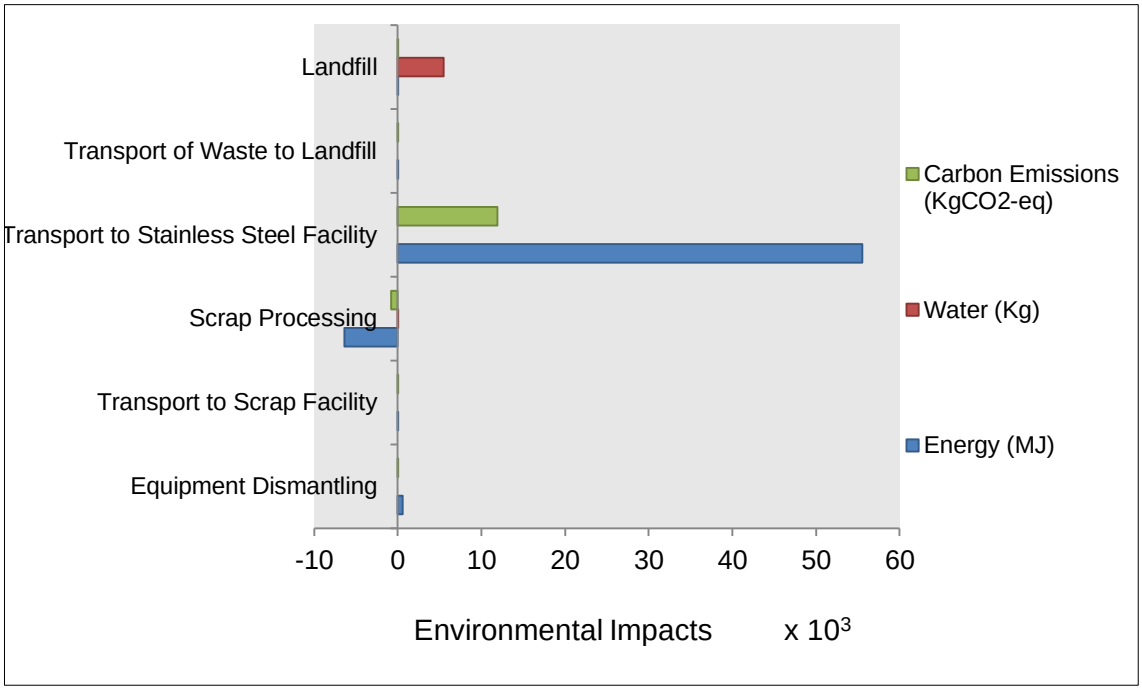


Figure 5.11c *Contribution of activities in the end-of-life phase to the overall polishing stage environmental impacts.*

5.3.3 Support equipment

As identified earlier, support equipment contributes to the highest levels of water consumption across all elements of a complete process. This stage comprises the general support systems such as waste tanks and CIP/SIP, PW and WFI skids that are shared across the traditional process. Figure 5.7 shows that the water consumption in this stage is considerably higher than in all the other stages across the traditional process. Although the water consumption is high, the energy and carbon impacts of this stage are low compared to the upstream and polishing stages.

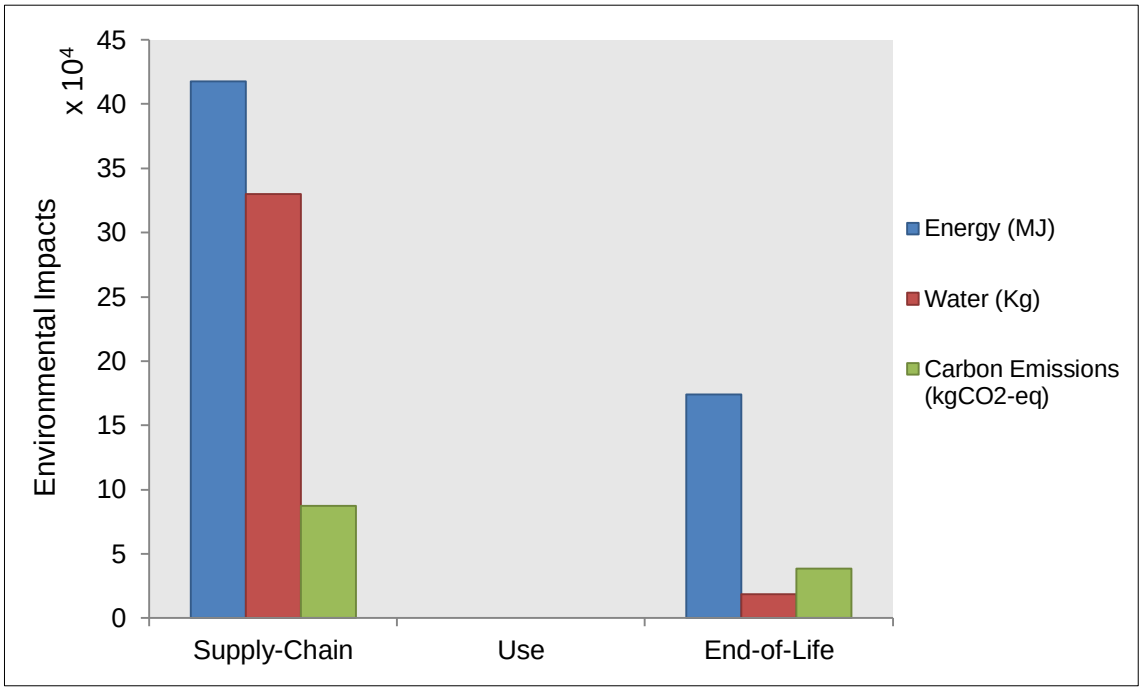


Figure 5.12 *Contributions of each of the life cycle phases to the overall environmental impact produced by support equipment.*

In the support equipment stage, the water consumption mainly takes place in the supply chain and end-of-life phases, as illustrated by Figure 5.12. The water consumption in the supply chain phase is associated with equipment fabrication. The reason for the support equipment stage having a high level of water consumption across the process is due to the fact that this stage consists of a number of large vessels which each need high levels of water for fabrication. The water consumption in the end-of-life phase is mainly for stainless steel scrap processing and landfill of scrap waste.

Replacing the traditional stainless steel vessels with single-use bags where possible (the largest volume of single-use bags is currently 2000 L) lead to a significant reduction in water consumption. The single-use alternatives required 80% less water compared to the process using traditional equipment.

The detailed evaluation carried out for the individual stages; upstream, polishing and support equipment identified the underlying issues which cause these stages to exhibit high environmental impacts. However, this analysis also shows that a shift from traditional to single-use alternatives can result in substantial reductions in a range of environmental impacts. This case study's main aim was to demonstrate the functionality of the framework developed for selecting biomanufacturing processes and solid waste disposal methods that are most environmentally acceptable. Although for this particular study, the framework identified the hybrid process as a better alternative to the traditional approach, no ideal solid waste management method could be identified in the analysis. This is because the differences in impacts between the solid waste disposal methods were insignificant at the 200 L scale.

Therefore, the aim of the next stage of analysis (Section 5.4) in this case study was to investigate the environmental impacts of solid waste management methods at larger scales (scales above 200 L of fermentation), and hence to determine whether the operational scale plays a significant role in selecting the best solid waste management method.

5.4 Solid Waste Management Studies

Previously, it was identified that the environmental impacts produced by the solid waste management methods of processes run at 200 L scale of fermentation were similar. The main focus of this next analysis therefore was to investigate the environmental impacts produced by solid waste management methods for a traditional process at larger operational scales of fermentation: 2,000 L and 20,000 L, and to see if the environmental impacts correlate with the scale of the operation. The

study will focus on treatment, transport and disposal of solid waste using three different disposal methods: landfill, incineration and pyrolysis (the description of these methods can be found in section 3.6.1). Presently, these are the options that can be considered by the biopharmaceutical industry to manage their solid waste effectively (Rawlings and Pora, 2009).

5.4.1 Energy consumption

Figure 5.13 shows the energy demand of the solid waste management methods at different scales. As the scale increases, the benefits of incineration and pyrolysis can be seen. Both the methods recover energy, however at a 200 L scale, the overall energy consumed for the treatment, transport and disposal activities of the solid waste route is higher than the energy recovered from the waste. The amount of energy recovered from the waste is small because of the low levels of solid produced at this scale, and at a 200 L scale, no appreciable additional energy is produced.

At a 2000 L scale, for pyrolysis, the overall energy consumed for the treatment, transport and disposal activities is higher than the energy recovered. However, for incineration at this scale, the energy recovered from the waste is slightly higher than the overall energy consumed for the treatment, transport and disposal activities. At a 2,000 L scale, net energy is produced from the incineration method.

Finally, at a 20,000 L scale, the energy recovered from incineration and pyrolysis is higher than the overall energy consumed for the treatment, transport and disposal activities. At this scale, higher levels of energy are produced from incineration and pyrolysis compared to operation at the previous scales. The incineration method

recovers higher levels of energy than the pyrolysis method at this scale. The energy recovered can be used by the local industry or sold to the local utility. At a 20,000L scale, there is a net gain energy produced from the incineration and pyrolysis methods.

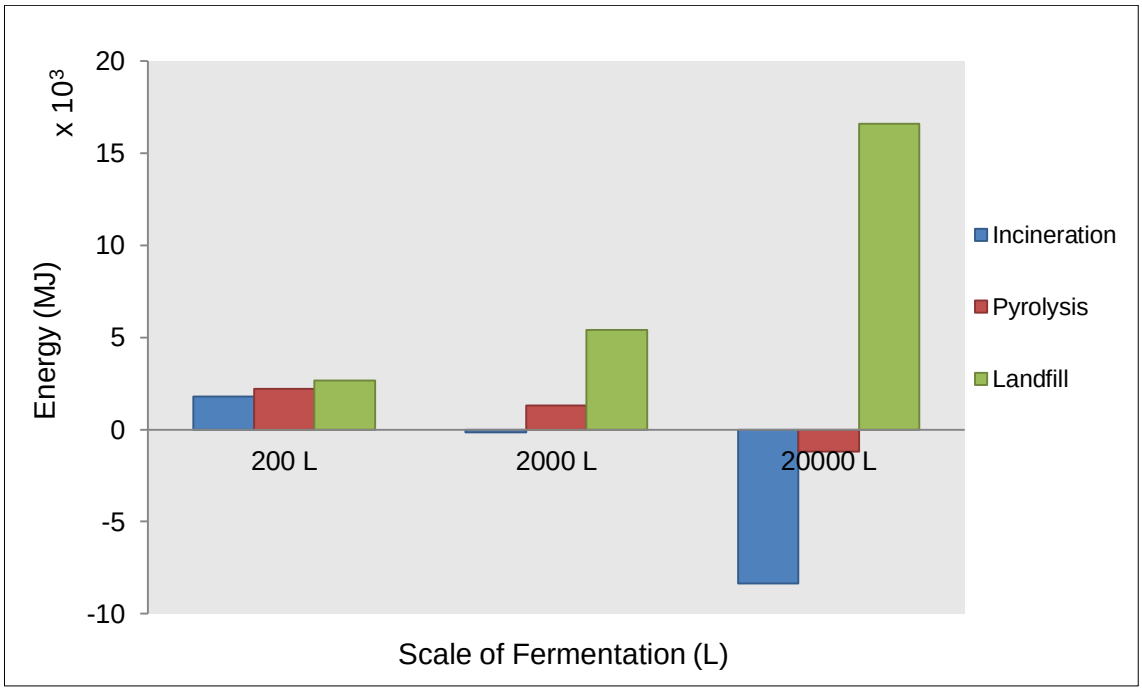


Figure 5.13 *The energy demand of the solid waste management methods at different scales of fermentation operation.*

To conclude, it is clear from Figure 5.13, that the incineration solid waste management method has the lowest energy demand, whilst the landfill solid waste management method has the highest energy demand at all operational scales studied (200-20,000 L). A minimum scale of operation appears to exist below in which net energy production is not possible. Operation at 2000 L and beyond offers possibilities for net energy production (even higher net energy production at 20,000 L scale). This is best achieved when incineration is used for solid waste treatment.

Therefore, there does appear to be a correlation between the levels of net energy production and operation scales, but this correlation is only valid for incineration and pyrolysis solid waste management methods. This correlation does not apply when a landfill route for solid waste is adopted.

5.4.2 Water consumption

Figure 5.14 shows the levels of water consumption of the solid waste management methods at different scales of fermentation operation. As the scale increases so the water consumption for each solid waste management method also rises. Amongst the solid waste management methods, landfill has the highest water consumption and pyrolysis the lowest. However, the differences in the levels of water consumption between incineration and pyrolysis are small at all scales studied. The levels of water consumption for landfill and incineration can be associated with the production of materials for leachate and sludge treatments and deposition (see Section 3.6.1). The water consumption for pyrolysis can be associated with the cleaning of syngas (Rabou *et al*., 2009).

To conclude, it is clear that incineration and pyrolysis are more suitable solid waste management alternatives if water use is a concern in the industry due to the high levels of water savings that can be achieved especially at the 20,000 L scale (approximately 1.3 x 10^5 kg of water can be saved by employing incineration and 1.4 x 10^5 kg of water by employing pyrolysis at the 20,000 L scale).

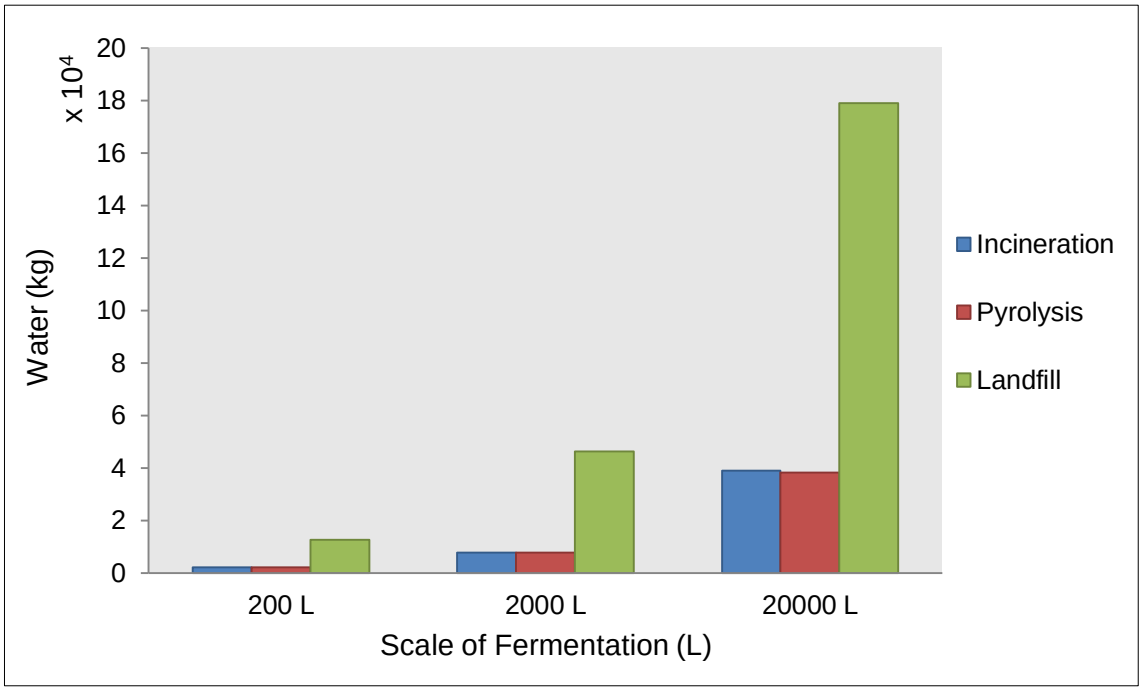


Figure 5.14 *The water demand of the solid waste management methods at different scales of fermentation operation.*

5.4.3 Carbon emissions

Figure 5.15 shows the levels of carbon emissions of the solid waste management methods at different scales. As the scale of operation increases so the levels of carbon emissions emitted by each solid waste management method increases. Since energy is recovered from waste for the incineration and pyrolysis methods, some carbon savings or "avoided" GHGs were achieved for these disposal methods. Despite this, incineration has the highest contribution to carbon emissions at all scales studied. Although the landfill method has the highest energy and water consumptions, this method contributes to the lowest levels of carbon emission. The analysis evaluated the environmental impacts of each solid waste management method at different operational scales. The analysis also identified the best waste disposal methods for the different impact categories studied. For example, the incineration method has the best energy performance at all scales. The analysis

however, did not identify a single solid waste management method that satisfies all the impact categories/metrics examined.

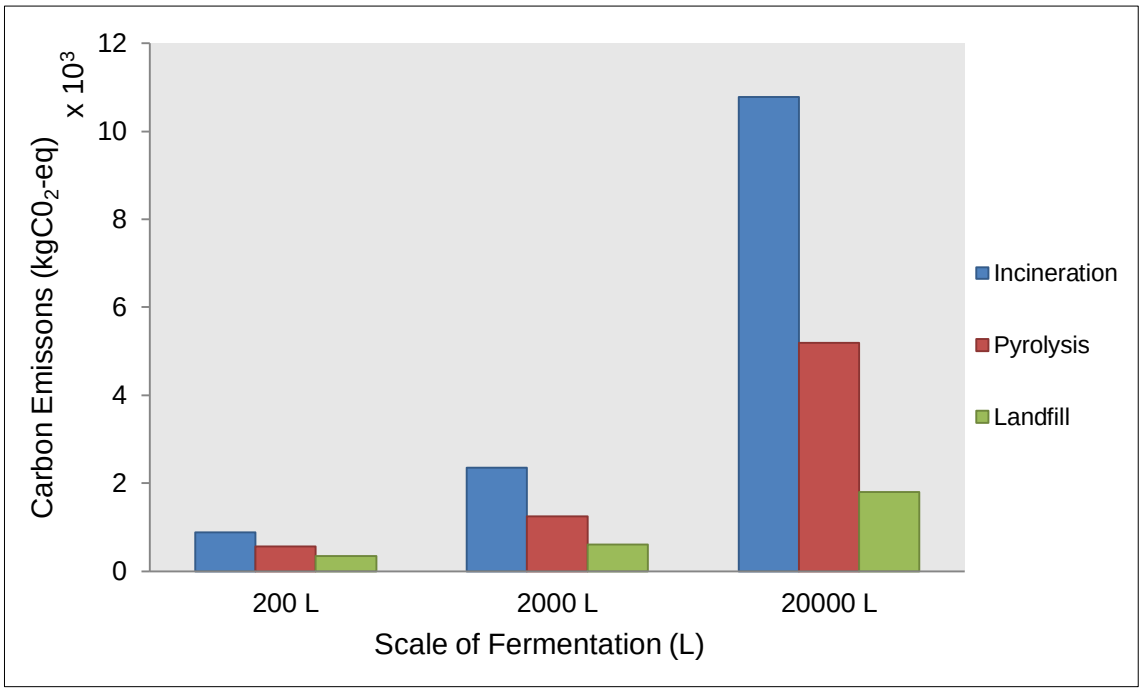


Figure 5.15 *The levels of carbon emitted by the solid waste management methods at different scales of fermentation operation.*

As expected there are complex trade-offs associated with each method of manufacturing and of waste management. A minimum scale of operation appears to exist below which energy recovery is not viable and offers little by way of an offset saving. Operation at beyond 2000 L offers some possibility for energy savings. Although energy recovery is possible with incineration and pyrolysis methods for scales 2,000 L and beyond, water requirements and carbon emissions for each waste disposal method increases with operation scales. The next step is to provide a level of guidance in selecting the best solid waste management method from an environmental perspective.

5.5 Decision Making Considerations for Selecting the Ideal Solid Waste Management Method in the Biopharmaceutical Industry

The best form of solid waste management is that of reducing or avoiding the production of waste at the point of generation (Morrissey and Browne, 2004). However, this is difficult when the industry employs single-use technologies, which the research in this thesis has shown will increase the production of solid waste. At present, a majority of biopharmaceutical facilities manage their solid waste using landfill, and only a very few use incineration (Rawlings and Pora, 2009). However, as this research has shown (see Section 5.2), landfill does not appear to be a sustainable solution in the long term for the biopharmaceutical industry due to the high levels of energy and water consumed by this method of disposal. Therefore, there is a pressing need for the industry to identify the best method to manage effectively their solid waste. To date, the choice of solid waste management approach in this industry remains ambiguous, and has not been the subject of any published rigorous study. Thus, this section aims to identify the possible solid waste management method that can be deployed by the biopharmaceutical industry to manage their solid waste.

In order to identify the best solid waste management method, the major environmental concerns faced by the biopharmaceutical industry must be recognised. One of the main environmental issues in the biopharmaceutical industry is that the manufacturing processes consume high levels of water (the analysis carried out in Section 5.2 clearly demonstrated that monoclonal antibody manufacturing processes consume high levels of water especially when traditional technologies are employed). Although this research has demonstrated that significant

water savings can be achieved through the adoption of single-use technologies, these technologies are however constrained by the operational scale envisaged (the highest volume of current single-use bioreactors is 2000 L). Thus, large-scale production of monoclonal antibodies is likely to continue to take place using traditional technology, making the processes water-intensive. The high levels of water consumed by these large-scale traditional technology processes are anticipated to become a major concern for the biopharmaceutical industry.

High levels of water consumption are an even bigger concern for the biopharmaceutical facilities located in areas where water is scarce. One example is California. California is one of the largest states in America and has the highest density of biopharmaceutical facilities (biopharmaceutical companies in California have more than 1000 drugs in their pipeline to treat various diseases such as cancer and infectious diseases) (Gollaher and Claude, 2013). California is facing a major water crisis as a result of constant and severe droughts. Since water usage is a major concern in this state, the biopharmaceutical manufacturers in California might find it expeditious to deploy manufacturing processes and solid waste management methods with lower water requirements. This will benefit the biopharmaceutical industry not only environmentally, but also economically (it is believed that the price of water will be raised as a water conservation step in the future) (Shelton and Mckenzie, 2014).

All these factors clearly suggest that at this point in time, water consumption is an interesting impact category to the biopharmaceutical industry. Therefore, this doctoral project proposes to use water consumption as a key indicator by which to

select the most suitable solid waste management method.

5.5.1 Using water consumption of the waste disposal options as the key indicator to select the best solid waste management method

Using water consumption of solid waste disposal options as a key indicator to select the most ideal solid waste management method, it is apparent that pyrolysis is the best option as it is associated with the lowest levels of water consumption (see Table 5.1). This is followed by incineration. Landfill is the least desirable option due to the high levels of water consumed by this method. However, it can be observed from Table 5.1 that the absolute or relative difference in water consumption between incineration and pyrolysis is small at all scales investigated.

As highlighted before, most biopharmaceutical facilities employ landfill to manage their solid waste, and only a very few facilities employ incineration. Landfill is the longest established solid waste management method and it is available in most locations. However, landfill (for waste management in the biopharmaceutical industry) does not provide other functions in addition to treatment of the waste, such as recovery of energy. These may be provided by advanced waste management methods such as incineration and pyrolysis (Eriksson *et al.*, 2005). In addition, the tax introduced on landfill by a number of countries in Europe should discourage the industry (industries located in Europe) from disposing their waste as landfill (Morris *et al.*, 1998).

Incineration is widely employed by many industries in Europe responding to the European Waste Management Directive (Monte *et al.*, 2009), which places greatest

importance on incineration than landfill (Eriksson *et al*., 2005). Incineration as a disposal method is also gaining popularity for reasons that include low operating costs, the fact that it is free from the odor that are typically associated with landfill methods, and the energy recovery possibilities of this method (in the form of heat/or power) (Environment Agency, 2012; Rawlings and Pora, 2009). However, it appears not to be a popular solid waste management choice in the biopharmaceutical industry, especially for facilities located in the UK. The Lonza and UCB facilities located in Slough, and the Fujifilm Diosynth Biotechnologies located in Billingham are some of the very few biopharmaceuticals in the UK that employ incineration to manage their solid waste (FHC, 2012).

Pyrolysis is a relatively new solid waste disposal method for the biopharmaceutical industry and has yet to be deployed by the industry. Like incineration, it also offers energy recovery possibilities (Environment Agency, 2012). Pyrolysis is likely to become an important solid waste disposal option in the future due to the capability of this method to generate useful products from burning waste such as bio-char, bio-oil and syngas that themselves have wide industrial applications (Al-Salem *et al*., 2009; Rawlings and Pora, 2009).

All of the above signify that the biopharmaceutical industry (mainly the facilities located in the UK) is still behind in terms of their solid waste management approach when compared with other EIIs such as the chemical, cement and automotive industries (WBCSD, 2014). This is not a sustainable solution for an industry that is growing rapidly with many therapeutic drugs in the pipeline. In Section 5.5, it was proposed that water consumption should be used as a key indicator by which to

select the most suitable solid waste disposal option. By using water consumption as the key indicator, it was determined that the biopharmaceutical industry should consider either pyrolysis or incineration (with pyrolysis being the first choice) to manage effectively their solid waste. These solid waste disposal methods not only consume low levels of water, but also offer energy recovering possibilities, making these methods environmentally favourable.

Table 5.1 *Level of water consumption of monoclonal antibody manufacturing processes as a function of the scale of operation.*

Scale	Landfill (kg)	Incineration (kg)	Pyrolysis (kg)
200 L	12900	2200	2100
2000 L	46400	7900	7700
20000 L	178800	39000	38400

5.6 Overall Assessment

The analysis presented in this chapter has identified how best manufacturing process and possible solid waste management method for the biopharmaceutical industry may be selected from an environmental perspective. Other key observations that can be made from this analysis include:

- The traditional and hybrid manufacturing processes each have significant energy, water and carbon footprints.
- Employing single-use technologies in the manufacture of biopharmaceutical products increases the amount of solid waste produced but reduces the level

of liquid waste generated (due to the elimination/reduction of CIP and SIP steps).

- Highest levels of energy consumption come from the use-phase for the traditional and hybrid process options.
- Highest levels of water consumption come from the supply-chain phase for both the process options.
- Highest levels of carbon emissions come from the use-phase for both the process options.

It is clear that monoclonal antibodies manufacture contributes to considerable levels of environmental impact. However, what is as yet unclear is how the environmental impacts produced by this industry compare with those of other industries; especially the pharmaceutical industry. The pharmaceutical industry shares a number of features with the biopharmaceutical industry (e.g. pharmaceuticals consume high levels of water for solvents used in the synthesis steps, and pharmaceuticals manufacturing processes require purification steps and formulations). An LCA study carried out by Wernet *et al* (2010) identified that the energy for the full synthesis of a pharmaceutical compound produced by F-Hoffmann-La Roche in Basel, Switzerland is around 1430 MJ/kg of product. The study only considered environmental impacts produced from solution manufacture, use-phase (equipment utilisation and waste management) and energy usage. Taking a similar system boundary for this analysis, the energy consumed by the monoclonal antibody manufacturing process was determined to be 11 times higher than for the pharmaceutical compound manufacturing process. It should be noted however that the annual production volumes of monoclonal antibodies are much smaller than those of most

pharmaceuticals. The annual production of monoclonal antibodies is estimated to be around 16 metric tons where else for pharmaceuticals, it is estimated to be 1000 metric tons (Wernet *et al.*, 2010). Therefore, when analysed from a global perspective, pharmaceuticals manufacture contributes higher levels of environmental impact than does monoclonal antibodies manufacture. This is not entirely unexpected due to the scale differences.

5.7 Conclusion

This chapter provides the results of an environmental assessment of a typical monoclonal antibody production process at 200 L operational scale and identifies the possible solid waste management methods for this. The analyses provide crucial information to decision-makers when seeking to select the best combination of manufacturing process and solid waste management methods.

The results of the analyses identified that the hybrid process (consisting of a mix of traditional and single-use equipment) as the more environmentally favourable option. The analysis also revealed that the majority of the impacts for the traditional and hybrid processes at a 200 L operational scale come from the supply-chain and use phases. However, identifying the best solid waste management method appears to be more difficult at the 200 L production scale. Thus, a separate solid waste management study was necessary to identify the possible solid waste management methods.

From the solid waste management study, it was clear that there is no single solid waste management method that performs best in all impact categories at all the

scales studied (200-20,000 L). From an energy efficiency perspective, incineration appeared to be the best option. At scales of 2,000 L and beyond, energy recovery (and carbon savings) are achievable with the incineration method. Energy recovery is also possible with pyrolysis, however only at the 20,000 L scale. Landfill offers no energy recovery possibilities at the scales studied. From the water consumption perspective, pyrolysis appears to be the best option at all scales. And finally from the carbon emissions perspective, landfill appears to be the best option at all scales studied. Thus, it is clear that deciding on a solid waste management method depends critically upon which impact category is deemed most important for the biopharmaceutical industry (the major environmental concern faced by the industry). On that basis, the research identified water consumption as the key impact category for the industry as their manufacturing processes consume high levels of water. Thus, water consumption was used as an indicator to select the best solid waste disposal option. Based on this indicator, pyrolysis appears to be the most suitable disposal option, followed by incineration as these methods consume significantly lower levels of water than landfill.

The results obtained from the analyses in this chapter will provide a level of guidance for the industry to help manufacture biopharmaceuticals in a more environmentally favourable fashion.

In this thesis, a number of assumptions were made in order to develop and utilise the framework based on LCA to identify the manufacturing process and the solid waste management method with the lowest contribution to the overall environmental impacts. The outcome of this chapter depended heavily on these assumptions. The

next chapter therefore presents sensitivity analysis studies to assess critically these assumptions, and to investigate the influence of these assumptions on the overall outcome of the research.

Chapter 6. Sensitivity Analyses

6.1 Introduction

In 1927, Werner Heisenberg, a German quantum physicist, stated that one can't assign with full precision values for certain pairs of observable variables, including the position and momentum, of a single particle at the same time (Wainwright and Mulligan, 2004). Although this statement was specific to quantum physics, the theory holds true for every scientific model. There is always an element of uncertainty when making an observation. Thus, the act of observation perturbs what we are measuring. Some systems may be particularly sensitive to these perturbations (Wainwright and Mulligan, 2004).

In this work, the uncertainty in the LCA mainly comes from the input parameters (inventory data) and the assumptions made. In order to understand how the overall results estimated are affected by changes in certain parameters/assumptions, sensitivity analyses were carried out. Sensitivity analysis is a systematic procedure to identify the key uncertainties of a model (Bjorklund, 2002). The main goals of the analysis are:

- To evaluate the robustness of the LCA studies
- To define the importance of model parameters used.

The main aim of this chapter is to identify the process parameters/assumptions that have the most influence on the estimates made of overall environmental impacts.

This will further increase the confidence on the predictions made in the study and improve the robustness of the study.

In Section 6.2, the descriptions of the sensitivity analysis studies are provided. The results of the sensitivity analyses are presented in Section 6.3. In Section 6.4, the findings of this chapter are analysed, and the parameters that contributes to the most and least impact are identified. Finally, a summary is provided in Section 6.5.

6.2 Setting up of Sensitivity Scenarios

Five scenarios were designed to investigate the influence of certain process parameters/assumptions on the environmental impacts of a traditional monoclonal antibody process operated at 200 L scale (the description of this process can be found in Section 3.6.1). These scenarios examined important process parameters such as process titre, downstream processing yield and number of batches. The scenarios also examined important parameters related to transport such as distance. The effect of the number of transportation trips as well as the mode of transport on the overall environmental impact produced was also examined. The process parameters were varied according to the typical operating parameters presently used for monoclonal antibodies manufacture in the biopharmaceutical industry. A one-way sensitivity analysis was employed for all the scenarios. The one-way sensitivity analysis is the simplest type of sensitivity analysis in which a single parameter is studied (Kjaerulff and Van der gaag, 2000).

6.2.1 Process titre

The first scenario considers the overall environmental impacts produced by a traditional monoclonal antibody manufacturing process operated at 200 L scale when the process titre is increased from 5 g/L (baseline case parameter) to 15 g/L. At present, the process titre of monoclonal antibody processes ranges from 2 g/L-5g/L (Farid, 2006). However, some studies have reported process titres of 10 g/L, and the trend is for even higher titres in the future (Farid, 2011; Kelly, 2009). Therefore, a process titre of 15 g/L was chosen for this scenario to investigate the environmental impacts produced by a potential scenario set some time in the future. The main question this scenario aimed to answer was whether significant environmental savings could be achieved if the industry adopted higher process titres. In this scenario, the process titre is the only parameter that was varied. All other parameters remained fixed at the baseline values (see Table 6.1).

Table 6.1 *The constant and varied parameters of this scenario-process titre.*

Constant Parameter	**Value**
DSP yield	75%
Number of Batches	20
Varied Parameter	**Value**
Process titre	From 5 to 15 g/L

6.2.2 Downstream processing (DSP) yield

The second scenario considered the influence of the DSP yield on the overall environmental impacts produced by a traditional monoclonal antibody

manufacturing process operated at 200 L scale. The DSP yield is a function of the individual step yields and the number of downstream processing steps (Farid, 2007). As mentioned in Section 3.6, the DSP yields for the traditional and hybrid manufacturing processes were determined to be 75% (the rationale for this is given in Section 3.6). Presently, the DSP yield for a monoclonal antibody process based on mammalian cell culture ranges from 60%-80% (Kelley, 2007). Therefore, this scenario investigated the environmental impacts of operation when a lower process yield of 60% is assumed (taking the lower end of the range). The DSP yield for the baseline case is 75%. The main aim of this scenario is to show the environmental savings that could be achieved if a higher DSP yield is realised for the monoclonal antibody processes. In this scenario, the DSP yield is the only parameter that was varied. All other parameters remained fixed at the baseline values (see Table 6.2).

Table 6.2 *The constant and varied parameters of this scenario-DSP yield.*

Constant Parameter	**Value**
Number of batches	20
Process Titre	5 g/L
Varied Parameter	**Value**
DSP Yield	Lowered from 75% to 60%

6.2.3 Number of batches

The third scenario considers the influence of the annual number of production batches on the overall environmental impacts produced by a traditional monoclonal antibody manufacturing process. In the baseline case, 20 batches per annum were

employed (see Section 3.6), assuming a 12-14 day production culture cycle and a short plant shutdown (Kelley, 2009).

In this scenario, higher levels of annual production batch numbers were employed to assess the changes in the environmental impacts when the number of batches is increased from the baseline case. The production batches employed in this study were 22 and 24 (presently, the range of number of batches that can be employed for mammalian cell based monoclonal antibodies manufacturing processes is between 20-24) (Kelley, 2009).

Table 6.3 *The constant and varied parameters of this scenario-number of batches.*

Constant Parameter	**Value**
Process Titre	5 g/L
DSP Yield	75%
Varied Parameter	**Value (s)**
Number of Batches	Ranged from 10-24

This scenario also aims to assess the environmental trade-offs between the scale of production and batch number (whether it is environmentally beneficial to employ a process with lower production scale and increased production batches or a process with higher production scales but a decreased number of production batches). A range of batch number per annum (10-24 batches per annum) was used to investigate the environmental trade-offs associated with batch number and scale of operation. In this scenario, the number of batches was the only parameter that was varied. All other parameters remained fixed at the baseline values (see Table 6.3).

6.2.4 Transport

In the environmental analysis reported in Chapter 5, a number of assumptions were made for transport, in particular on the distance and mode of transport. These assumptions will be examined in this section.

6.2.4.1 Transport distance

The main aim of this scenario was to examine the influence of transport distance on the overall environmental impacts of a traditional manufacturing process operated at 200 L scale. The transport involved in the stainless steel equipment fabrication was used to study the effect of transport distance on the overall environmental impacts produced. The route that was examined is the transport of raw material from the processing facility to the equipment fabrication facility (route A), using a cargo ship. Figure 6.1 illustrates the transport involved in the stainless steel equipment manufacturing.

As mentioned in Section 4.3.1, the raw material facility was assumed to be located in China and the stainless steel equipment fabrication facility was assumed to be located in Sweden. The raw material facility was assumed to be located in China because they are the largest producer of stainless steel (Millbank, 2013). The stainless steel equipment fabrication was assumed to be located in Sweden because a large number of biopharmaceutical processing equipment are fabricated/assembled around that region (Levine *et al.*, 2012).

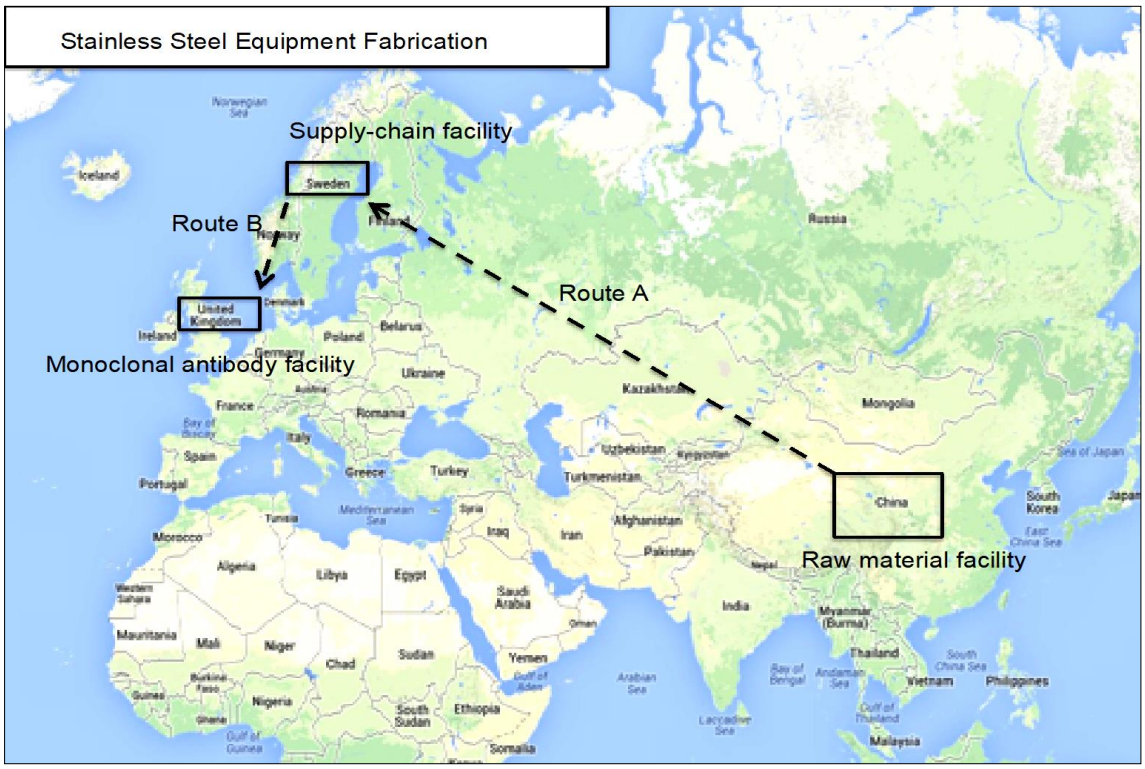


Figure 6.1 *Transportation involved in the stainless steel equipment fabrication.*

The baseline case transport distance from China to Sweden was assumed to be 6500 km (see Table 4.11). An increase or decrease in the transport distance by 50% from the baseline case were made to investigate the sensitivity of the transport distance parameter. Other transportation distances involved in the analysis remained fixed at the baseline value (see Table 4.11).

6.2.4.2 Transport logistics

From the analysis in Chapter 5, it can be determined that transport accounts for 23% of the total energy consumption and 25% of the total carbon emissions. This clearly shows that although transport is not the main contributor to the overall environmental impacts, it still contributes to considerable levels of impact.

Transport is an important aspect for the biopharmaceutical industry. Poor transport planning can cause product wastage, resulting in loss of millions of dollars in revenues for the biopharmaceutical industry (Biopharma Cold-Chain Sourcebook, 2012). According to a study conducted by the Medicines and Healthcare Products Regulatory Agency (MHRA), 43% of critical and major product deficiencies are related to poor transportations (Biopharma Cold-Chain Sourcebook, 2012). Poor transportation planning can also lead to increased levels of environmental impact. Therefore, proper transportation planning is an imperative for the biopharmaceutical industry in order to reduce the impacts from transportation. Thus, a case study was developed in this analysis to evaluate the impacts from transportation included in this work, and the possible ways to reduce them. The transport involved in the consumables' fabrication (route D) was used in this case study to evaluate the impact from transportation (transport from the supply-chain facility to monoclonal antibody manufacturing facility). Figure 6.2 shows the routes involved in the consumables' fabrication. As highlighted in Section 4.3.1, the consumables manufacturing facility was assumed to be located in Sweden as a high level of production of biopharmaceutical consumables take place there (Plastics Europe, 2011; Sandstròm *et al*., 2011).

The main focus of this case study was to identify the possible environmentally effective ways to transport the consumables needed in manufacture from the supply-chain (consumables fabrication) facility to the monoclonal antibody manufacturing facility. Thus, two scenarios; A and B were developed to investigate whether it is better to transport material needed for manufacturing just before the start of a production batch (just-in-time) from the supply-chain facility to the monoclonal

antibody manufacturing facility (scenario A), or annually where all the materials needed for a 20-batch per annum are transported in a single trip (scenario B). From this case study, an ideal way to transport the consumables (from the supply-chain facility to the monoclonal antibody manufacturing facility) in order to minimise the environmental impacts can be determined. This case study will also identify an ideal transport mode that can be employed to transport these consumables from the supply-chain facility to the monoclonal antibody manufacturing facility.

Figure 6.2 *Transportation involved in the consumables' fabrication.*

6.3 Results and Discussion

The results of the sensitivity analyses are presented and discussed in this section.

6.3.1 Process titre

This section presents the outcome of the sensitivity analysis carried out to examine the influence of process titre on the overall environmental impacts of a traditional monoclonal antibody manufacturing process operated at 200 L scale. Figure 6.3 shows the environmental savings that could be achieved per annum when the process titre is increased from the base line value to a higher process titre; that is from 5 g/L to 15 g/L (negative values show savings achieved).

It is evident from Figure 6.3 that, not surprisingly, a process with 15g/L titre produces lower environmental impacts. The process with a titre of 15 g/L consumes 30% less energy, 18% less water, and generates 32% less carbon than the base case. This is because with higher process titres, the scales of manufacture will decrease, resulting in lower environmental impacts. Equation 6.1 was used to determine the production scales of such monoclonal antibody processes. It shows that the relationship between production scale and process titre is an inverse one; increasing the process titre will lower the production scale of manufacture. By increasing the process titre, the industry is essentially producing the same amount of monoclonal antibody product but employing a lower production scale; it is more intensive. Therefore, by tripling the process titre from the base case, it can be estimated that around 1.6 million MJ of energy, 0.15 million kg of water and 0.27 million kgCO_2-eq of carbon can be saved (savings equivalent to average energy used by 94 houses in the UK per year, average water consumed by 53 houses in the UK per year and average carbon generated by 800 houses in the UK per year) (see Chapter 4, Section 4.5).

$$\textbf{Production scale (L)} = \frac{\textbf{Monoclonal antibody to be produced (Kg)} \times \frac{1}{\textbf{Process titer}} \left(\frac{\textbf{L}}{\textbf{Kg}}\right) \times \left(\frac{1}{\textbf{DSP yield}}\right)}{\textbf{Number of batches}}$$

(Equation 6.1)

Hence, it is evident that increasing the process titre can lead to significant environmental savings. Currently, the highest process titre in the biopharmaceutical industry is 10 g/L. However, it is estimated that a higher process titre (around 15g/L) should be attainable in the future (Farid, 2011). Thus, the results of this analysis should motivate the biopharmaceutical industry to work towards achieving higher process titres, as it will not only lead to major costs savings, but also significant environmental savings.

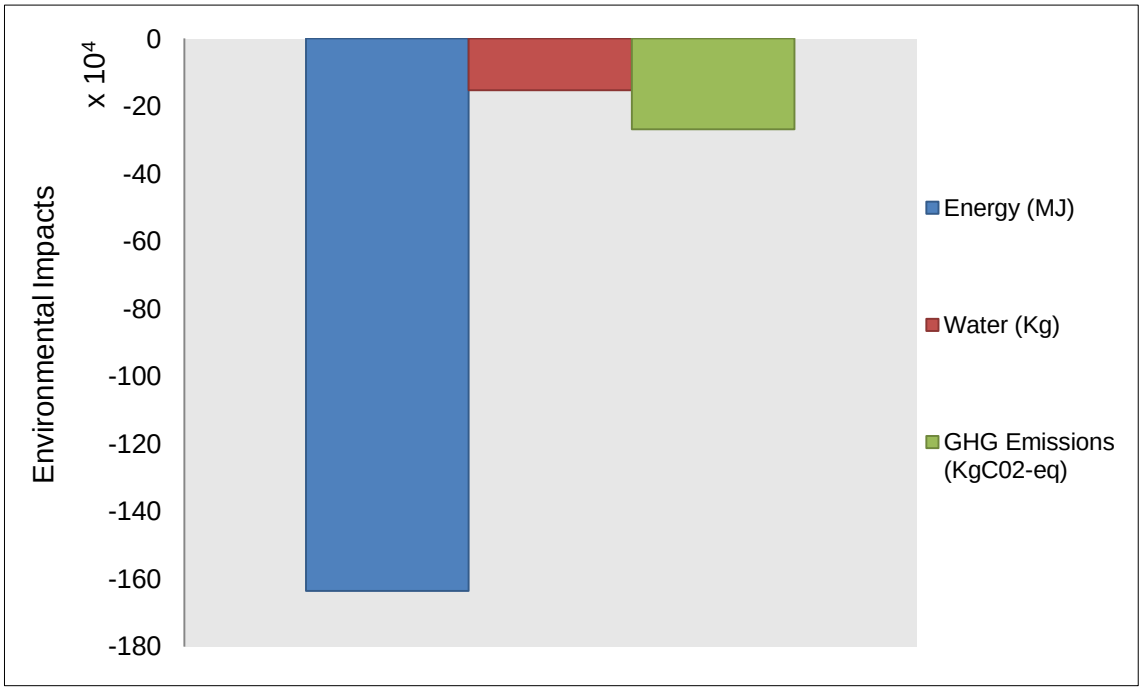


Figure 6.3 *Environmental savings achieved per annum when the process titre is increased from 5g/L (baseline case) to 15g/L.*

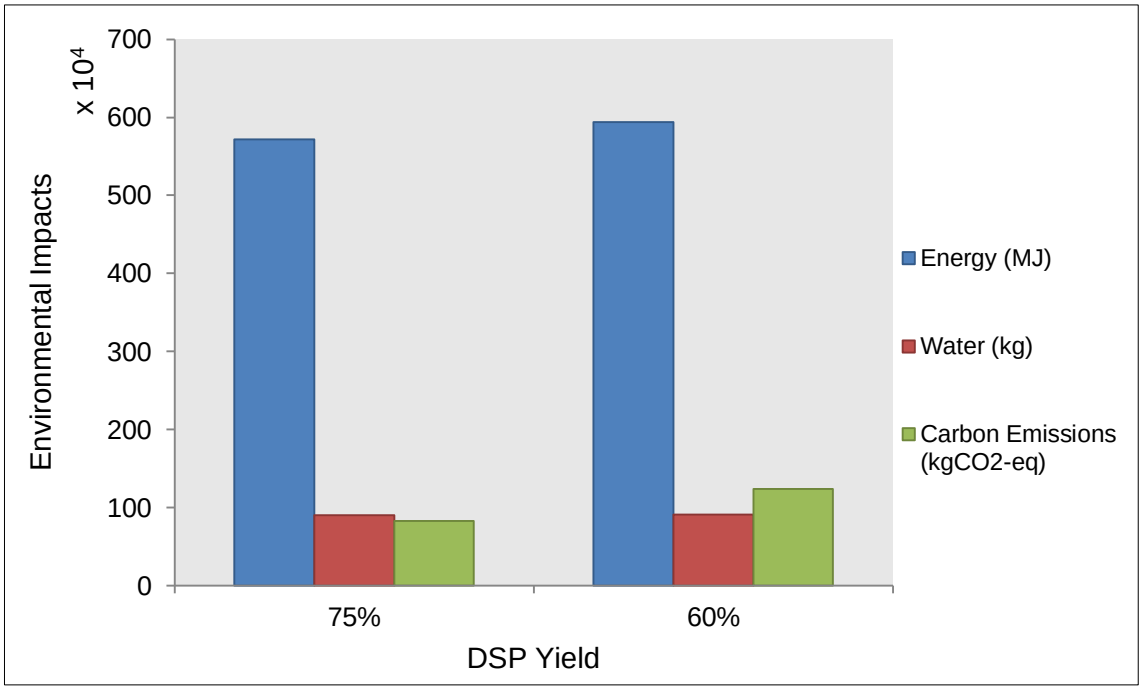
6.3.2 Downstream processing (DSP) yield

This section presents the outcome of the sensitivity analysis carried out to examine the influence of the DSP yield on the environmental impacts of a traditional manufacturing process operated at 200 L scale. Figures 6.4 shows the annual environmental impacts produced for processes with DSP yields of 75% and 60%, selected as the representative of the study. From this figure, it was identified that the manufacturing process with a DSP yield of 75% consumes around 571 x 10^4 MJ of energy and 90 x 10^4 kg of water, and generates around 83 x 10^4 kgCO_2-eq of carbon per annum (this is equivalent to the average energy used by 335 houses in the UK per year, average water consumed by 3 houses in the UK per year and average carbon generated by 415 houses in the UK per year). Meanwhile, the manufacturing process with a DSP yield of 60% consumes around 594 x 10^4 MJ of energy and 91 x 10^4 kg of water, and generates around 124 x 10^4 kgCO_2-eq of carbon per annum (this is equivalent to the average energy used by 349 houses in the UK per year, average water consumed by 3 houses in the UK per year and average carbon generated by 620 houses in the UK per year).

The process with a DSP yield of 60% contributes to higher levels of environmental impact. This is because at a lower DSP yield of 60%, the fermentation volumes and the production scales of manufacture will increase, resulting in higher environmental impacts. Again, Equation 6.1 can be used to explain this. It shows the link between the production scales and the DSP yields to be inversely proportional; lowering the DSP yield will increase the fermentation volume of manufacture. It was determined that the lowering the DSP yield of the traditional process from 75% to 60% will increase the bioreactor scale from 200 L to 500 L (assuming other process

parameters such as process titre remain constant), and will lead to around 2.3 x 10^5 MJ of more energy and 7 x 10^3 kg of more water being consumed, and around 41 x 10^4 $kgCO_2$-eq of more carbon being generated. This is clearly a significant difference.

The findings demonstrate that a higher DSP yield is beneficial in reducing the environmental impacts of monoclonal antibody manufacturing processes. Therefore, there is a need for the industry to focus on the efficiency of monoclonal antibody manufacturing processes and reduce the loss of product from each unit operation. This will not only lead to environmental savings as demonstrated in this analysis, but also result in savings of cost of goods and investment, and allowing for higher facility throughputs (Farid, 2007).



Figures 6.4 *Environmental impacts produced by the monoclonal antibody manufacturing process per annum when DSP yields of 75% and 60% are assumed.*

6.3.3 Number of batches

This section presents the outcome of a sensitivity analysis carried out to examine the effect of the number of production batches on the overall environmental impacts of a traditional monoclonal antibody manufacturing process on an annual basis.

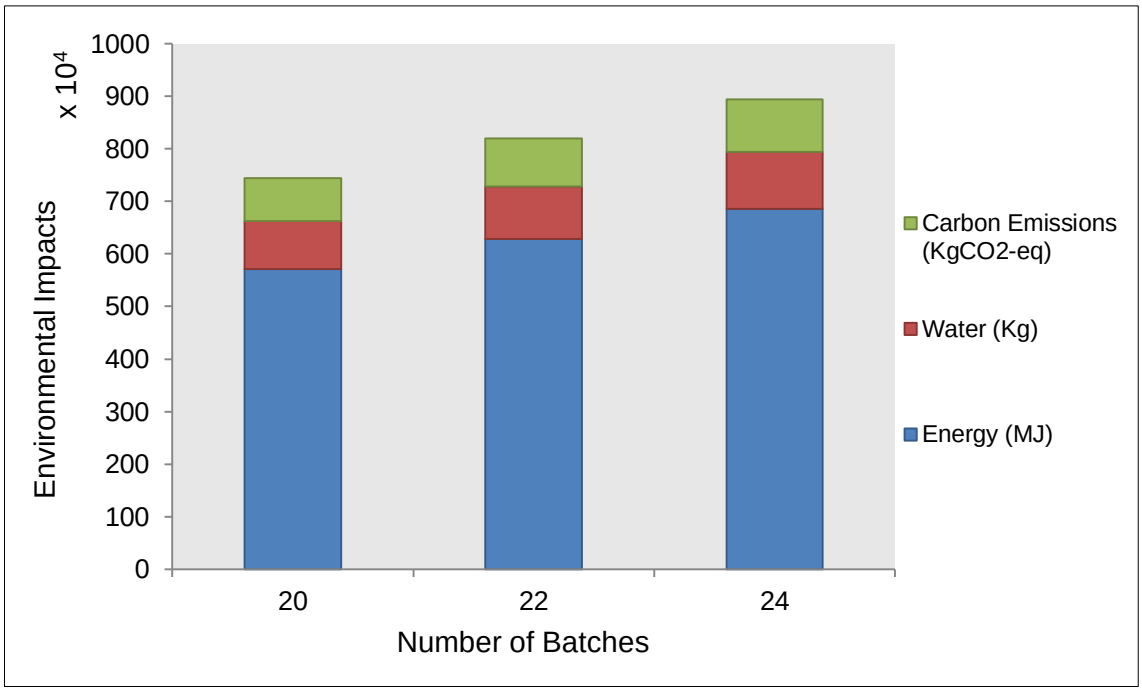


Figure 6.5 *Annual environmental impact of a monoclonal antibody manufacturing process when 20 (baseline case), 22 and 24 batch numbers are employed.*

Figure 6.5 shows the environmental impacts of the traditional process with three different levels of production assumed per annum: 20 (baseline case), 22 and 24 batches. From this figure, it can be seen that the process requiring the smallest number of batches contributes the least environmental impact whilst the highest number of batches contributes to the most; which is unsurprising. Equation 6.1 demonstrated that employing a higher number of batches for a manufacturing process lowers the fermentation volumes (and potentially reduces the environmental impacts). However, the scenario has also shown that the annual environmental

impact arising from an increase in the number of production batches outweighs the environmental benefits obtained from a decreased operation volume. This is mainly due to the fixed production scales of 200 L for the three batches investigated (the production scales for 20, 22 and 24 batch numbers were determined to be 200 L).

The remainder of this section focuses on the environmental trade-offs associated with choice of batch number and operational (bioreactor) scale. As highlighted by Equation 6.1, varying the number of batches will lower/increase the operation scale of the traditional monoclonal antibody manufacturing process. The following analysis will focus on identifying the batch number that contributes the least environmental impact.

A wider range of annual production batches from 10-24 was employed (10, 15, 17, 20 and 24 batches were selected). Again, a frequency of 10 batches per annum is not commonly used in monoclonal antibodies manufacture, however it was used in this study to investigate how smaller batch numbers influence the environmental impacts of the monoclonal antibody manufacturing process that is being studied (Kelley, 2009). As stated in Section 6.2.3, the maximum number of batches per annum that can be employed for monoclonal antibody manufacturing is 24 before process limitations take place (Guldager, 2009).

The bioreactor scales for all these batches investigated are shown in Figure 6.6. The main purpose of Figure 6.6 is to illustrate the relationship between batch numbers and bioreactor scales. The bioreactor scales for different batch numbers were established using operational scales (which were determined using Equation 6.1),

and volume of fermentation to the volume of bioreactor ratio (V_F/V_B). The accepted range of V_F/V_B is between 0.7-0.8 (Doran, 1995). Thus, the bioreactor scales for the different number of batches were ensured to satisfy this ratio.

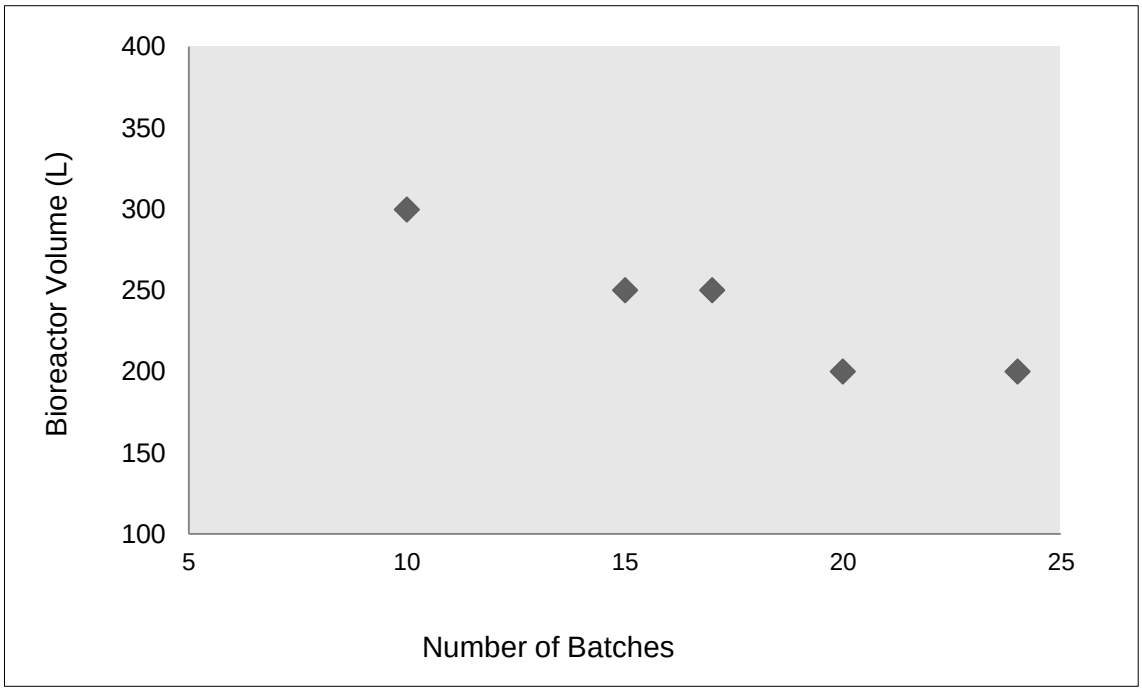


Figure 6.6 *Scale of bioreactor of a monoclonal antibody manufacturing process for different number of batches per annum for a fixed output (monoclonal antibodies).*

From Figure 6.6, it can be observed that the bioreactor scale of the traditional monoclonal antibody process reduced from 300 L to 250 L at a batch number of 15 and further reduced to 200 L at a batch number of 20, and remained at this scale until the maximum number of batches, 24. To understand the environmental trade-offs associated with the number of batches and bioreactor scales, the environmental impacts per year of different number of batches were estimated and are shown in Figure 6.7.

Figure 6.7 shows the energy and water consumption and carbon emissions per annum of a traditional manufacturing process for different batch numbers. From this figure, it can be deduced that the batch number that contributes to the least environmental impact is 10 (the bioreactor scale at this batch size is 300 L) and the most is 24 (the bioreactor scale at this batch number is 200 L). The level of impact increases with batch number although the bioreactor scale reduces (see Figure 6.6). In this analysis, the impacts arising from an increased number of batches outweigh the benefits obtained from the reduction in bioreactor volumes (this is mainly due to the weak dependency with bioreactor scale). Therefore, at a batch number of 10, the maximum environmental savings for monoclonal antibodies manufacture can be achieved. It has been estimated that reductions of 2.8 million MJ of energy, 0.3 million kg of water and 0.4 million kgCO_2-eq of carbon emissions from the base case scenario can be achieved.

From this analysis, it was identified that a traditional monoclonal antibody manufacturing process with a larger bioreactor scale and hence a lower number of batches performs better environmentally than a process with a lower bioreactor scale and higher number of batches. The analysis also identified a batch number of 10 as the best annual batch number for the traditional manufacturing process where maximum environmental savings can be achieved.

So far in this thesis, only the annual impacts associated with the monoclonal antibody manufacturing process have been provided. This information is useful for the industry to understand the overall impacts of monoclonal antibody manufacturing processes to the environment. However, the impact produced per patient using a

particular monoclonal antibody is unknown. Thus, Figure 6.8 is provided to depict the environmental impacts per patient of a monoclonal antibody drug. Avastin, was used as the example monoclonal antibody to populate the impact produced per patient (the dosage information of Avastin was used to populate this) (see Appendix 2). The environmental impacts in Figure 6.8 are populated for different number of batches to demonstrate how the impacts per patient vary for different batch sizes. The range of number of batches employed is similar to that of above, that is from 10-24.

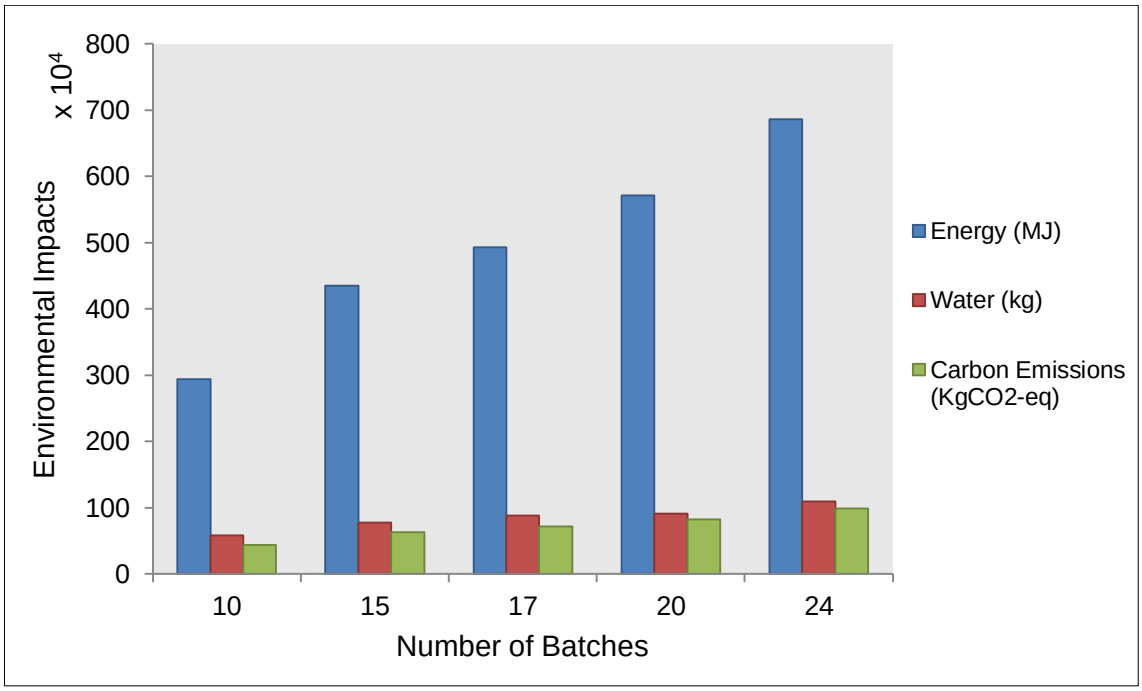


Figure 6.7 *Annual environmental impact of a monoclonal antibody manufacturing process when 10-24 batch numbers are employed.*

Figure 6.8 shows the environmental impacts per patient for different number of batches. As expected, the batch size of 10 contributes to the least impact per patient and the batch size of 24 contributes the most. By identifying the environmental impacts per capita for a drug, the overall impacts associated with the use of that drug can be identified (by determining the number of patients currently on the drug).

Consequently, the impacts associated with each of the monoclonal antibody currently on the market can be populated.

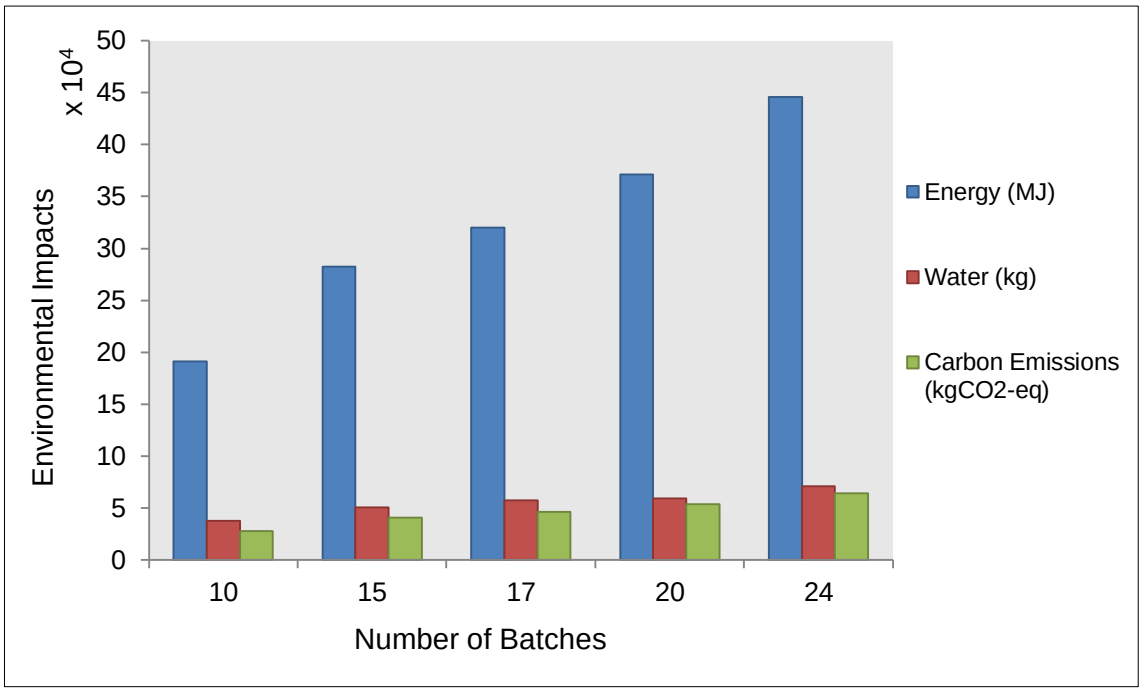


Figure 6.8 *Environmental impacts produced per patient for different batch numbers.*

It is clear that the number of production batches employed for the manufacture of a given monoclonal antibody plays a critical role in the overall environmental impacts produced. Selecting the best number of batches for manufacturing processes is crucial in order for the manufacture to take place in a more sustainable fashion. The outcome of the analysis should be useful for the biopharmaceutical industry, as studies investigating the effect of production batches on the overall environmental impacts of manufacturing processes do not exist. This analysis will help the industry to better understand the influence of batch number on the overall environmental impacts of monoclonal antibody manufacturing processes and the potential savings that could be achieved by selecting the best annual production strategy.

6.3.4 Transport distance

The main aim of this scenario is to examine the influence of transport distance on the environmental impacts produced. Figure 6.9 shows the environmental impacts of stainless steel equipment fabrication when the transport distance of route A is decreased/increased from the baseline case. Note that water consumption will remain fixed as water is not used in transport.

When the transport distance is decreased from the base case, it can be seen that lower environmental impacts are produced. The case with reduced distance consumes 2% less energy and generates 5% less carbon from the base case. When the transport distance is increased from the base case, it can be seen that higher levels of environmental impact were produced. The case with increased distance consumes 1% more energy and generates 4% more carbon from the base case.

Although there were differences in overall environmental impacts when the distance of route A is altered from the base case, these changes were not significant. Decreasing the distance by 50% from the base case resulted in savings of 1.1×10^5 MJ of energy and 3.4×10^4 $kgCO_2$-eq of carbon. Increasing the transport by 50% from the base case resulted in increase of 2.7×10^4 MJ of energy and 3.3×10^4 $kgCO_2$-eq of carbon. Therefore, it can be concluded that although transport distance plays a role in the overall environmental impacts produced, it is not a sensitive parameter in the analysis. This finding agrees with a study carried out by Pietrzykowski *et al* (2013) where the transportation activities involved in the biopharmaceutical manufacture did not contribute to significant environmental impacts.

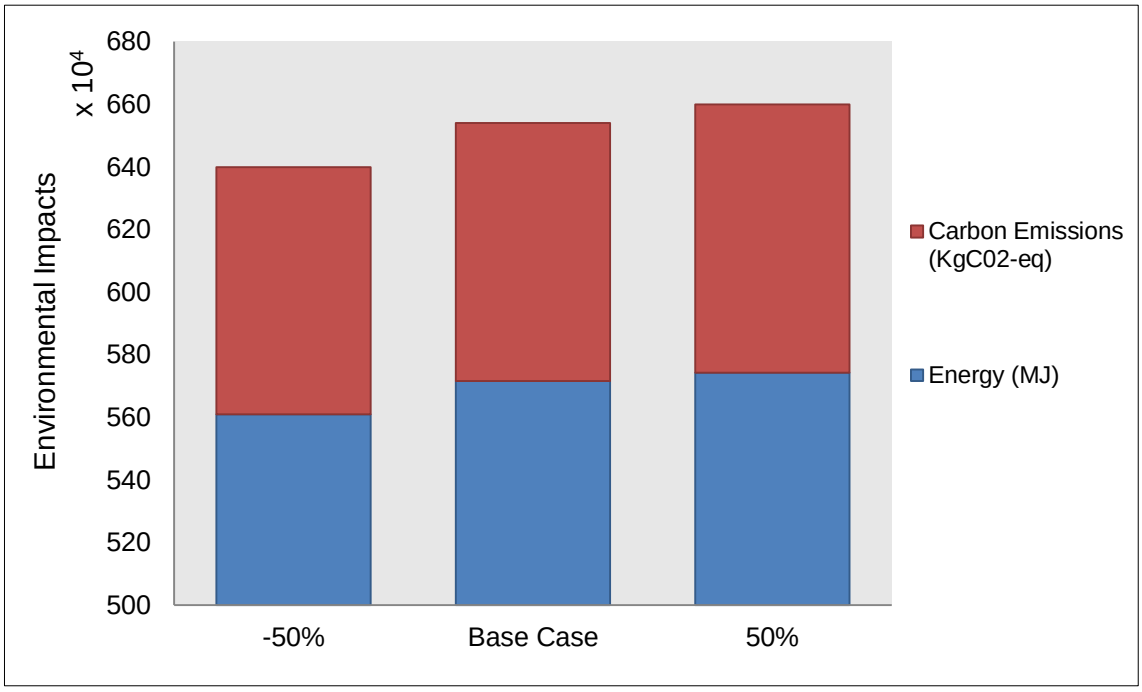


Figure 6.9 *Environmental impacts per annum of the monoclonal antibody process when the transport distance of route A is decreased/increased from the baseline case.*

6.3.5 Transport logistics

The main aim of this scenario was to identify an effective method to transport the materials needed for manufacture in order to minimise the environmental impacts produced. Thus, two scenarios: A and B were developed (see Section 6.2.4.2). Scenario A evaluates the impacts of transporting consumables just-in-time for production, and scenario B evaluates the impact of transporting consumables needed for the entire 20-batch per annum in a single trip.

Since scenario A requires the consumables in time for production, suppliers must find an effective way to transport these consumables to the monoclonal antibody manufacturing facility. Delays in transporting these consumables may cause severe interruptions in production, resulting in economical loss for the biopharmaceutical industry. Thus, choosing the right mode of transport is an imperative. For scenario A,

the most ideal transport mode is a cargo plane. Cargo planes are typically employed when the materials to be transported are time-sensitive (when quick delivery of the goods are required). However high costs are associated with this mode of transport.

In scenario B, since all the consumables needed for a 20-batch per annum production were transported together in a single trip, regular and fast delivery of consumables were not necessary. Thus, suppliers can opt for slower transportation modes such as cargo ship. Transport mode such as a cargo ship represents an economically feasible option for the biopharmaceutical industry as the cost of transport for this mode is significantly cheaper than the cargo plane (WIR, 2009). In this case study, the mode of transport assumed for scenario A was a cargo plane and for scenario B was a cargo ship.

Figure 6.10 shows the environmental impacts produced for consumables transport (route D) when scenarios A and B were assumed. From this figure, it can be seen that scenario B (single trip using cargo ship as the mode of transport) contributes to lower levels of environmental impact than scenario A. However, it can be observed that the differences in the levels of environmental impact between the scenarios are small (around 2000 MJ of energy).

Although scenario A is not a favourable option in terms of environmental impacts for consumables transport (as observed in Figure 6.10), this scenario is suitable for industries with space limitations in their facility that prevent them from storing the consumables for the entire production year. This scenario is also ideal in clinical studies where there is a high rate of drugs failure. Storing the consumables for an

extended period of time is a risk for the industry as failure of a drug will almost inevitably result in all the consumables not being fully utilised. This will lead to financial loss for the biopharmaceutical industry. By opting for scenario A, the industry might minimise the extent of consumables from going to waste.

The biopharmaceutical industry should also consider other factors besides the environmental factor for transportation planning. These factors include storage space limitations (availability of space) to store all the consumables needed for manufacture, storage conditions to maintain the optimal temperature especially when the products stored are temperature-sensitive (if a storage doesn't meet the temperature requirements, the products can be potentially deemed as unfit for use by the FDA or equivalent non-US agency), storage costs (utility costs for the storage such as electricity cost), and the short consumables shelf-life (the shelf life of the consumables is said to be 3 years before the consumables stored goes to waste) (Weber *et al.*, 2014; Biopharma Cold-Chain Sourcebook, 2012). These factors must be carefully evaluated before the industry can make decisions related to transportation.

From this case study, it was identified that both the scenarios have their benefits and limitations. Thus, decision-makers must consider all these factors for transportation planning to select the most appropriate type and mode of transport for the biopharmaceutical industry. This will further improve the industry's environmental performance.

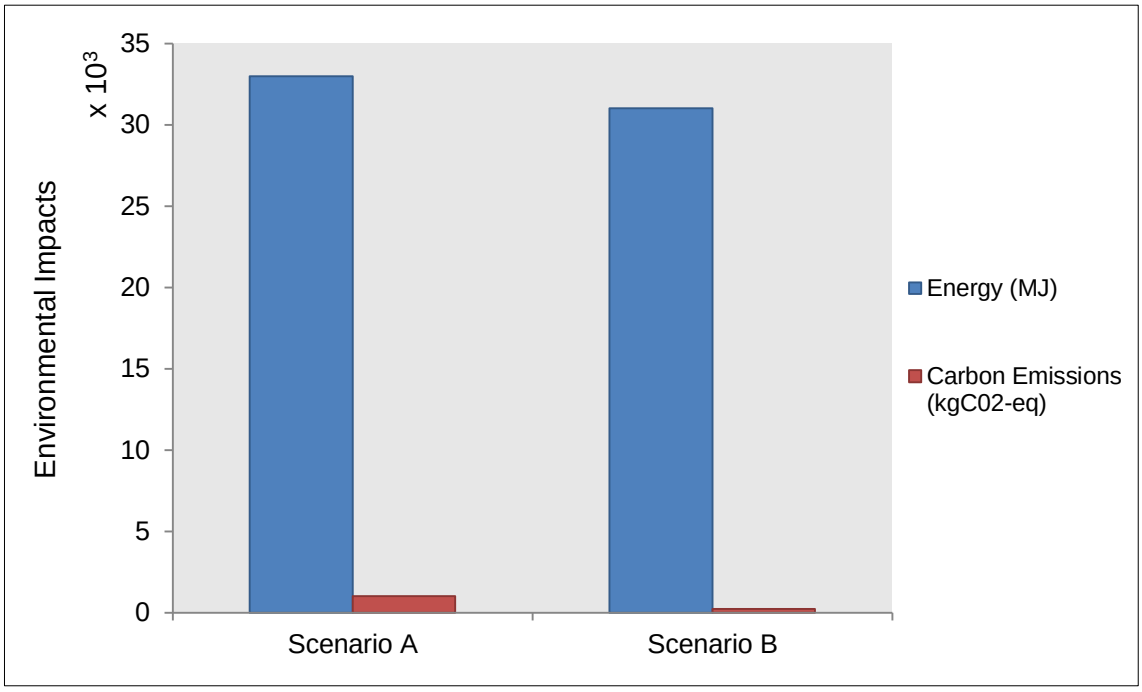


Figure 6.10 *Environmental impacts of the consumables transport for two scenarios: A and B to transport the consumables from the fabrication facility to the monoclonal antibody manufacturing facility.*

6.4 Analysing the Sensitivity of Parameters

At this point in the study, the influence of certain process parameters on the environmental impacts produced by a monoclonal antibody manufacturing process have been examined. The possible environmental savings that could be achieved by the biopharmaceutical industry have been highlighted. This section focuses on identifying a set of process parameters most sensitive to the environmental impacts of a traditional monoclonal antibody process. The analysis was carried out by varying the value of each of the process parameter by 10% (increasing/decreasing) from the baseline case. For each parameter, the possible range in the environmental impacts was calculated, assuming all other variables remain fixed at the baseline values. The results of this analysis are displayed in Figure 6.11. This figure allows

for rapid visualisation of the parameters that are most sensitive, identified by the longer bars in the figure.

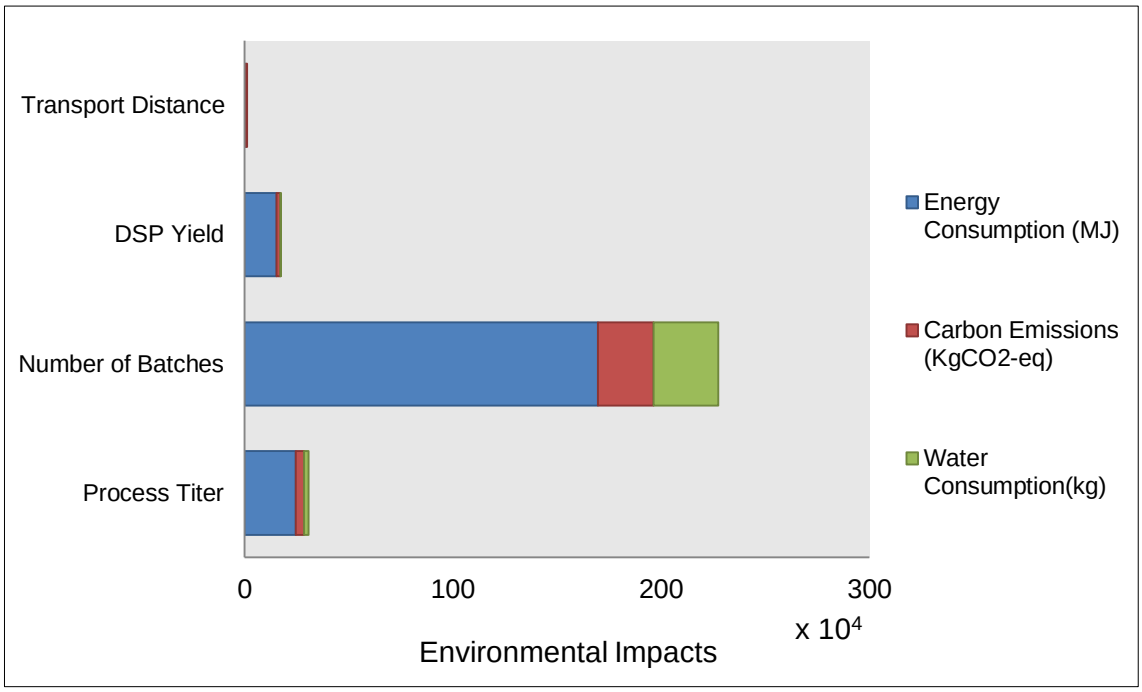


Figure 6.11 *Parameters that are most sensitive (indicated by the long bars).*

The figure 6.11 indicates that the most critical parameter that is affecting the overall energy and carbon levels is the number of batches. This is followed by the process titre and the DSP yield. It is interesting to note that transport distance has only relatively minor impacts on the energy and carbon levels. The parameter with the most significant impact on the overall levels of water consumption was found to be the number of batches, followed by the process titre.

The information obtained from this analysis is crucial for the sustainable manufacture of biopharmaceuticals where the industry can employ the best parameters (as identified in this research) to increase productivity whilst controlling their environmental contributions.

6.5 Conclusion

This chapter sets out to examine the impact of key process parameters on the levels of environmental impact. In this study, it was identified that the degree of uncertainty in the number of batches and the process titre have significant impacts on the environment burdens. The degree of uncertainty in the transport distance has relatively minor impact on the environmental burdens. This study also identified that transporting the consumables annually in a single trip using a cargo ship contributes to lower levels of environmental impact than transporting the consumables just before the start of a production batch using a cargo plane. The information generated by the sensitivity analysis studies can help determine the role of certain process parameters/assumptions in reducing the overall environmental impacts, and hence enhance the robustness of the decision-making process. This will ultimately improve the industry's environmental performance, helping the industry to be a step closer towards achieving industrial sustainability.

At this point in the thesis, only the environmental impacts associated with the manufacture of monoclonal antibodies to meet the product demand have been evaluated. However, to truly reduce the overall environmental contributions of monoclonal antibodies manufacture, the impacts in the clinical development phase should also be considered. Thus, the next chapter will evaluate the environmental impacts produced by the need to materialise monoclonal antibodies for meeting all of the clinical phases as well as production over a period of time.

Chapter 7. Applying the Framework to Evaluate the Cumulative Environmental Impacts of Therapeutic Monoclonal Antibody Manufacturing Processes

7.1 Introduction

The success of therapeutic monoclonal antibodies in the treatment of indications such as cancer and autoimmune diseases has helped make them the fastest growing segment within the biopharmaceutical industry. Presently, there are nearly 300 monoclonal antibodies in clinical development, with many more to enter clinical studies (Ecker *et al*., 2015). At the current approval rate of four new monoclonal antibodies per year, it has been estimated that around 70 monoclonal antibody products will be on the market by 2020, with the combined world-wide sales of $125 billion (Ecker *et al*., 2015).

At this point in the thesis, only the environmental impacts associated with the manufacture of monoclonal antibodies to meet the product demand have been evaluated. The environmental impacts of monoclonal antibody manufacture in clinical trials are unknown, and have not been the subject of any published study. It is anticipated that with a large number of monoclonal antibodies in clinical trials, a significant level of environmental impact will be associated with production for meeting even the clinical development phase. Therefore, in order for the monoclonal antibody manufacturers to truly reduce the environmental contributions of their processes, the cumulative impacts represented by the materialisation for the clinical phases should also be evaluated. This forms the impetus for the work reported in this chapter.

In this chapter, a hypothetical case study was designed. The main aim of the case study was to evaluate the environmental impacts produced by the need to materialise monoclonal antibodies for meeting all of the clinical phases as well as production over a period of time. The focus of this case study was not only to evaluate the cumulative environmental impacts associated with transitioning monoclonal antibodies from clinical studies to production, but also to demonstrate the benefits of employing single-use technologies in the clinical development phase for monoclonal antibodies manufacture. The potential savings that could be achieved by the manufacturers from employing such technology in the clinical studies will be assessed in this case study. The framework developed in this research (Chapter 3, Section 3.5) was applied to the case study to evaluate the cumulative environmental impacts of monoclonal antibodies manufacture.

In order to carry out the case study, a drug portfolio consisting of monoclonal antibodies that entered clinical study for a duration of time was developed. These monoclonal antibodies will be used in the case study to populate the cumulative environmental impacts. The information on the number of monoclonal antibodies entering clinical development was obtained from the data collected by the Tufts Center for the Study of Drug Development (the Centre collects such information by surveying pharmaceutical and biotechnology firms, from public documents and commercially available databases specific to these industries) (Nelson *et al.*, 2010). The number of monoclonal antibodies entering each phase of clinical study in the portfolio developed was estimated using monoclonal antibody transition probabilities (Reichert, 2008). The information on the total number of monoclonal antibodies

approved from 1997-2008 was obtained from a literature source (Nelson *et al.*, 2009). This will be further described in Section 7.2.3.

This chapter is divided into four sections. Section 7.2 describes the development of a case study to estimate the cumulative environmental impacts of monoclonal antibodies manufacture. In this section, the descriptions of the analyses carried out in this case study are provided. In section 7.3, the results of the analyses described in Section 7.2 are presented. Section 7.4 demonstrates how process intensifications of biopharmaceutical process will help improve the industry's environmental performance. In this section, specific examples are provided to demonstrate how process intensifications reduce the environmental impacts of manufacturing processes. In section 7.5, the significance of the framework in the biopharmaceutical industry is highlighted. Section 7.6 demonstrates whether the biopharmaceutical industry is an energy-intensive industry. The definition of an energy-intensive industry is provided in this section. Finally, a summary is provided in Section 7.7.

7.2 Setting up the Case Study

This section describes the development of a hypothetical case study using the framework based on the LCA tool described in Chapter 3 (Section 3.5) to estimate the cumulative environmental impacts of monoclonal antibodies manufacture.

7.2.1 Objectives of the case study

As stated in Section 7.1, the framework developed in this doctoral project was applied to a hypothetical case study to evaluate the cumulative environmental

impacts of monoclonal antibodies manufacture. The specific aims of the case study are:

a) To populate the cumulative environmental impacts of the production of monoclonal antibodies for clinical development over a period of time (1997-2008).
b) To estimate the potential environmental savings that could be achieved when single-use technologies (single-use bioreactors/bags) are introduced in the clinical development phase.
c) To compare the cumulative environmental impacts between the clinical development phase and production over a period of time.

To this point, the framework developed in this doctorate has been employed to select the manufacturing process and the solid waste management method that are environmentally favourable. This chapter will demonstrate how the framework may be applied to a wider scope of analysis within the biopharmaceutical industry.

The applicability of the framework to evaluate the environmental impacts of monoclonal antibodies manufacture in both the clinical development and manufacturing phases will demonstrate the effectiveness of this framework in the biopharmaceutical industry's decision-making processes.

The next section will provide a brief description of the clinical development phase and the importance of quantifying the environmental impacts produced in this phase.

7.2.2 The clinical development phase

The typical clinical development phase for a therapeutic monoclonal antibody is a lengthy process which can range from five to twelve years, with the estimated cost ranging from $0.8-$2 billion (Collier, 2009). For a monoclonal antibody molecule to gain market approval from a regulatory body (the Food and Drug Administration (FDA) in the United States and European Medicines Agency (EMA) in the United Kingdom or Europe), it is subjected to 3 phases (Phases I, II and III) of clinical studies (ABPI, 2007). The clinical studies can last up to 10 years (Dimasi and Grabowski, 2007). Figure 7.1 illustrates the clinical development phase of a monoclonal antibody drug.

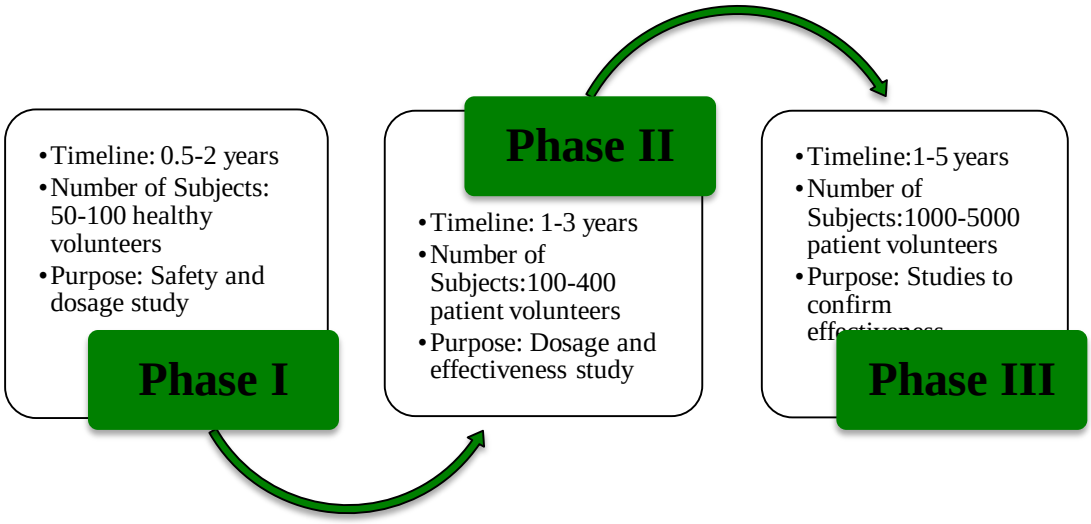


Figure 7.1 *The clinical development phase of a monoclonal antibody drug. As shown in the figure, the are 3 main phases of study involved in the clinical development of a monoclonal antibody; Phase I, Phase II and Phase III (ABPI, 2007).*

It is clear that with such a lengthy clinical development phase and high demand of product to meet the clinical development phase (mainly due to the large number of patients used in the Phase II and Phase III clinical studies), production of significant

levels of environmental impact may be anticipated. Therefore, it is imperative for the industry to consider the impacts produced by the clinical development phase as well as production in order to capture accurately the environmental impacts associated with monoclonal antibody manufacture.

7.2.3 Developing a portfolio

A drug portfolio was created in this case study using the monoclonal antibodies entering clinical studies over a period of time (Nelson *et al*., 2010). The objectives of the case study (as listed in Section 7.2.1) were met using the monoclonal antibodies in the portfolio.

It was estimated that from 1997-2008, approximately 131 monoclonal antibodies entered the clinical development phase (Phase I). The number of monoclonal antibodies entering Phase II and Phase III were determined using monoclonal antibodies phase transition probabilities (see Table 7.1), from a study carried out by Reichert (2008). Using these probabilities and literature sources, it was estimated that around 110 monoclonal antibodies entered Phase II, 48 entered Phase III and 11 obtained marketing approval and transitioned to production from 1997-2008. It was assumed that the rate with which monoclonal antibodies transition through clinical development and production phases is at a steady state.

Table 7.1 *Monoclonal antibody phase transition probabilities and the number of monoclonal antibodies entering each phase (Reichert, 2008; Nelson et al., 2010).*

Stage	Phase Transition Probability (%)	Number of Monoclonal Antibodies Entering from 1997-2008
Phase I	83	131
Phase II	44	110
Phase III	82	48

The top 7 best-selling monoclonal antibodies with different approved indication (s), dosage, market size, and annual production demand were used in this case study to represent the monoclonal antibodies in the portfolio. These monoclonal antibodies were used as their information is widely available, especially on the annual product demand (see Table 7.2). Thus, using the annual product demand information and several valid assumptions (listed in Section 7.2.4.2), the production scales of the representative drugs were estimated. Production scales were estimated for this case study using the drug's annual product demand information and process assumptions, and these may not compare to the actual production scales employed in the industry for the manufacture of these particular monoclonal antibodies but overall provide a good estimate of total production.

Table 7.2 *The top 7 best-selling therapeutic monoclonal antibodies (Huggett, 2013; Genentech, 2017; EMC, 2016; Janssen Biotech, 2016; MedImmune, 2014; Rituxan, 2017).*

Drug Name	**Manufacturer**	**Approved Indication**	**Annual Demand (kg)[1]**	**Dosage Requirements[2]**
Avastin	Roche	Metastatic colorectal cancer	2780	20 mg/kg for every 3 weeks once in a span of a year (dosage per person is 18 g)
Humira	(Abbott +Eisai)	Crohn's disease	2100	40 mg/kg for every month (for a year) (dosage per person is 34 g)
Rituxan	Roche	Rheumatoid arthritis	1900	2000 mg every 6 months for 2 years (dosage per person is 8 g)
Herceptin	Roche	Breast cancer, gastric cancer	1600	12 mg/kg weekly dosing for 6 months (dosage per person is 20 g)
Remicade	SGP+ J&J+ Mitsubishi Tanabe	Ulcerative colitis (UC)	700	5 mg/kg for the first 0, 2 and 6 weeks followed by 8 weeks once (for a year) (dosage per person is 3 g)
Lucentis	Roche	Wet age-related macular degeneration	100	0.5 mg for 4 weeks once (for a period of 3-6 months) (dosage per person is 1 g)
Synagis	MedImmune	Respiratory syncytial virus	70	15 mg/kg for every 4 weeks once for 5 months (dosage per person is 5g)

[1]*In the table above, only one approved indication of the drug is highlighted although these drugs may be approved by the regulatory bodies (e.g. FDA) for several indications. The dosage requirements are provided for the given approved indication.*

[2]*The dosage requirements are the average dosage requirements. However, this may differ from person to person. This case study will use only the standard dosage prescribed.*

7.2.4 Modelling the operational scales for the monoclonal antibodies in the portfolio

To evaluate the cumulative environmental impacts of manufacture, the operational scales to produce the materials needed for clinical studies, and to meet the annual product demands of the 7 monoclonal antibodies used to represent the drugs in the portfolio were required.

7.2.4.1 Clinical trials scale

The operational scales to produce materials needed for the Phase I, Phase II and Phase III clinical studies were determined by an examination of the clinical study data of a high and a low dosage requirements mAbs (see below for the assumptions made to determine these operational scales). The assumed clinical operational scales of a high dosage requirements mAb and a low dosage requirements mAb were used to represent the clinical development phase operational scales of the drugs in the portfolio (FDA, 2003; EMA, 2011).

In this case study, Humira was used to represent a high dosage requirements monoclonal antibody. This is because amongst the 7 monoclonal antibodies used to represent the drugs in the portfolio, Humira has the highest dosage requirements (see Table 7.2). Meanwhile, Remicade was used to represent a low dosage requirements monoclonal antibody, as this drug has one of the lowest dosage requirements amongst the 7 monoclonal antibodies used to represent the drugs in the portfolio (Lucentis has the lowest dosage requirements however the clinical studies information wasn't easily accessible). In the absence of the clinical study information for the other representative drugs, the assumed clinical operational scales determined

for Humira and Remicade, were used as the basis by which to set the operational scales for all the representative monoclonal antibodies (see Table 7.2 for the list of the representative monoclonal antibodies). The operational scales of the representative drugs are necessary to populate the cumulative environmental impacts of monoclonal antibodies manufacture in the clinical development phase.

Clinical study operational scale calculation for Humira (an example of a high dosage monoclonal antibody):

The monoclonal antibody Humira was used to represent a high dosage monoclonal antibody. In order to determine the operational scales employed to produce materials for Phase I, Phase II and Phase III clinical studies, the monoclonal antibody requirements for each phase should be determined. The Table 7.3 highlights the number of patients, dosage used and number of trials in each phase. The duration of the study in each phase is also highlighted. These information were obtained from the published clinical study report of Humira for the treatment of Crohn's disease (FDA, 2013). The information provided in Table 7.3 is necessary to determine the monoclonal antibody requirements for each phase in the clinical study. The monoclonal antibody required for each phase can be determined using Equation 7.1.

When the monoclonal antibody requirements of all the phases (Phase I, II and III) are known, the operational scales employed in each phase of the clinical study can be determined (see Equation 7.2). Equation 7.2 shows how the operation scales needed to produce sufficient materials for clinical studies can be determined. A number of assumptions were made in order to calculate the operational scales for Phase I, Phase II, and Phase III. It was assumed that the process titre is 5g/L and the overall DSP

yield is 70%. These assumptions remain consistent in the research. It was also assumed that the average body weight in the UK is 70 kg (NHS, 2015).

Table 7.3 *Number of patients employed and dosage requirements in the clinical studies of Humira for the treatment of Crohn's disease (FDA, 2013; Abbvie, 2017).*

Phase	Information
Phase I	Number of patients: 100 patients. In Phase I study, the patients were separated into three groups where patients in group 1 were administered with 20 mg/kg of Humira, the patients in group 2 were administered with 40 mg/kg of Humira and the patients in group 3 were administered with 80 mg/kg of Humira (safety and dosage study) Duration of the study: around 1 year
Phase II	Number of patients: 500 patients. In phase II study, the patients were separated into 3 groups where the patients in group 1 were administered with 20 mg/kg of Humira for 26 weeks, the patients in group 2 were administered with 40 mg/kg of Humira for 26 weeks, and the patients in group 3 received placebo treatment (dosage and effectiveness study) Duration of the study: around 2 years
Phase III	Number of patients: 1000 patients. In Phase III study, the patients were separated into four groups, where the patients in group 1 were administered with 20 mg/kg of Humira for 52 weeks, patients in group 2 were administered with 40 mg/kg of Humira for 26 weeks, the patients in group 3 were administered with 40mg/kg of Humira for 13 weeks and the patients in group 4 received placebo treatment (effectiveness study) Duration of the study: around 5 years

Table 7.4 highlights the operational scales assumed for the high dosage monoclonal antibodies in the clinical development phase (Phase I, Phase II and Phase III). The operational scales provided in this table were determined through the method provided (Equations 7.1 and 7.2) by using the information provided in Table 7.3. Assumptions were made for the production batches typically employed in the clinical development phase. It was assumed the Phase I clinical materials for a high dosage monoclonal antibody were produced in a single batch, Phase II materials were produced in 2 batches and Phase III materials were produced in 6 batches. The batches in the clinical studies were set in line with the typical production of materials for clinical studies for monoclonal antibodies in the biopharmaceutical industry, based on the information obtained through consultation with industrial experts (see Chapter 4, Section 4.2). Such dialogue helped in understanding the logistics of clinical studies operation and supply within the biopharmaceutical industry.

Product demand (mg) = Number of patients × dosage $\left(\frac{\text{mg}}{\text{kg}}\right)$ × average body weight (kg) × number of trials

(**Equation 7.1)**

Operation scale (L) =Annual product demand (kg) × $\left(\frac{1}{\text{Process Titre}}\right)\left(\frac{\text{L}}{\text{kg}}\right)$ × $\left(\frac{1}{\text{Overall DSP Yield}}\right)$

(Equation 7.2)

Table 7.4 *Clinical studies operational scales employed for a high dosage monoclonal antibody.*

	Phase I	**Phase II**	**Phase III**
Scale of Operation (L)	1, 000	2, 000	2, 000
Number of Batches	1	2	6

Clinical study operational scale calculation for Remicade (an example of a low dosage monoclonal antibody):

The monoclonal antibody Remicade was used to represent a low dosage monoclonal antibody. Similar to Humira's clinical study operational scales calculations, the monoclonal antibody required for each phase in the clinical study must be identified to calculate the operational scales. Table 7.5 highlights the number of patients, dosage used and number of trials in each phase. The duration of the clinical study is also highlighted in the table. The information on dosage used and number of trials for each phase was obtained from the published clinical study report of Remicade for the treatment of ulcerative colitis (UC) (Reinisch, *et al*., 2011; NIH, 2011; Rutgeerts *et al*., 2005*).* The information on the number of patients used in the clinical study was not clear, thus, the average number of patients typically used in clinical studies were adopted (ABPI, 2007). The information on the drug's dosage, number of trials and number of patients are necessary to determine the product required in each phase of the clinical study (FDA). The product demand and the operational scales of Phase I, II and III for Remicade can be determined using Equations 7.1 and 7.2.

Table 7.5 *Number of patients employed and dosage requirements in the clinical studies of Remicade for the treatment of ulcerative colitis (Reinisch, et al., 2011; NIH,2011; Rutgeerts et al., 2005).*

Phase	Information
Phase I	Number of patients: around 200 patients. In Phase I study, the patients were separated into two groups where patients in group 1 were administered with 5 mg/kg of Remicade and the patients in group 2 were administered with 10 mg/kg of Remicade at weeks 0, 2 and 6 and then every eight weeks through week 46. Phase I study is a safety and dosage study Duration of the study: around 1 year
Phase II	Number of patients: around 600 patients. The patients were separated into three groups where patients in group 1 were administered with 5 mg/kg of Remicade, the patients in group 2 were administered with 10 mg/kg of Remicade, and the patients in group 3 received placebo treatment at weeks 0, 2, 6 and every 8 weeks for 54 weeks. Phase II study is a dosage and effectiveness study Duration of the study: around 2 years
Phase III	Number of patients: around 1000 patients. The patients were separated into three groups where patients in group 1 will be administered with 5mg/kg of Remicade, the patients in group 2 were administered with 10 mg/kg of Remicade, and the patients in group 3 received placebo treatment at weeks 0, 2, 6 and then once every 8 weeks thereafter through week 46 for up to 3 years (effectiveness study) Duration of the study: around 3 years

Table 7.6 highlights the operational scales determined for the low dosage monoclonal antibodies in the clinical development phase. The operational scales provided in this table were determined through the method provided (Equations 7.1

and 7.2) by using the information provided in Table 7.5. The average body weight in the UK is 70 kg (NHS, 2015). Assumptions were made for the production batches typically employed in the clinical development phase. It was assumed that the materials needed for the Phase I, II and III trials for a low dosage monoclonal antibody were produced in a single batch, and the Phase III materials were produced in 2 batches. Again, the batches in the clinical studies were set in line with the typical production of materials for clinical studies for monoclonal antibodies in the biopharmaceutical industry, based on the information obtained through consultation with industrial experts (see Chapter 4, Section 4.2). The differences in the number of batches required are due to the differences in the annual requirements of the drugs.

Table 7.6 *Clinical study operational scales employed for a low dosage monoclonal antibody.*

	Phase I	**Phase II**	**Phase III**
Scale of Operation (L)	1000	2,000	2, 000
Number of Batches	1	1	2

As mentioned previously, in the absence of the clinical study information for the other representative monoclonal antibodies, the clinical operational scales determined for a high dosage monoclonal antibody (Humira) and a low dosage monoclonal antibody (Remicade), were used as the basis by which to set the operational scales for all the representative monoclonal antibodies. Typically, the operational scales of the clinical studies are dependent on a number of factors such as cumulative dose of the drug, the number of patients in the trial and the projected product demand of the drug for the clinical development phase (Farid *et al*., 2005). Therefore, the clinical studies operational scales of a high dosage monoclonal antibody were assumed to apply for Avastin, Humira, Rituxan, Herceptin and

Remicade as these drugs have similar annual product demands and dosage per patient (Levine, 2012). The clinical studies operational scales of a low dosage monoclonal antibody were assumed to apply for Remicade, Synagis and Lucentis as these drugs have similar product demand and dosage per person. Also, it was assumed that in this case study, each of the monoclonal antibodies that are employed to represent the drugs in the portfolio were only approved by the regulatory bodies (either FDA or EMA) for one indication (to simplify the clinical operational scales calculations).

7.2.4.2 Assumed scales of operation

The scales of operation for all the 7 drugs investigated were designed to meet the projected annual product demands based upon the envisaged clinical trials, determined through a number of assumptions outlined below. The formula that was used to obtain the production scales is given below:

$$\text{Production Scale (L)} = \frac{\text{Annual product demand (kg)} \times \left(\frac{1}{\text{Process Titre}}\right)\left(\frac{\text{L}}{\text{kg}}\right) \times \left(\frac{1}{\text{Overall DSP Yield}}\right)}{\text{Production Batches}}$$

(**Equation 7.3)**

The annual product demand information of the 7 representative monoclonal antibodies is available (see Table 7.2). With this piece of information, and assumptions made on the process titre, overall DSP yield and production batches, the production scales for all the 7 representative monoclonal antibodies were estimated (using Equation 7.3) for this case study. These production scales are provided in Table 7.7.

In this case study, the duration of monoclonal antibodies manufacture (production batches) was designed to be 20 weeks. The fermentation time for each production batch was assumed to be between 7-14 days, operated through a batch campaign. The process titre was assumed to be 5 g/L, and the process yield was assumed to be 70% (Kelley, 2007).

From Table 7.7, it is clear that the monoclonal antibodies have different production scales; ranging from 1000 L to 40, 000 L scales. In the case study, it was assumed that the largest stainless steel bioreactor volume in the facility is 25, 000 L (based on the equipment sizing in BioSolve Software). Therefore, the drugs with fermentation volumes beyond 20, 000 L employed multiple bioreactors for manufacture.

Table 7.7 *Estimated production scales of the monoclonal antibodies in the portfolio for this case study.*

Drug	Estimated Scale of Operation (L)
Avastin	40,000
Humira	30,000
Rituxan	30,000
Herceptin	25,000
Remicade	10,000
Lucentis	2,000
Synagis	1,000

7.2.5 Populating the cumulative environmental impacts of monoclonal antibodies in the clinical development phase

This section provides the description of the analysis designed to meet the first objective (listed in Section 7.2.1). The main focus of the case study is to populate the environmental impacts of monoclonal antibodies manufacture in the clinical development phase. Through this analysis in the case study, a number of questions will be addressed, such as:

1. What are the cumulative environmental impacts produced by Phase I, Phase II and Phase III trials?
2. Whether the clinical development phase contribute to significant levels of environmental impact?
3. Which clinical trial phase contributes to the highest levels of environmental impact?

To this point, no such studies exist in the biopharmaceutical industry. This case study is the first study attempting to populate the cumulative environmental impacts associated with the clinical development phase for monoclonal antibodies manufacture. The results of this analysis are presented in Section 7.3.1.

This framework based on the LCA tool developed in this doctoral project was used to carry out this analysis (see Chapter 3, Section 3.5 for the description of the framework). The framework was used to model the environmental impacts of manufacturing processes employing traditional fixed-in-place technologies with different operational scales (1000 L and 2000 L operational scales) employed in the clinical development phase. The assumed clinical operational scales of a high and

low dosage monoclonal antibodies were used to represent the clinical operational scales of the monoclonal antibodies used in this case study (see Table 7.2). The energy and water consumption and carbon emissions produced by the 131 monoclonal antibodies in the Phase I trial, 110 monoclonal antibodies in the Phase II trial and 48 monoclonal antibodies in the Phase III trial were then estimated (the monoclonal antibodies in the portfolio that enter Phase II, Phase III and production were randomly selected). The rationale for the number of monoclonal antibodies entering each phase is provided in the drug portfolio (see Section 7.2.3).

In this analysis, the life cycle inventory data for the different operational scales employed in the clinical studies were obtained from a number of sources. This will be explained in detail in Section 7.2.9.

7.2.6 Evaluating the environmental benefits of single-use technologies in the clinical development phase

The analysis in this section was designed to meet the second objective of the case study (as listed in Section 7.2.1). The main aim of the analysis is to assess the environmental benefits of introducing single-use technologies in the clinical development phase. Previously, in the analysis in Section 7.2.5, the manufacture of monoclonal antibodies for clinical trials took place using processes based on traditional technologies, where fixed-in-place stainless steel equipment were employed. Since this doctoral project has identified that there are environmental benefits associated with single-use technologies, this analysis will quantify the environmental savings that could be achieved when the monoclonal antibodies manufacture for the clinical studies take place using hybrid processes, where a mix

of traditional and single-use equipment are employed (adoption of a fully single-use process was not pursued because for some of the unit operations, single-use equipment are not yet well-established). This analysis will also identify the clinical trial phase with the highest level of environmental savings. The life cycle inventory data used in this analysis are described in Section 7.2.9.

This framework based on the LCA tool developed in this work was used to model the manufacturing processes (1000 L and 2000 L operational scales) employing hybrid technologies in the clinical development phase. The energy and water consumption, and carbon emissions produced by the 131 monoclonal antibodies in the Phase I trial, 110 monoclonal antibodies in the Phase II trial and 48 monoclonal antibodies in the Phase III trial were then estimated. The analysis is similar to the analysis carried out in Section7.2.5, however with hybrid manufacturing processes. In this analysis, the single-use equipment that were mainly considered included disposable bioreactors and bags. The results generated from this analysis are presented in Section 7.3.2.

7.2.7 Comparing the cumulative environmental impacts between the clinical development phase and production stage

The final objective of the case study was to compare the cumulative environmental impacts between the clinical development phase and production to understand the contribution of the clinical development phase to the overall environmental impacts of monoclonal antibodies manufacture. In this analysis, the cumulative environmental impacts between the clinical development phase and production per

year were compared. The framework based on the LCA tool developed in the doctoral project was used to carry out the analysis.

In the drug portfolio, it was assumed that between 1997-2008, only 11 monoclonal antibodies obtained product approval (Reichert, 2008). Therefore, in this analysis, it was assumed that only one monoclonal antibody obtained product approval per year (assuming a steady state trend in monoclonal antibody product approval). Since only one drug gets product approval per year, it was not possible to select randomly the drug that will get product approval out of the 7 monoclonal antibodies used to represent the drugs in the portfolio. Thus, the average energy and water consumption and carbon emissions produced by these 7 monoclonal antibodies with different production demands were used to represent the environmental impacts of the production stage. These were then compared with the environmental impacts produced in the clinical development phase per year. The results generated in this analysis are presented in Section 7.3.3.

7.2.8 Inventory data for the clinical development and manufacturing phases

In this case study, the life cycle inventory data were mainly obtained from the BioSolve software, GaBi software, literature surveys and expert interviews (see Chapter 4, Section 4.2). The BioSolve software was used to model the monoclonal antibody processes at the clinical development and production scales (as highlighted in Section 7.2.4) to obtain information such as (i) list of stainless steel equipment and consumables employed in the manufacturing processes, (ii) amount of reagents (e.g. buffer) used in manufacturing, and (iii) type (solid or liquid) and amount of waste produced. The list of stainless steel equipment, consumables and media requirements

of manufacturing process at clinical and production scales for the traditional and hybrid processes are provided in Appendix 4.5.

In the supply-chain phase, the energy and water consumption and carbon emissions data for equipment fabrication in the clinical development phase and production at various scales were populated from the already existing supply-chain data for monoclonal antibodies manufacture at 200 L operation scale (see Section 4.3 for the equipment fabrication process and inventory data). The energy and water consumption and carbon emissions data for the consumables fabrication, reagent production, use phase and end-of-life phase activities were determined using the methodology described in Chapter 4, Section 4.3. The inventory data for these activities are provided in Section 4.3, and they were mainly obtained from vendors/industrial experts (the industrial experts that were interviewed for inventory collection are listed in Chapter 4, Section 4.2), literature surveys and GaBi software (more information on this software and the database used for inventory collection are listed in Section 4.2). The assumptions on the life inventory data listed in Chapter 4, Section 4.4 were also assumed to apply for this case study. The cumulative energy and water demand, and carbon emissions per annum of monoclonal antibodies manufacture in the clinical development phase and production are provided in Appendix 4.6.

7.3 Case Study Results and Discussion

The results of the case study are reviewed in this section to demonstrate the further insights that can be gained from the framework developed in this research. The

results from the analyses (Sections 7.2.5, 7.2.6 and 7.2.7) are discussed in this section.

7.3.1 Cumulative environmental impacts in the clinical development phase

This section discusses the environmental impacts associated with the clinical development phase. The environmental impacts produced in the clinical development phase of monoclonal antibodies manufacture have not been the subject of rigorous environmental analysis in the biopharmaceutical industry. However, this phase is anticipated to contribute to significant levels of environmental impact due to the production of materials needed for studies carried out on a large number of subjects in the clinical trials. By excluding the impacts produced in this phase, the industry faces the risk of not accurately capturing the environmental impacts of monoclonal antibodies manufacture.

Figure 7.2 shows the cumulative energy and water consumption and carbon emissions of the clinical development phase for monoclonal antibodies manufacture over a period of 12 years. This figure highlights the environmental impacts of each phase of the clinical study: Phase I, Phase II and Phase III. From Figure 7.2, it is apparent that the clinical development phase contributes to high levels of environmental impact. The cumulative energy consumption of the clinical development phase was estimated to be around 398 million MJ of energy, the cumulative water consumption was estimated to be around 128 million kg of water and the cumulative carbon emissions were estimated to be around 62 million kgCO_2-eq of carbon. This is equivalent to the average energy used by 23, 412 houses in the

UK per year, average water consumed by 4267 houses in the UK per year, and average carbon generated by 31, 000 houses in the UK per annum.

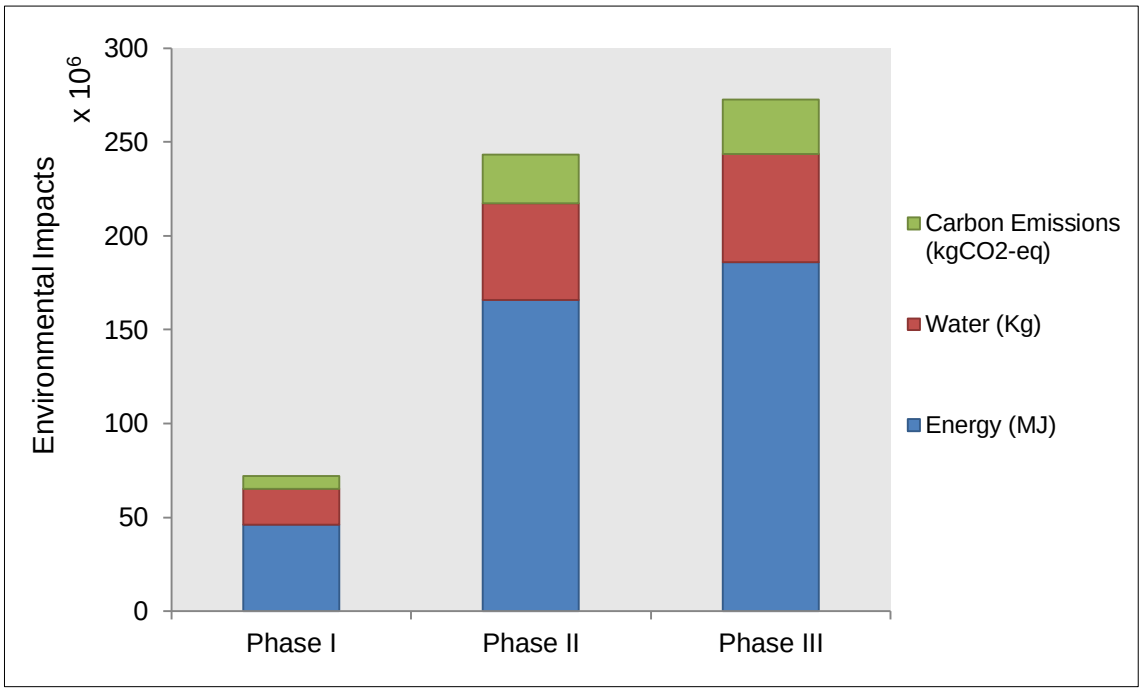


Figure 7.2 *Cumulative environmental impacts produced in the clinical development phase over a period of 12 years.*

From Figure 7.2, it is also evident that the majority of environmental impacts occur in Phase III trial. The Phase III trial alone contributes to 47% of the total energy consumption, 45% of the total water consumption and 47% of the total carbon emissions in the clinical development phase (the Phase III trial itself contributes to almost half of the total environmental impacts produced in the clinical development phase). The high levels of environmental impact produced in Phase III trial can be associated with the large operational scales employed to meet the product demand of the clinical study (larger materials are needed for Phase III trial than the Phase I and Phase II trials due to larger number of subjects and higher dosage of monoclonal antibodies used in this phase).

7.3.2 Environmental benefits of applying single-use technologies in the clinical development phases

The flexibility in manufacturing offered by single-use technologies such as no CIP and SIP operations between production batches, low capital investment and reduction in equipment lead time (the benefits of single-use technologies are described in Chapter 2, Section 2.4.8) can be beneficial in the clinical development phase where there is a high failure rate of drugs, particularly in the Phase II of the trial (Reichert, 2008). The current scales available for the single-use technologies (up to 2000 L) are also well-suited for the clinical development phase where small scales of operation are typically employed (Guldager, 2009). Thus, this section evaluates the benefits of employing hybrid manufacturing processes in the clinical development phase from an environmental perspective.

Figure 7.3 shows the total environmental savings that could be achieved over a period of 12 years when hybrid processes (a mix of traditional and single-use equipment such as single use bioreactors and bags) are employed. From this figure, it can be seen that significant levels of environmental saving are achieved in each phase of the trial. The total energy, water and carbon savings were around 160 million MJ of energy, 47 million kg of water and 25 million kgCO_2-eq of carbon (around 58% savings in energy consumption, 63% savings in water consumption and 57% savings in carbon emissions). The energy savings achieved is equivalent to the average energy used by 9700 houses in the UK per year, the water savings achieved is equivalent to the average water used by 160 houses in the UK per year, and the carbon emissions savings achieved is equivalent to the average carbon emitted by 10,300 houses in the UK per year (see Chapter 4, Section 4.5). By showing this

comparison, the magnitude of savings achieved by employing a mix of traditional and single-use technologies in the clinical development phase can be clearly envisaged. It also can seen in Figure 7.3 that most of the environmental savings occur in the Phase III trial (level of savings increase as the operational scale increases). It is evident that the benefit of single-use technologies in monoclonal antibody manufacture becomes apparent as the operation scale increases (major reduction in the volumes of CIP and SIP reagents are achieved).

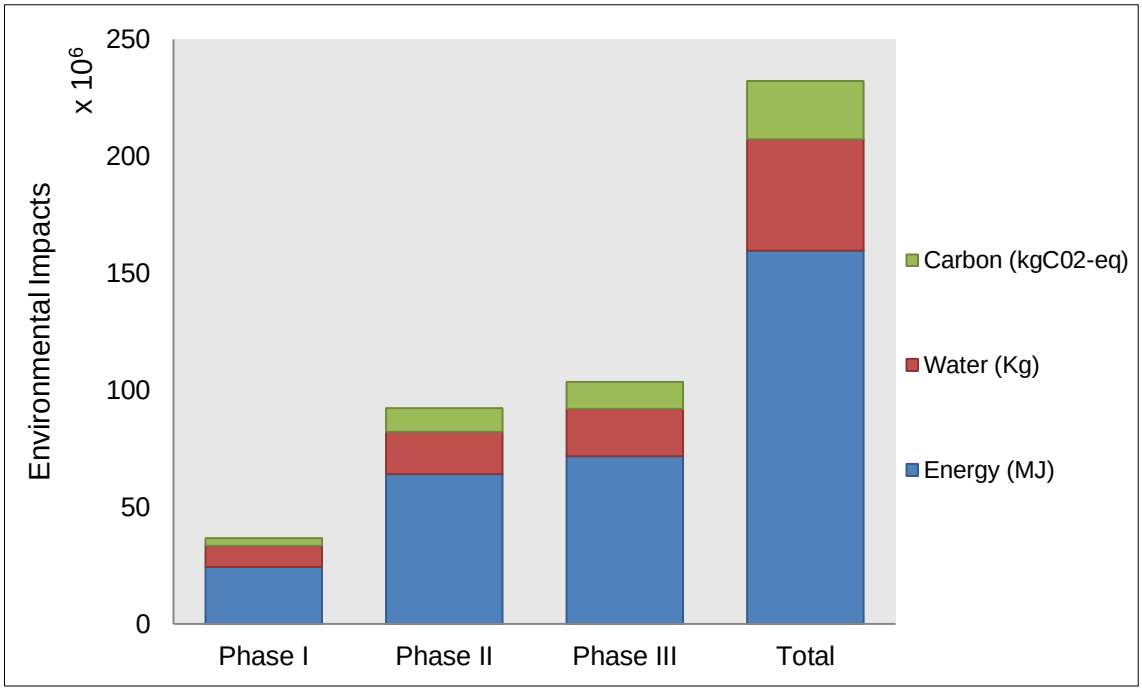


Figure 7.3 *Environmental savings achieved over a period of 12 years in the clinical development phase when hybrid processes were employed.*

As highlighted before, single-use technologies are ideal in the clinical development phase due to the flexibility it offers (see Chapter 2, Section 2.4.8 for the benefits of single-use technologies). This research has demonstrated that considerable levels of environmental savings can be achieved by deploying disposable technologies in the clinical development phase. Therefore, the industry should consider employing such

technologies for the production of monoclonal antibodies in the clinical development phase.

7.3.3 Comparing the cumulative environmental impacts between the clinical development phase and the production stage

This section discusses the final objective of the case study; that is to compare the average environmental impacts between the clinical development phase and the production stage. The main focus of this analysis is to assess how the environmental impacts from the clinical development phase compare with the impacts from production of monoclonal antibodies to meet the market demand per annum.

Figure 7.4 shows the average environmental impacts produced by the clinical development phase and the production stage over a period of a year. From this figure, it can be deduced that the production stage contributes to higher levels of environmental impact; the production phase contributes to 85% of the total energy consumed, 76% of the total water consumed, and 82% of the total carbon emissions generated. This can be mainly attributed to the large scales of operation and high number of batches (20-24 production batches) used in this stage (scales of operation in this study ranged between 1000 L-40, 000 L). Although the clinical development phase was anticipated to contribute to substantial environmental impacts and even more than the production stage, the analysis demonstrated that the levels of environmental impact generated in the production stage are far greater in magnitude when compared to impacts from the clinical study phase per year. Although a large number of studies were carried out in the clinical development phase, the scales of operation employed to produce materials needed for this phases and production

batches employed are small (due to the low volumes of monoclonal antibodies needed in clinical studies) (Walter, 2012). It is predicted that the environmental impacts in the clinical development and production stages will decrease as the biopharmaceutical processes go through process intensification within the next decade to improve their overall efficiency.

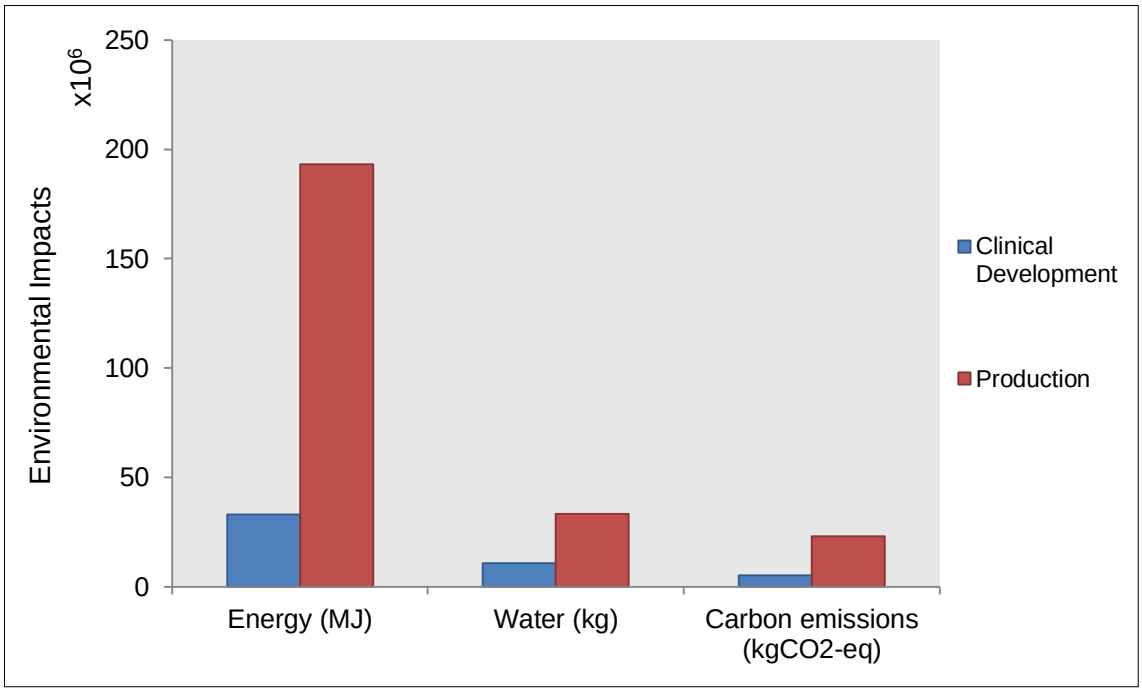


Figure 7.4 *Comparison of average environmental impacts between the clinical development phase and production stage per year.*

Process intensification is a term used to describe the strategy of making reductions in the physical size of a facility whilst achieving a given production objective (Dautzenberg and Mukherjee, 2001). Process intensification was first introduced in the chemical industry to reduce investment and operating costs of chemical plants to increase profitability and reduce greenhouse gas emissions (Stankiewicz and Moulijn, 2002; Dautzenberg and Muherjee, 2001). The benefits of process intensification in reducing the operating and capital cost in the chemical industry

have sparked interest in the biopharmaceutical industry in applying this strategy for their own processes (Rathore and Sengar, 2011). Presently, huge emphasis is being placed on process intensification in the biopharmaceutical industry (Simaria and Farid, 2010; Guldager, 2009).

Process intensification in the biopharmaceutical industry can be achieved through a number of ways such as improvements in process titre and DSP yield, reduction in process time of unit operations, employment of disposable manufacturing technology and employment of continuous manufacturing strategy to manufacture biopharmaceuticals (all these will reduce the footprint of the facility) (Rathore and Sengar, 201; Buchholz, 2010). Although process intensification was mainly introduced to reduce capital and operating costs, this strategy can be also applied as a step to reduce the overall environmental impacts produced by manufacturing processes (achieved through reduction in equipment footprint) (Dautzenberg and Muherjee, 2001; Buchholz, 2010; Guldager, 2009). It can be expected that with process intensification, the biopharmaceutical industry may undergo significant transformations, resulting in smaller and flexible facilities. This is likely the direction the biopharmaceutical industry will be heading towards within the next decade (Guldager, 2009).

The next section explores how the environmental impacts of the clinical development phase and production stage change when process intensification is carried out for monoclonal antibody manufacturing processes. The process titre will be used as an example in this analysis to study how intensification of manufacturing processes influence the overall environmental impacts of monoclonal antibodies

manufacture. As highlighted above, improving the process titre is one way of carrying out process intensification for biopharmaceutical processes. More suggestions on process intensification for biopharmaceutical processes will be provided in Chapter 8 under future work and directions (see Section 8.3).

The next section will also explore the effect of process intensification on the operational scales of monoclonal antibody manufacturing processes, and the changes in the levels of environmental impact resulting from this. It is anticipated that as processes become more efficient, the operational scales of these processes will become smaller as a result of reduction in the equipment footprint (Guldager, 2009). Thus, the analysis in the next section will investigate how the environmental impacts in the production stage change when manufacturing processes with lower scales of operation are employed.

7.4 Process Intensification of Monoclonal Antibody Processes

This section's main aim is to explore the effect of process intensification of monoclonal antibody manufacturing process on the overall environmental impacts produced.

7.4.1 Improvements in process titres

The process titre of monoclonal antibody processes is expected to increase over the next decade. Presently, a majority of monoclonal antibody manufacturing processes achieve a process titre of 5g/L, with some studies reporting a process titre of 10 g/L (Stonier *et al*., 2012). It has been forecasted that a process titre of 15g/L is possible before process limitations come into play (Guldager, 2009). Thus, in this analysis,

two process titres: 10g/L and 15g/L are assessed and compared with the original process titre of 5g/L (baseline value) to evaluate how improvements made to the process titre affect the environmental impacts of the clinical development phase and production stage per annum for monoclonal antibodies manufacture. Process intensifications will not only take place for the processes in the production stage, but also for the processes in the clinical development phase. The FDA considers changes in cell line (higher product expression cell line) as a major process change, thus, clinical studies must be carried out to determine the efficacy and safety of these processes (Li *et al*., 2010).

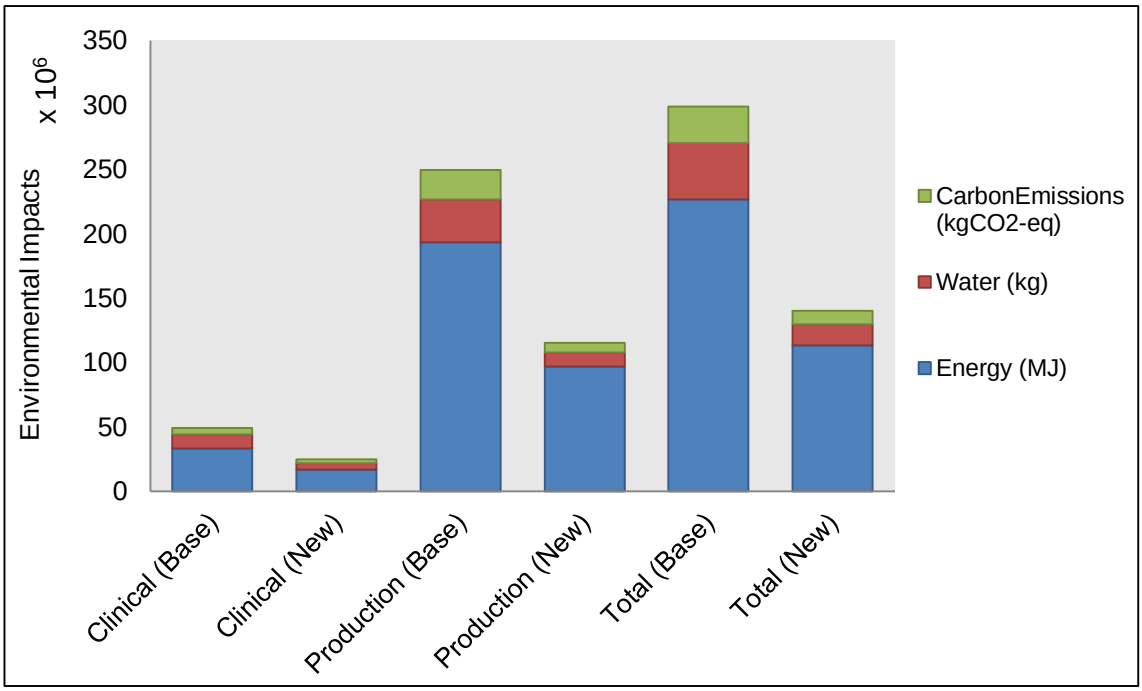


Figure 7.5 *Difference in environmental impacts between the clinical development phase (indicated as clinical in the figure) and the production stage per year when the process titre is increased from 5g/L (Base case) to 10g/L (New).*

Figures 7.5 and 7.6 show that the differences in environmental impacts between the clinical development phase and the production stage per year when the process titre

is increased from the base case scenario (5 g/L) to 10 g/L and 15 g/L. As expected, the processes in the clinical development phase and production stage with increased process titres (10 g/L and 15 g/L) contribute to lower levels of environmental impact. From this analysis, it was determined that by increasing the process titre from 5 g/L to 10 g/L, a reduction of 17 million MJ of energy, 5 million kg of water, and 2 million $kgCO_2$-eq of carbon emissions from the base case scenario can be achieved per year in the clinical development phase, and a reduction of 97 million MJ of energy, 17 million kg of water, and 11 million $kgCO_2$-eq of carbon emissions from the base case scenario can be achieved in the production stage.

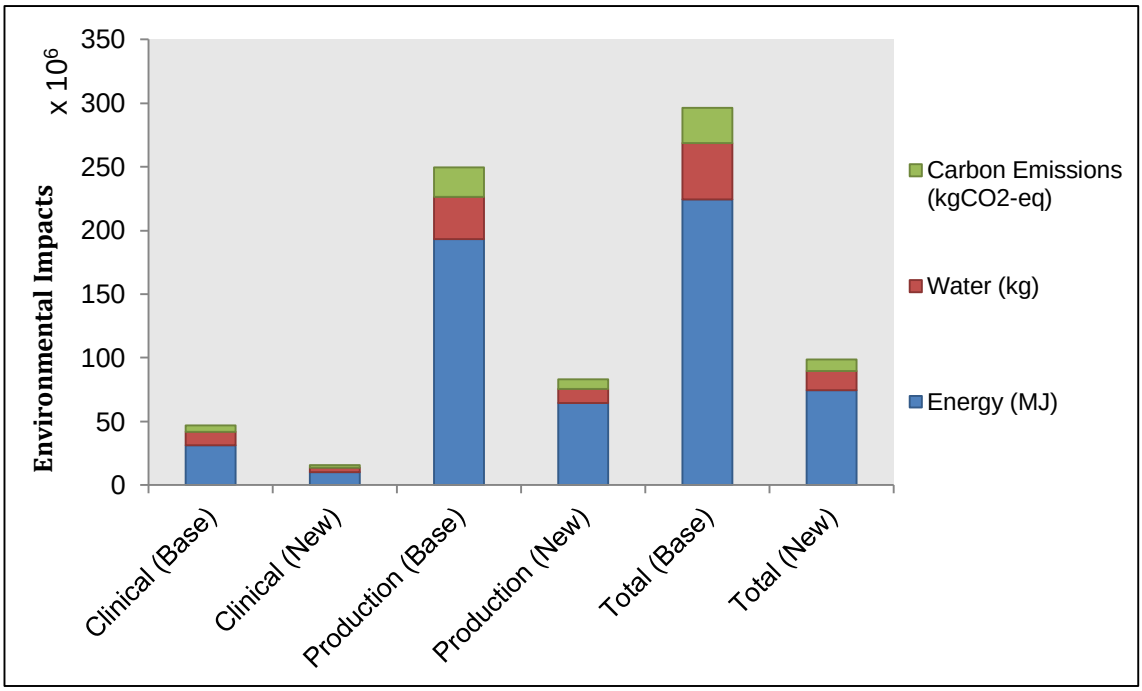


Figure 7.6 *Difference in environmental impacts between the clinical development phase (indicated as clinical in the figure) and the production stage per year when the process titre is increased from 5 g/L (Base case) to 15 g/L (New).*

Similarly, increasing the process titre from 5 g/L to 15 g/L will result in a total reduction of 21 million MJ of energy, 7 million kg of water, and 3 million $kgCO_2$-eq

of carbon emissions from the base case scenario in the clinical development phase, and a reduction of 129 million MJ of energy, 22 million kg of water, and 15 million $kgCO_2$-eq of carbon emissions from the base case scenario in the production stage. It is clear from this analysis that a majority of environmental savings from process intensifications to improve the process titre occur in the production stage.

This analysis demonstrates that environmental savings can be achieved when process intensification is applied to the monoclonal antibody processes. This analysis only demonstrated one method towards achieving process intensification for biopharmaceutical processes to reduce the environmental impacts. As highlighted in Section 7.3.3, a number of methods for process intensification exist, and should be explored by the biopharmaceutical industry to further improve the environmental performance of biopharmaceutical processes.

7.4.2 Lower scale of operation

Previously, in order to populate the environmental impacts in the production stage, the average energy and water consumption and carbon emissions of the 7 representative monoclonal antibodies were used (see Section 7.2.3). These representative monoclonal antibodies have different production demands, thus their assumed scales of operation ranged from 1000 L- 40,000 L. However, as highlighted in Section 7.3.3, the operational scales of biopharmaceutical processes are expected to become smaller as process intensification advances in the industry, resulting in smaller facilities (Rathore and Sengar, 2011). Thus, this analysis will assess the changes to the environmental impacts in the production stage when the scales of operation in the production stage are at the lower end of the range; that is 1000 L.

This analysis will also assess how the environmental impacts produced in the clinical development phases compare with the impacts from the production stage per year when processes with small operational scales are employed at that stage.

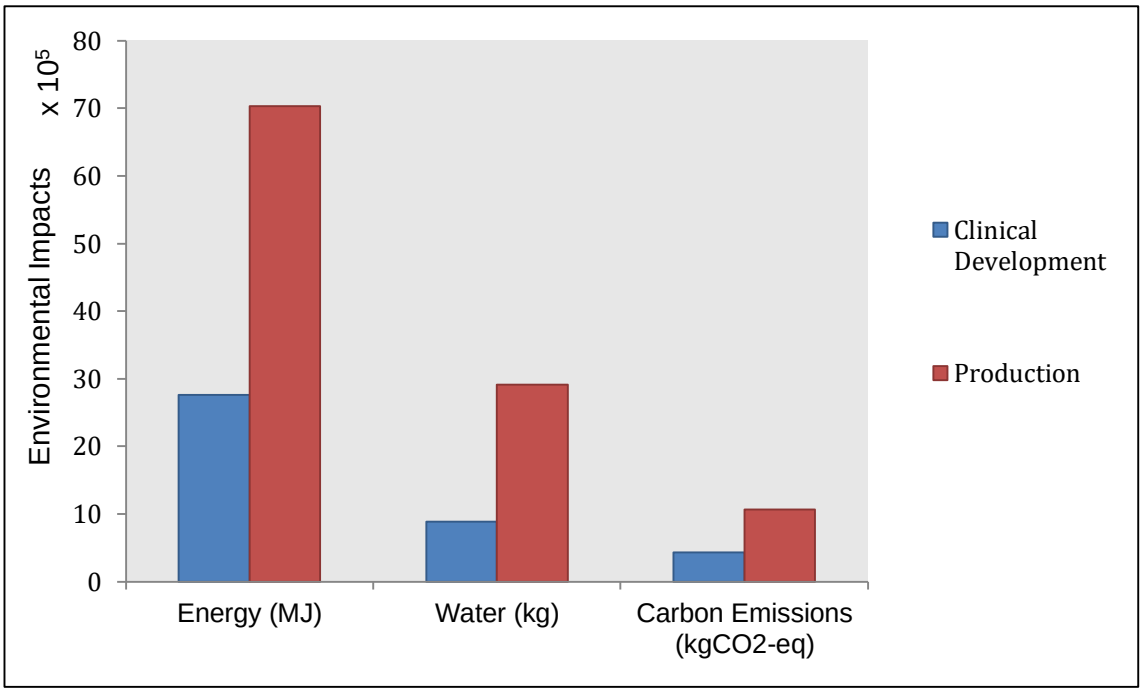


Figure 7.7 *Difference in environmental impacts between the clinical development phase and the production stage per annum when a 1000 L operational scale is employed for the manufacturing processes in the production stage.*

Figure 7.7 shows the differences in environmental impacts between the clinical development phase and the production stage when a 1000 L operational scale is employed for the manufacturing processes in the production stage. When compared with the original scenario, where scales of operation ranged from 1000 L-40, 000 L (Figure 7.4), the environmental impacts produced in Figure 7.7 are observed to be much lower. As shown in Figure 7.7 and earlier figures (Figures 7.5 and 7.6), the production stage contributes to higher levels of environmental impact than the

clinical development phase (when the impacts per annum are compared between these two stages).

The environmental savings achieved by opting for processes requiring lower operational scales at the production stage were estimated to be around 186 x 10^6 MJ of energy, 30 x 10^4 kg of water and 22 x 10^6 kgCO_2-eq of carbon per year. These savings clearly highlight the importance of process intensification and the environmental benefits it brings to this industry. Future manufacturing in this industry may hence be expected to take place in equipment with smaller footprints but improved levels of efficiency. Such operations will help improve the industry's environmental performance.

The next section (Section 7.6) will highlight the significance of the framework in the biopharmaceutical industry and its application in the industry's decision-making process.

7.5 Significance of the Framework Developed in the Biopharmaceutical Industry

As highlighted above, the framework based on the LCA methodology was deployed in this research to evaluate the environmental impacts produced in biopharmaceutical manufacturing. Using the framework developed, it was determined that incorporating single-use technologies in the biopharmaceutical manufacturing can be less environmentally impactful than the use of traditional process equipment, and employing pyrolysis as a solid waste management approach can help the industry to reduce the environmental impacts (especially water consumption) produced by solid

waste management activities. These findings clearly highlight that the framework developed in this research allows the industry to evaluate and compare the environmental impacts of different manufacturing and solid waste management strategies, and provides guidance to the industry to select technologies that are more environmentally favourable (low carbon technologies). This will help the industry to manufacture their products in the most sustainable fashion.

The use of the framework in evaluating the environmental impacts of monoclonal antibodies manufacture in the clinical development stage (Phase I, II and III) was also demonstrated. This clearly shows that the framework's scope is extensive, and it can be deployed by the industry to assess the environmental impacts produced in the clinical development stage for drug manufacture. This will guide the industry to employ lean manufacturing initiatives in the clinical development stage.

The findings clearly suggest that the framework provides a comprehensive method for environmental assessments in the biopharmaceutical industry. As sustainability concerns become more relevant in business decisions in the biopharmaceutical industry, the framework developed in this research can be helpful in the planning and maintenance of biopharmaceutical manufacturing. Ideally, the industry should deploy the framework during the initial process planning and development stages. Employing a comprehensive framework to carry out environmental studies along with process economic and efficiency studies during this stage can help the industry identify the most environmentally and economically feasible manufacturing strategy. Environmental studies at this stage can help the industry identify the key stages in the process where the environmental impacts are the greatest. This will enable the

industry to employ the right technologies to reduce the impacts at stages where the environmental impacts are high. Thus, using the framework early in the process planning and development stage can help the industry to design a sustainable manufacturing process that is less impactful to the environment.

The framework developed in this research can also be deployed by the industry to "green" their existing manufacturing processes. Using this framework, the industry can identify the environmental 'hot-spots' (stages/unit operations with high environmental impacts) in the manufacturing processes. The industry can then improve the environmental performance of the 'hot-spots' through replacing the energy-intensive technologies with technologies that are more environmentally favourable. This will help the industry with their lean manufacturing initiatives.

As a final analysis in this chapter, an investigation to determine if the biopharmaceutical industry is an EII was carried out. The following section (Section 7.6) determines whether the biopharmaceutical processes consume sufficient energy to be considered an EII. An industrial scale monoclonal antibody manufacturing process, based on mammalian cell culture was taken as representative of a biopharmaceutical manufacturing process. Monoclonal antibodies are the main and best-selling products of this industry.

7.6 Are Biopharmaceutical Processes Energy-Intensive?

As defined in the report by the CBI, the UK's premier lobbying organisation for business, EIIs are companies in the EU 2000 Regulation and Pollution Prevention and Control, and within the 2006 EU Energy Products Directive whose energy

intensity is more than 3% (i.e. energy costs must be 3% or more of their production costs) (CBI, 2011).

An analysis to determine whether biopharmaceutical processes are energy intensive was carried out in which the energy costs of a monoclonal antibody manufacturing process were compared with the production costs (see Table 7.8). The outcome of the analysis revealed that based on the definition provided above, the monoclonal antibody manufacturing processes are energy intensive since their energy costs are 7% of the total monoclonal antibody production costs.

Table 7.8 *Energy and production cost of monoclonal antibodies per annum.*

Type	Costs (£)
Production costs[1]	$\approx 54 \times 10^6$
Energy costs[2]	$\approx 4 \times 10^6$

[1]Production costs includes capital investment and cost of goods of a monoclonal antibody manufacturing process based on mammalian cell culture per annum. The costs were based for an industrial scale (10, 000 L operational scale) process with titre of 5g/L (Farid, 2007). A 10, 000 L process with a process titre of 5g/L, production batches of 15, and an overall DSP yield of 70% is estimated to produce around 250 kg of Mab per annum (Farid, 2007; Sinclair et al., 2008). [2]Energy costs (energy used in the supply, use and end-of-life phases by a monoclonal antibody manufacturing process) were determined based on the energy used per annum by a manufacturing process (process titre of 2 g/L) with an operation scale of 10, 000 L multiplied by the electricity costs per unit KWh for the UK (DECC, 2015).

Thus, as anticipated, the biopharmaceutical processes are energy intensive. The total level of energy consumption of biopharmaceuticals manufacture is likely to increase as the industry grows. Therefore, serious efforts should be taken to reduce the levels of environmental impact produced by this industry. The work in this thesis provides a level of guidance for the biopharmaceutical industry to evaluate and control the

levels environmental impact arising from the biopharmaceutical manufacturing processes.

7.7 Conclusion

This chapter provides the outcomes of a case study carried out to assess the cumulative environmental impacts of monoclonal antibodies manufacture in the clinical development phase and production for a period of time. The case study was carried out applying the framework developed in this doctoral project.

The outcomes of this case study provide crucial information on the cumulative environmental impacts of monoclonal antibodies manufacture. The outcomes obtained in this case study meet the objectives listed at the beginning of this chapter, which were;

a) To populate the cumulative environmental impacts of the production of monoclonal antibodies for the clinical development phase over a period of time.
b) To estimate the potential environmental savings that could be achieved when single-use technologies are introduced in the clinical development phase.
c) To compare the cumulative environmental impacts between the clinical development phase and production over a period of time.

The likely levels of environmental impact produced during the clinical development phase of monoclonal antibodies manufacture were observed to be greatest in the Phase III clinical study period.

This chapter also demonstrated that considerable levels of environmental savings were achieved in the clinical development phase when the industry shifted from the traditional route of manufacture to hybrid manufacture, where a mix of traditional fixed-in-place and disposable equipment were employed. Again, the highest levels of environmental savings were observed in the Phase III of the clinical study.

The analysis to meet the final objective of the case study focused on comparing the environmental impacts produced between the clinical development phase and production for monoclonal antibodies manufacture over a period of a year. This analysis highlighted that the production stage contributes higher levels of environmental impact than the combined clinical development phases per year. However, this analysis also highlighted that with an increasing focus on process intensification in the biopharmaceutical industry which is designed to improve the overall efficiency, as well as the levels of economic and environmental performance of biopharmaceutical processes, the extent of impact felt during the clinical development phase and the production stage will most likely decrease. This chapter also highlighted that process intensification could bring about major transformations to the biopharmaceutical industry, resulting in smaller and more flexible facilities. The results of the case study will provide decision-makers with crucial information required for the clinical development and production planning phases of monoclonal antibodies manufacture in order to reduce the overall levels of environmental impact produced. This in turn will help the industry to be a step closer towards achieving sustainable development.

Chapter 8. Conclusion and Future Work

8.1 Introduction

Increasing concerns about sustainable development and climate change amongst the public, investors, and international governments are driving many industries to focus more actively on the environmental contributions of their manufacturing processes. The biopharmaceutical industry is increasingly recognised as a contributor although this work has shown it to be less energy-intensive than other industries such as steel and chemical. Whilst the efficiency and benefits of biopharmaceutical manufacturing processes have been studied and compared through decision-support tools, the environmental impacts of these processes have not been the subject of such analysis. This chapter summarises the insights gained in this thesis to evaluate the environmental impacts of biopharmaceuticals manufacture as an aid in the decision-making process when assessing manufacturing and solid waste management alternatives. This chapter also provides recommendations and directions for future work that will further advance the understanding of these topics.

8.2 Overall Conclusions

The main focus of this work has been the design and implementation of a decision support framework that captures the environmental aspects of biopharmaceutical manufacture to enhance the quality of decision-making processes in the industry. The framework was used to evaluate the manufacture of monoclonal antibodies, using mammalian-based processes. A review of the manufacturing technologies and strategies currently implemented for monoclonal antibody production led to the definition of a single generic process for the subsequent analyses including for

developing comparative flow sheets; traditional and hybrid processes. As illustrated in Chapter 3, the main difference between the two flow sheets is that the traditional option employs entirely fixed-in-place stainless steel equipment whilst the hybrid option employs a mix of disposable and fixed-in-place stainless steel equipment.

One of the key aims of the research was to identify whether the monoclonal antibody manufacturing processes are energy intensive. Investigation revealed that the monoclonal antibody manufacturing processes are energy intensive since their energy costs are 7% of the total monoclonal antibody production costs. For a manufacturing process to be considered energy intensive, the energy costs of the process must be 3% or more of the production costs (CBI, 2011). The analysis was performed using the framework developed in this work.

The framework created and then deployed in this thesis allows the environmental aspects of biopharmaceutical manufacture to be addressed explicitly. The LCA tool was applied in the framework to carry out environmental assessments. The LCA tool was deemed appropriate to be used in this framework as this is the only tool that allows comprehensive studies of the entire life cycle of products, processes or activities. In this thesis, a number of assumptions (e.g. process titre) were made to carry out the environmental assessments. These assumptions were validated using expert knowledge. The framework was also deployed to identify the most suitable solid waste disposal option for the biopharmaceutical industry.

The use of the framework in evaluating the environmental impacts of biopharmaceuticals manufacture was demonstrated through a case study, presented

in Chapter 5, which used the framework to assess and to compare the environmental impacts of manufacturing alternatives and different solid waste treatment strategies when applied to a monoclonal antibody manufacturing process. The results of this case study identified the hybrid process as the more environmentally favourable option to manufacture monoclonal antibodies at a 200 L operational scale (Chapter 5). Employing the hybrid process option lowered the environmental impact of manufacture, especially the levels of water consumption. It was estimated that the hybrid process consumes around 43% less water than the traditional process. From this case study however, a single and generic solid waste management method suitable for the biopharmaceutical industry could not be identified. Thus, a separate study was carried out in which water consumption was used as an indicator by which to select the most appropriate solid waste management method for the biopharmaceutical industry. Using this indicator, it was identified that pyrolysis and incineration are the most suitable solid waste management methods as they consume the least water (it was estimated that incineration and pyrolysis solid waste management methods consume around 78% less water than the landfill method). These methods also offer energy recovery possibilities making them better alternatives to the landfill method.

Using this framework, it was identified that the use-phase contributes the highest levels of environmental impact for both the manufacturing processes, and the end-of-life phase contributes the lowest. The environmental framework was also used to identify the “hot spots” of the monoclonal antibody manufacturing processes at 200 L operational scale. The upstream, polishing and support equipment stages were identified as the “hot spots”. The lighting and HVAC, liquid waste treatment and

supply-chain activities contributed the most to the environmental impacts. This kind of information provided by the framework is crucial for the biopharmaceutical industry if it has as a goal to improvise the current manufacturing processes to be more energy efficient.

As highlighted before, a number of assumptions/parameters were used to carry out the environmental assessments. Sensitivity analysis studies were carried out (presented in Chapter 5) to assess critically the robustness of certain assumptions/parameters used in this work. These sensitivity analysis studies helped increase the levels of confidence in the predictions made in the study by identifying the process parameters/assumptions that have the most influence on the estimates made of overall environmental impacts. It was determined that the batch size of the manufacturing process is the most sensitive parameter and that employing the optimum number of batches for a manufacturing process will result in significant savings in the levels of environmental impact produced. The analysis also determined that transport is not a major contributor to the overall environmental impacts produced. Cumulatively, such information can be used by decision-makers to make improvements to monoclonal antibody manufacturing processes.

A hypothetical case study to populate the cumulative environmental impacts of monoclonal antibodies manufacture was presented in Chapter 7. This was an attempt to extend the use of the framework based on the LCA tool from evaluating the environmental impacts of monoclonal antibodies manufacture in the production phase to the clinical development phase, so as to capture accurately the total environmental impacts associated with monoclonal antibody development and

manufacture. An illustration of the environmental impacts comparison between the clinical development phase and manufacturing was provided. The chapter also highlighted the environmental savings that could be achieved in the clinical development phase of monoclonal antibody manufacture by employing single-use technologies. Ways to reduce the environmental impacts of monoclonal antibodies manufacture through process intensification of the manufacturing processes were also presented. This analysis demonstrated that up to 60% of savings in energy consumption (both in the clinical development and manufacturing) can be achieved through intensification of monoclonal antibody manufacturing processes.

The work in this thesis highlights the benefits of adopting a consistent engineering framework by which to capture the environmental aspects of biopharmaceutical manufacture. This has been realised through the design and application of an environmental framework based on the LCA tool. The outcomes of the thesis (Chapters 5, 6 and 7) can be used in the decision-making process to help guide the selection of manufacturing and disposal routes that are environmentally favourable. This in turn will help the industry to predict and to control their environmental performance.

8.3 Future Work

The systematic framework developed in this thesis is a contribution to the emerging area of environmental sustainability. It also provides a foundation for future work; several of such examples are discussed below.

In this thesis, only the primary processes (upstream and downstream manufacturing stages) were considered. The secondary processes (e.g. drug product manufacturing stage) were not included as it was beyond the scope of the research. Obtaining the life cycle inventory for the secondary processes will also create an additional challenge. However, including this stage will increase the level of detail of the research (making the research thorough and as exact as possible). Hence, the secondary processes should be included in the future work if the study aims to capture the complete environmental impacts of biopharmaceutical manufacture.

The environmental framework developed in this research was only applied to one type of biopharmaceutical manufacture-the monoclonal antibody manufacturing process. It would be useful to apply this framework to other biopharmaceutical manufacturing processes such as vaccines. The results could provide decision-makers with essential information on the whole life cycle impact of the biopharmaceutical industry. Also, this thesis reports only the complete environmental assessment for a monoclonal antibody manufacturing process. Comprehensive economic and social life cycle assessments are necessary for the sustainability evaluation of biopharmaceutical manufacture.

The environmental impacts considered in this thesis refer only to levels of energy consumption, water consumption, and carbon (GHGs) emissions, which are the most common measures of environmental performance. It would be beneficial to include other impact categories in the study, such as ozone depletion potential and acidification potential to determine other, and increasingly relevant, environmental

impacts. Presently, the availability of such data is very limited and often uncertain due to the application of varying measuring tools and reporting techniques.

In Chapter 5, it was demonstrated that water consumption can be used as an indicator to select the most appropriate solid waste management method. A more comprehensive method that can be considered by which to select the solid waste management method for the biopharmaceutical industry is by the application of a Multi-Attribute Decision Making (MADM) approach. This method is employed under situations where an alternative must be chosen based on a set of attributes and where the attributes might conflict. The MADM method deals with the process of making decisions in the presence of multiple objectives, and includes qualitative aspects of performance in the evaluation (Farid *et al*., 2005; Pohekar and Ramachandran, 2004). The most common approach in the MADM method is the additive weighting technique, which requires assigning weights to the attributes evaluated (approach is based on weighted average) (Farid *et al*., 2005; Afshari *et al*., 2010). The weights for the attributes are typically obtained by carrying out surveys involving a large group of decision-makers (e.g. policy makers and industrial experts). This tends to be complex and time consuming, and was hence felt to be outside the scope of this thesis. On the other hand, the method (using water consumption to select the most appropriate solid waste management method) proposed in this thesis offers a less complicated and quicker way of identifying the most appropriate solid waste disposal option based on the most pressing environmental problem currently faced by the biopharmaceutical industry; is simple to adopt and interpret.

The framework developed in this research was used to quantify the water footprint of biopharmaceuticals manufacture. However, the impact of water usage to the environment is presently unknown, and has never been explored in the biopharmaceutical industry. Thus, a study should be carried out to explore the effects of water impacts using formal impact assessment methodology, once they become available (methodology is currently under development). This would benefit an industry such as the biopharmaceutical industry (that consumes high levels of water in their production) to understand the environmental impacts from water consumption, and to identify sustainable methods/ways to reduce water consumption in the manufacturing.

The life cycle data used in this thesis ideally needs to be compiled and organised into a database. As noted previously, this database should be extended to incorporate life cycle data for different manufacturing processes besides monoclonal antibody manufacture. This will speed up LCA-related studies in the biopharmaceutical industry as the users can easily access the data needed for their analyses.

Optimisation studies are typically carried out in the biopharmaceutical industry to improve process performance and resource utilisation, and also reduce cost of goods. Investigation into the use of optimisation models to reduce further the environmental impacts should be also considered as future work. Optimisation studies can be carried out both on the upstream (e.g. studies on optimising the bioreactor sizing) and the downstream stages (e.g. studies on optimising the chromatography column sizing and the number of columns employed) (Liu *et al*., 2014).

Finally, the process intensification study presented in Chapter 7 could be expanded to include improvements to the DSP yield of manufacturing processes, process time reductions of unit operations, and deployment of continuous manufacturing strategies. This will help the industry to reduce the scales of operation of biopharmaceutical manufacture and also to lower the levels of environmental impact (analysis in Chapter 7 demonstrated that reduction in the scales of operation can lead to environmental savings), making the processes more flexible, efficient and environmentally sustainable; a very desirable set of goals for future generations.

As sustainability concerns become more relevant in business decisions, the evidence presented in this research can be helpful in planning for sustainable manufacturing of biopharmaceuticals. The detailed insights gained in this comprehensive study offer the potential for further improvements in environmentally conscious product and process developments for biopharmaceutical manufacturing. It is imperative to ensure that these findings are properly communicated to the biopharmaceutical industry through publishing the research findings in reputable journals that are widely recognised and utilised by the biopharmaceutical industry.

References

AbbVie, 2017. Medication Guide Humira.
http://www.rxabbvie.com/pdf/humira_medguide.pdf (accessed 09.11.2016).

ABPI, 2007. Guidelines for Phase I clinical trials.
http://www.abpi.org.uk/ourwork/library/guidelines/Documents/guidelines_phase1_clinical_trials.pdf (accessed 04.06.2015).

Accenture, 2012. Sustainable energy for all: Opportunities for the pharmaceutical and biotechnology industries. United Nations Global Impact.

Adams, G.P. and Weiner, L.M., 2005. Monoclonal antibody therapy of cancer. Nature biotech 23(9), 1147-115.

Afshari, A., Mojahed, M., Mohd Yussuf, R., 2010. Simple additive weighting approach to personnel selection problem. International J. of Innovation, Management and Tech. 1(5), 511-515.

AGARGEL, 2013. Energy.
http://www.agargel.com.br/agar-tec-en.html (accessed 02.01.2014).

Aggarwal, S., 2010. What's fueling the biotech engine-2009–2010. Nat. Biotechnol. 28 (11), 1165–1171.

Allison, N., Richard, J., 2014. Current status and future trends for disposable technology in the biopharmaceutical industry. J. of Chem. Tech and Biotech 89(9), 1283-1287.

Al-Salem, S., Lettieri, P., Baeyens, J., 2009. Recycling and recovery routes of plastic solid waste (PSW): A review. Waste Management 29, 2625–2643.

Andersen, J.K., Boldrin, A., Christensen, T.H., Scheutz, C., 2012. Home composting as an alternative treatment option for organic household waste in Denmark: An environmental assessment using life-cycle assessment-modelling. Waste Management 32, 31-40.

Andersen, D.C., Reiley, D.E. Production technologies for monoclonal antibodies and their fragments. Current Opinion Biotechnol. 15(5), 456-462.

ANON, 2012a. Electric car site.
http://www.electriccarsite.co.uk/ (accessed 11.11.2013).

ANON, 2012b. Toyota website.
https://www.toyota.co.uk/ (accessed 11.11.2013).

Azapagic, A., 1999. Life cycle assessment and its application to process selection, design and optimisation. Chem. Eng. J. 73(1), 1-21.

Baumann, H., Tillman, A., 2004. The Hitch Hiker's Guide to LCA, 1st ed. Studentlitteratur, Lund.

Beaver, E., 2000. LCA and total cost assessment. Environ. Prog. 19(2).

Bebbington, J., Brown, J., Frame, B., 2007. Accounting technologies and sustainability assessment models. Ecological Economics, 61, 224-236.

Becker, M., 2010. Monoclonal antibody companies command premiums.
http://seekingalpha.com/article/214040-monoclonal-antibody-companies-command-premiums (accessed 23.08.2014).

Bernstad A., la Cour Jansen, J., 2012. Review of comparative LCAs of food waste management systems – Current status and potential improvements. Waste Management 13-12, 2439-2455.

Biopharma Cold-Chain Sourcebook, 2012. Logistics protects-bringing more control to temperature-sensitive logistics.
http://www.ups.com/media/en/Cold_Chain_CRT_ebook.pdf (accessed 03.02.2015).

Birch, J.R., Racher, A.J., 2006. Antibody production. Advanced Drug Delivery Reviews 58(5-6), 671-685.

Bjorklund A.E., 2002. Survey of approaches to improve reliability in LCA. Int. J. LCA 7(2), 64-72.

Blue Spring, 2011. WFI-R series double-pass reverse osmosis systems. http://www.bluspr.com/reverse_osmosis_wfi.html (accessed 08.10.2011)

Bonollo, F., Carturan, I., Cupito, G. and Molina, R. Life cycle assessment in the automotive industry: Comparison between aluminum and cast iron cylinder block. Metallurgical Science and Tech. 3, 1-25.

Boyd, J.H., 2005. By the numbers: What is costs to operate a biopharmaceutical facility. BioPharm International 18(10).

Brander, M., Tipper, R., Hutchison, C., Davis, G., 2008. Consequential and attributional approaches to LCA: a guide to policy makers with specific reference to greenhouse gas LCA of biofuels: Technical Paper. http://ecometrica.com/assets/approachesto LCA3 technical.pdf (accessed 15.12.12).

Bredveld, F., 2000. Therapeutic monoclonal antibodies. The Lancet 355 (9205), 735-740.

Brownsort, P. A., 2009. Biomass pyrolysis processes: Review of scope, control and variability. http://www.biochar.org.uk/(accessed 08.06.2013).

Buchholz, S., 2010. Future manufacturing approaches in the chemical and pharmaceutical industry. Chem. Eng. and Processing 49, 993–995.

Bullock, S., 2009. Energy-intensive industry and climate change http://www.foe.co.uk/resource/briefings/energy intensive industry.pdf (accessed 4.01.13).

Cain, A., Disch, S., Twaroski, C., Reindl, J., Case, C.R., 2007. Substance flow analysis of mercury intentionally used in products in the United States. J. of Industrial Ecology 11(3), 61-75.

Carbon Independent, 2015. Home energy sources. http://www.carbonindependent.org/sources_home_energy.html (accessed 09.05. 2015).

CBI, 2011. Protecting the UK's foundation. A blue print for energy intensive industries.

http://www.cbi.org.uk/media/1057969/cbi_eii_report_0811.pdf (accessed 07.01.2015)

CC Water, 2015. Average water use.

http://www.ccwater.org.uk/savewaterandmoney/averagewateruse/ (accessed 09.05.2015).

Chadd, H.E., Chamow, S.M., 2001. Therapeutic antibody expression technology. Current opinion in biotech 12(2), 188-194.

Challener, C., 2014. Biopharma moves to integrated, single-use, downstream processing. Biopharm Int. 27(6).

Chew, C.M., Aroua, M.K., Hussain, M.A., Was Ismail, W.M., 2014. Practical performance analysis of an industrial-scale ultrafiltration membrane water treatment plant. Journal of the Taiwan Institute of Chemical Engineers 46, 132-139.

Chu, L., Robinson, D.K., 2001. Industrial choices for protein production by large scale culture. Current Opinion in Biotech. 12(2), 180-187.

CIPS, 2007. How to develop a waste management and disposal strategy.

https://www.cips.org/ (accessed 23.11.2013)

Clift, R., Doig, A., Finnveden, G., 2000. The application of life cycle assessment to integrated solid waste management: Part 1 –Methodology. Trans IChemE Part B, Proc. Safety Environ. Prot.78, 279–287.

Cochet, O., Corbiere, J., Sinclair, A., Monge, M., Brown, A., Geraldine, E., 2013. A sustainable, single-use facility for monoclonal antibody production. BioProcess International.

Cohen, S., Demeritt, D., Robinson, J. and Rothman, D., 1998. Climate change and sustainable development: towards dialogue. Global Environmental Change 8(4), 341-371.

Collier, R., 2009. Drug development cost estimates hard to swallow. CMAJ 180 (3), 277-278.

Curran, M.A., 2006. Life cycle assessment: Principles and practice. EPA Report. http://brevard.ifas.ufl.edu/communities/pdf/chapter1_frontmatter_lca101.pdf (accessed 12.12.2017)

Curran, M.A., Mann, M. and Norris, G., 2005. The international workshop on electricity data for life cycle inventories. J. of Cleaner Produc. 13(8), 853-862.

Da Benedetto, L., Klemes, J., 2009. The environmental performance strategy map: an integrated LCA approach to support the strategic decision-making process. J. of Cleaner Produc. 17(10), 900-906.

Dautzenburgh, F.M., Mukherjee, M., 2001. Process intensification using multifunctional reactors. Chem. Eng. Science 56, 251-267.

DEAT, 2004. Life cycle assessment. https://www.environment.gov.za/sites/default/files/docs/series9_lifecycle_assessment.pdf (accessed 03.04.2012).

DECC, 2012. The renewables obligation (RO). http://www.decc.gov.uk/ (accessed 24.09.2013)

DEFRA, 2014. UK Government conversion factor for Company Reporting. http://www.ukconversionfactorscarbonsmart.co.uk/ (accessed 30.01.2014).

DEFRA, 2010. A strategy for hazardous waste management in England. http://archive.defra.gov.uk/environment/waste/topics/hazwaste/documents/policy.pdf (accessed 08.08.2013).

Deloitte, 2014. High value, high uncertainty: Measuring risk in biopharmaceutical research and other industries. file:///D:/Downloads/gx-lshc-measuring-risk-in-biopharmaceutical-research.pdf (accessed 09.11.2014).

Dimasi, J.A., Grabowski, H.A., 2007. The cost of biopharmaceutical R&D: Is biotech different?.Managerial and Decision Economics 28, 469-479.

Donaldson, 2013. Surface filtration versus depth filtration. http://www.tetratexlatam.com/literatura/filtracionprofunda.pdf (accessed 01.09.2014).

Doran, P.M., 1995. Bioprocess engineering principles (1st ed.). Academic Press, Oxford, UK.

Drexhage, J., Murphy, D., 2012. Sustainable development: From brundtland to Rio 2012. United Nations Headquarters, New York.

Dunn, P., Wells, A., Williams, M.T., 2010. Green chemistry in the pharmaceutical industry. John Wiley & Sons, Inc., Hoboken, New Jersey.

Ecker, D.W., Dana Jones, S., Levine, H., 2015. The therapeutic monoclonal antibody market. mAbs 7(1), 9-14.

Ecotricity, 2015. UK Grid Live.
https://www.ecotricity.co.uk/our-green-energy/energy-independence/uk-grid-live (accessed on 25.05.2015).

EEA and Norwegian Financial Mechanism, 2006. Sustainable Development Policy and Guide.

EEA, 1998. Life cycle assessment (LCA): a guide to approaches, experiences and information sources. European Environment Issues Series No.6, European Environment Agency, Copenhagen.

Eibl, R., Kaiser, S., Lombriser, R., Eibl, D., 2010a. Disposable bioreactors: the current state-of-the-art and recommended applications in biotechnology. Appl. Microbiol. Biotechnol. 86(1), 41–49.

Eibl, D., Peuker, T., Eibl, R., 2010b. Single-use equipment in biopharmaceutical manufacture: A brief introduction, in single-use technology in Biopharmaceutical Manufacture (eds R. Eibl and D. Eibl), John Wiley & Sons, Inc., Hoboken, NJ, USA.

EMA, 2011. Assessment report for Lucentis. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000715/WC500107807.pdf (accessed 17.12.2014).

EMC, 2016. Lucentis 10mg/ml solution for injection.
https://www.medicines.org.uk/emc/medicine/19409 (accessed 17.12.2016)

Environment Agency, 2012. Waste incineration directive.
http://www.environment-agency.gov.uk/ (accessed 06.06.2013).

EPA, 2010. Climate change indicators in the United States.
http://epa.gov/climatechange/indicators/pdfs/ClimateIndicators_full.pdf (accessed 16.12.2011).

Eriksson, O., Carlsson Reich, M., Frostell, B., Bjorklund, A., Assefa, G., Sundqvist, J.O., Granath, J., Baky, A., Thyselius, L., 2015. Municipal solid waste management from a systems perspective. J. of Cleaner Production 13(3), 241-252.

Eurofer, 2012. Stainless steel roofing systems. Eco-Design Package.

European Commission, 2014. The Lima Climate Change Conference.
http://ec.europa.eu/clima/events/articles/0098_en.htm (accessed 22.02.2015).

European Commission, 2009. The financing of biopharmaceutical product development in Europe.
http://ec.europa.eu/enterprise/sectors/biotechnology/files/docs/financing_biopharma_product_dev_en.pdf (accessed 23.04.2014).

Farid, S.S., 2011. Economic drivers and trade-offs in antibody purification processes. BioPharm Int., 37-42.

Farid, S.S., 2007. Process economics of industrial monoclonal antibody manufacture. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 848 (1), 8–18.

Farid, S.S., 2006. Established bioprocess for producing antibodies as a basis for future planning. Advanced Biochem. Eng. Biotechnol. 101, 1-42.

Farid, S.S., Washbrook, J., Titchener-Hooker, N.J., 2005.Decision-support tool for assessing biomanufacturing strategies under uncertainty: stainless steel versus disposable equipment for clinical trial material preparation. Biotechnol. Prog. 21(2), 486–497.

FDA, 2013. Humira.
http://www.fda.gov/ohrms/dockets/ac/03/briefing/3930B1_02_B-Abbott-Humira%20Prescribing%20Info.pdf (accessed 17.12. 2014).

Feldon, J., 2007. The Black Hole in the Kyoto Protocol: Was the Exclusion of Black Carbon Regulation a" Fatal Flaw"? Sustainable Develop. Law & Policy 7(2), 24.

Ferrer-Miralles, N., Domingo-Espin, J., Corchero, J., Vazquez, E.,Villaverde, A., 2009. Microbial factories for recombinant pharmaceuticals. Microbial Cell Factories 8(1), 17.

FHC, 2012.Fujifilm sustainability report 2012.
http://www.fujifilm.com/sustainability/report/pdf/index/ff_sr_2012_en_all.pdf (accessed January, 2015).

Fiksel, J., 2010. Evaluating Supply Chain Sustainability. Chem. Eng. Prog. 106(5), 28.

Finnveden, G., Hauschild, M.Z., Ekvall, T., Guinee, J., Heijungs, R.,Hellweg, S., Koehler, A., Pennington, D., Suh, S., 2009. Recent developments in life cycle assessment. J. Environ. Manage. 91(1), 1–21.

Finnveden, G., 2008. A world with CO_2 caps. The Int. J. of Life Cycle Assessment, 13(5), 365-367.

Frankl, P., Rubik, F., 1999. Life-cycle assessment (LCA) in business. An overview on drivers, applications, issues and future perspectives. Global Nest: the Int. J. 1(3), 185-194.

Freshfields Bruckhaus Deringer, 2010. Efficient routes to CO2 reduction.
http://www.freshfields.com/publications/pdfs/2010/oct10/28876.pdf (accessed 07.09.2013).

GaBi, 2014. GaBi 6.0 Product Sustainability software and database. PE International.

Gagnon, P., 1995. The secret life of Protein A. BioProcess International.
http://www.bioprocessintl.com/downstream-processing/chromatography/the-secret-life-of-protein-a/ (accessed 12.12.2017).

Gameiro, D.N., 2016. The top 10 best-selling monoclonal antibodies. http://labiotech.eu/top-10-best-selling-biologicals-blockbusters-2015/ (accessed 10.12.2016)

GE Healthcare, 2012a. AxiChrom colums. http://www.gelifesciences.co.jp/catalog/pdf/28929041aj.pdf (accessed 02.01.2013)

GE Healthcare, 2012b. Wave bioreactor. https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314807262343/litdoc28952058_20120713211914.PDF (accessed 19.01.2013)

GE Healthcare, 2016a. Fixed stainless steel chromatography energy consumption. https://www.gelifesciences.com/shop/chromatography/columns/process-columns/axichrom-300-to-1600-mm-chromatography-columns-p-05981 (accessed 19.01.2013).

GE Healthcare, 2016b. Wave bioreactor system 500/1000. https://www.gelifesciences.com/shop/cell-culture-and-fermentation/rocking-bioreactors/systems/wave-bioreactor-system-500-1000-p-05684 (accessed 13.12.2017)

Genentech, 2015. Lung Cancer: Dosing and usage. http://www.avastin-hcp.com/indications/nsclc/dosing-usage#dose_and_duration (accessed 01.05.2015).

Gidding, B., Hopwood, B., O'Brien, G., 2002. Environment, economy and society: Fitting them together into sustainable development. Wiley Inter-Science 10, 187 - 196.

Gill, N.K., Appleton, M., Baganz, F., Lye, G., 2008. Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up. Biotechnology and Bioengineering 100(6), 1144-1155.

Gollaher, D., Claude, P., 2013. 2014 California biomedical industry medical report. https://www.pwc.com/en_US/us/health-industries/pharma-life-sciences/publications/assets/pwc-california-biomedical-industry-2013.pdf (accessed 03.04. 2014).

Gorter, A., Van De Griend, R.J., Van Eendenburg, J.D., Haasnoot, W.H., Fleuren, G.J.,1993. Production of bi-specific monoclonal antibodies in a hollow-fibre bioreactor. J. Immunol. Methods 161, 145-150.

Gronemeyer, P., Ditz, R., Strube, J., 2014. Trends in upstream and downstream process development for antibody manufacturing. Bioengineering 1, 188-212.

Guinee, J.B., Gorree, M., Heijungs, R., Huppes, G., Kleijn, R., Van Oers, L., Wegener Sleeswijk, A., Suh, S., Udo de Haes, H.A., De Bruijn, H., Van Duin, R., Huijbregts., 2012. Life cycle assessment: An operational guide to the ISO standards. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Guinee, J.B., Heijungs, R., 2005. Kirk-Othmer encyclopedia of chemical technology. John Wiley & Sons, Hoboken, New Jersey.

Guldager, N., 2009. Next-Generation facility for monoclonal antibody production. Pharmaceutical Tech. 33(7), 68-73.

Hamer, G., 2003. Solid waste treatment and disposal: effects on public health and environmental safety. Biotechnol. Advances 22, 71-79.

Heaven, S., 2011. Disc stack centrifugation and sell disruption of microalgae: A technical note. Environment and Natural Resources Research 1(1), 17-24.

Hill, C., Mcdonagh, G., Brown, A., 2010a. BioSolve Reference Manual. Biopharm Software Solutions.

Hood, E.E., Woodard, S.L., Horn, M.E., 2002. Monoclonal antibody manufacturing in transgenic plants — myths and realities. Current opinion in biotechnology 13(6), 630-635.

Ho, S.V., McLaughlin, J.M., Pollock, J., Farid, S.S., 2011. Towards greener therapeutic proteins. In: Tao, J., Kazlaukas, R, editor. Biocatalysis for Green Chemistry and Chemical Process Development. John Wiley & Sons, Inc., Hoboken, New Jersey.

Huggett, B., 2013. Public biotech 2013-the numbers. Nature Biotech. 31, 697-703.

Humphreys, D.P., 2003. Production of antibodies and antibody fragments in Escherichia coli and a comparison of their functions, uses and modification. Current Opinion Drug Discovery Develop. 6(2), 188-196.

ICCA, 2012. Responsible care.
http://www.icca-chem.org/en/Home/Responsible-care/ (accessed 09.10.2012).

ICCA, 2009. Innovations of greenhouse gas reductions.
http://www.icca-chem.org/ICCADocs/ICCA_A4_LR.pdf (accessed 09.08. 2012).

IPPC, 2007. Climate change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York.

ISO, 2006a. ISO 14040 Environmental management- Life cycle assessment- Principles and frameworks. Geneva: ISO.

ISO, 2006b. ISO 14044 Environmental management – Life cycle assessment- Requirements and guidelines. Geneva: ISO.

Jain, E., Kumar, A., 2008. Upstream processes in antibody production: Evaluation of critical parameters. Biotech. Advances 26, 46-72.

Janssen Biotech, 2016. Remicade.
http://www.remicadehcp.com/ (accessed 17.12.2016)

Jayal, A.D., Badurdeen, F., Dillon Jr, O.W., Jawahir, I.S., 2010. Sustainable manufacturing: Modeling and optimization at the product, process and system levels. CIRP J. of Manufacturing Science and Tech. 2, 144-152.

Jenck, J.F., Agterberg, F., Droescher, M.J., 2004. Products and processes for a sustainable chemical industry: a review of achievements and prospects. Green Chem. 6(11), 544-556.

Jeswani, H.K., Azapagic, A., Schepelmann, P., Ritthoff, M., 2010. Options for broadening and deepening the LCA approaches. Journal of Cleaner Production, 18, 120-127.

Jimenez-Gonzalez, C., Pondert, C., Broxterman, Q., Manley, J., 2011. Using the right green yardstick: Why process mass intensity is used in the Pharmaceutical industry to drive more sustainable processes. American Chemical Society 15(4), 912-917.

Johnston, R., 2010. The dinosaur reborn-evaluating stainless steel and disposables in large scale biomanufacturing. Bioprocess Int., 28-32.

Jolliet, O., Margni, M., Charles, R., Humbert, S., Payet, J., Rebitzer, G., Rosenbaum, R., 2003. IMPACT 2002: A new life cycle impact assessment methodology. The Int. J. of Life Cycle Assess. 8(6), 324-330.

Kara, S., Manmek, S., Herrmann, C., 2010. Global manufacturing and the embodied energy of products. CIRP Annals-Manufacturing Technol 59, 29-32.

Kelley, B., 2009. Industrialization of mAb production technology. The bioprocessing industry at a crossroad. *mAbs* 1.5, 443–452.

Kelley, B., 2007. Very large scale monoclonal antibody purification: the case for conventional unit operations. Biotech. Progress 23(5), 995-1008.

Kissinger, M., Rees, W.E., 2010. An interregional ecological approach for modelling sustainability in a globalizing world- Reviewing existing approaches and emerging directions. Ecological Modelling, 221, 2615-2623.

Kjaerulf, U., van der Gaag, L.C., 2000. Making sensitivity analysis computationally efficient. Uncertainty In Artificial Intelligence Proceedings.

Kovarcik, D.P., 2016. Critical factors for fill-finish manufacturing of biologics. BioProcess Int. http://www.bioprocessintl.com/manufacturing/fill-finish/critical-factors-for-fill-finish-manufacturing-of-biologics/ (accessed 12.12.2017)

KPMG, 2010. The transformation of the automotive industry: The environmental regulation effect. https://www.kpmg.com/US/en/IssuesAndInsights/ArticlesPublications/Documents/transformation-automotive-industry.pdf (accessed 16.06.2014).

Langer, E.S., Price, B.J., 2007. Biopharmaceutical disposables as a disruptive future technology. BioPharma Int. 20(6).

Lash, J., Wellington, F., 2007. Competitive advantage on a warming planet. http://capitalmarketspartnership.com/ (accessed 18.12.13).

Lashof, D.A., Ahuja, D.R., 1990. Relative contributions of greenhouse gas emissions to global warming. Nature 344, 529-531.

Lemly, A.D., 1997. PROFILE: Risk assessment as an environmental management tool: Considerations for freshwater wetlands. Environ. Management 21(3), 343-358.

Levine, H.L., Lilja, J.E., Stock, R., Hummel, H., Dana Jones, S., 2012. Efficient, flexible facilities for the 21st century. Bioprocess Int. 10(11), 20-30.

Li, F., Vijayasankaran, N., Shen, A., Kiss, R., Amanullah, A., 2010. Cell culture processes for monoclonal antibody production. MAbs 2(5), 466-477.

Li, F., Zhou, J.X., Yand, X., Tressel, T. and Lee, B., 2005. Current therapeutic antibody production and process optimization. BioProcessing J., 1–8.

Lim, J.A.C., Sinclair, A., Kim, D.S., Gottschalk, U., 2007. Economic benefits of single-use membrane chromatography in polishing—a cost of goods model. BioProc. Int., 48–56.

Lin, T.J., 1978. Low-energy emulsification. Principles and applications. J.Soc. Cosmet. Chem. 29, 117-125.

Linacre, N.A., 2005. Strategic environmental assessment: assessing the environmental impact of biotechnology.
http://www.lacbiosafety.org/wp-content/uploads/2011/09/assessing-the-environmental-impact-of-biotechnology1.pdf (accessed 26.05.2014).

Lipman, N.S., Jackson, L.R., Trudel, L.J. and Weis-Garcia, F., 2005. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. Ilar J. 46(3), 258.

Liu, J., 2014. The history of monoclonal antibody development-Progress, remaining challenges and future innovations. Ann Med Surg (London) 3(4), 113-116.

Lombardi, L., Carnavale, E., Corti, A., 2015. A review of technologies and performances of thermal treatment systems for energy recovery from waste. Waste Management 37, 26-44.

Low, D., O'Leary, R. PUJAR, N.S., 2007. Future of antibody purification. J. of chroma. B 848(1), 48-63.

Maggon, K., 2011. New drug approvals FDA 2011.
http://knol.google.com/k/krishan-maggon/new-drug-approvals-fda-2011/ (accessed 03.09.2014).

Makrides, S.C., 1996. Strategies for achieving high-level expression of genes in Escherichia coli. Microbiol Rev 60, 512-538.

Manfredi, S., Scharff, H., Jacobs, J., Christensen, T.H., 2009. Environmental assessment of low-organic waste landfill scenarios by means of life-cycle assessment modelling (EASEWASTE). Waste Management & Research. 2010, 130-140.

Martins, M.L., Mata, T.M., Martins, A.A., Neto, B., Costa, C.A.V., Salcedo, C.V.R., 2010. LCA tool adaptations to pharmaceutical processes.
http://www.ecopharmabuilding.com/ (accessed 27.01.13).

Mata, T., Neto, B., Martins, A.., Costa, C., 2012. LCA Tool for Sustainability Evaluations in the Pharmaceutical industry. Chemical Engineering Transactions 26, 261-266.

Mathiesen, B.V., Munster, M., Fruergaard, T., 2009. Uncertainties related to the identification of the marginal energy technology in consequential life cycle assessments. J. Cleaner Prod. 17, 1331–1338.

Mauter, M., 2009. Environmental life-cycle assessment of disposable bioreactors. BioProc. Int. 7 (4), S18–S29.

McGiness, S., 2001. Climate Change and Kyoto Protocol.
http://www.parliament.uk/documents/commons/lib/research/rp2001/rp01-106.pdf (accessed 14.01.2012).

Mckibben, W.J., Wilcoxen, P.J., 2002. The role of economics in climate change policy. The j. of econ. Perspectives 16(2), 107-129.

Meade, H.M., Echelard, Y., Ziomek, C.A.,Young, M.W., Harvey, M., Cole, E.S., Groet, S., Smith, T.E., Curling, J.M., 1998. Expression of recombinant proteins in the milk of transgenic animals. In: Fernendez, J.M., Hoeffler, J.P. (Eds.), Gene Expression Systems: Using Nature For The Art of Expression, *Academic Press*, San Diego.

Mease, P.J., Kivitz, A.J., Burch, F.X., Siegal, E.L., Cohen, S.B., Ory, P., Salonen, D., Rubenstein, J., Sharp, J.T., Dunn, M., Tsuji, W., 2006. Continued inhibition of radiographic progression in patients with psoriatic arthritis following 2 years of treatment with etanercept. J. Rheumatol 33(4), 712-721.

MedImmune, 2014. Synagis dosage.
https://www.synagis.com/content/dam/website-services/us/308-synagis-com/SPP/Pdf/synagis.pdf (accessed 12.12.2016).

Mehta, S.S., 2008. Commercialising successful biomedical technologies. Cambridge University Press, Cambridge.

Miettinen, P., Hamalainen, R.P., 1997. How to benefit from decision analysis in environmental life cycle assessment(LCA). Eur. J. Oper. Res. 102 (2), 279–294.

Millbank, P., 2013. Stainless steel faces subdued global growth. Insight 31, 28-31.

Milledge, J.J., Haven, S., 2011. Disc stack centrifugation separation and cell disruption of microalgae: A technical note. Environment and Natural Resources Research 1(1), 17-24.

Mirasol, F., 2008. Disposable bioreactor use grows in commercial production. http://www.icis.com/ (accessed 07.0.2012).

Møller, J., Boldrin, A., Christensen, T.H. 2009. Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contributions. Waste Management & Research 27, 813-824.

Monte, M.C., Fuente, E., Blanco, A., Negro, C., 2009. Waste management from pulp and paper production in the European Union. Waste Management 29(1), 293–308.

Moran, E., Heenan, M., McGowan, S., Cullen, S., Lobby, M., 2005. Biowaste management during biopharmaceutical plant start-up: From regulatory guidance to verified inactivation methods. BioPharm. Int. 18(10).

Morrisey, A.J., Browne, J., 2004. Waste management models and their application to sustainable waste management. Waste Management 24(3), 297-308.

Morris, J.R., Philips, P.S., Read, A.D., 1998. The UK landfill tax: an analysis of its contribution to sustainable waste management. Resources, Conservation and Recycling 23, 259-270.

Morrow, D., Rondinelli, D., 2002. Adopting corporate environmental management systems: motivations and results of ISO 14001 and EMAS certification. Eur. Manage. J. 20(2), 159–171.

Muchova, L., Eder, P., 2010. End-of-waste criteria for iron and steel scrap. Technical Proposal. JRC Scientific and Technical Reports.

Nelson, A.L., Dhimolea, E., Reichert, J.M., 2010. Development trends for human monoclonal antibody therapeutics. Nature Reviews 9, 767-774.

Nelson, A.L., Reichert, J.M., 2009. Development trends for therapeutic antibody fragments. Nat. Biotechnol. 27(4), 331-337.

Nesta, D., Puri, M., Morar-Mitrica, S., Crotts, G., 2015. Evaluating Freeze–Thaw Processes in Biopharmaceutical Development – Small-Scale Study Designs. BioProcess International.
http://www.bioprocessintl.com/manufacturing/fill-finish/evaluating-freeze-thaw-processes-biopharmaceutical-development-small-scale-study-designs/ (accessed 12.12.2017).

Nielsen, P.H., Oxenboll, K.M., Wenzel, H., 2006. Cradle-to-gate environmental assessment of enzyme products produced industrially in Denmark by Novozymes A/S. The Int. J. of LCA 12 (6), 432–438.

NIH, 2011. A safety and efficacy study for Inflixmab in patients with active ulcerative colitis.
https://clinicaltrials.gov/ct2/show/NCT00036439 (accessed 11.12.2017).

Nireesha, G.R., Divya, L., Sowya, C.,Venkateshan, N., Babu, M., Lavakumar, V., 2013. Lyophilization/freeze drying- A Review. IJNTPS 3(4), 2277-2782.

Newman, D., Isner, V., 2008. Environmental considerations for bio manufacturing Processes. French Industri Pharma page numbers not available 32.

Noorgate, T.E., 2004. Metal recycling; An assessment using life cycle energy consumption as a sustainability indicator. CSIRO Report.

Oberthur, S., Ott, H.E., 1999. The Kyoto Protocol. International climate change for the 21st century. Springer, Berlin; Heidelberg; New York.

OECD, 1998. Biotechnology for clean industrial products and processes: Towards industrial sustainability. http://dbtbiosafety.nic.in/guideline/OACD/Towards_Industrial_Sustainability.pdf (accessed 13.02.2013).

Ofgem, 2014. Renewables obligation: Guidance for generators 2014. https://www.ofgem.gov.uk/ofgem-publications/87997/renewablesobligation-guidanceforgenerators1june2014.pdf (accessed 10.02.2013).

Pall Life Sciences, 2006. Centramate 500 S tangential flow filtration system. http://www.pall.com/pdfs/Biopharmaceuticals/USD2448_-_Centramate_500S_brochure.pdf (accessed 05.03.2012)

Palmer, J., Cooper, I., 2012. United Kingdom housing fact file. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/201167/uk_housing_fact_file_2012.pdf (accessed 07.12.2014).

Pandey, D., Agrawal, M., Pandey, J.S., 2010. Carbon footprint: current methods of estimation. Environ. Monit. Assess., 1–26.

Parkes, O., Lettieri, P., Bogle, D., 2015. Life cycle assessment of integrated waste management systems for alternative legacy scenarios of the London Olympic park. Waste Management 40, 157-166.

Patriarca, P., 2007. Use of cell lines for the production of influenza virus vaccines: an appraisal of technical, manufacturing, and regulatory considerations. Immunization, Vaccines and Biologics, WHO.

Pennington, D., Potting, J., Finnveden, G., Lindeijer, E., Jolliet, O.,Rydberg, T., Rebitzer, G., 2004. Life cycle assessment. Part 2: Current impact assessment practice. Environ. Int. 30 (5), 721–739.

Petrides, D., Siletti, C., Jimenez, J., Psathas, P., Mannion, Y., 2011. Optimizing the design and operation of fill-finish facilities using process simulation and scheduling tools. Pharmaceutical Engineering 31(2), 1-10.

Pharmahorizons, 2001. The biopharmaceutical industry: Overview, prospect, and competitive challenges.
http://www.iyfnmi.com/?dn=pharmahorizons.com&pid=9POA0QGY6 (accessed 09.09.2013).

Pierce, L.N., Shabram, P., 2004. Scalability of a disposable bioreactor from 25L-500L run in perfusion mode with a CHO-based cell line: A tech review. BioProc. J., 3(4), 51.

Pietrzykowski, M., Flanagen, W., Pizzi, V., Brown, A., Sinclair, A., Monge, M., 2013. An environmental life cycle assessment comparison of single-use and conventional process technology for the production of monoclonal antibodies. J. Cleaner Prod. 41, 50–162.

Pietryzykowski, M., Flanagen, W., Pizzi, V., Brown, A., Sinclair, A., Monge, M., 2011. An environmental life cycle assessment comparing single-use and conventional process technology. BioPharm Int. Supplements, 30-38.

Plastics Europe, 2012. An analysis of European plastics production, demand and waste data for 2011.
http://www.plasticseurope.org/documents/document/20121120170458-final_plasticsthefacts_nov2012_en_web_resolution.pdf (accessed 17.12.2014).

Pohekar, S.D., Ramachandran, M., 2004. Application of multi-criteria decision making to sustainable energy planning-A review. Renewable and Sustainable Energy Reviews 8, 365-381.

Pollock, J., Ho, S.V., Farid, S.S. 2013. Fed-batch and perfusion culture processes: economic, environmental and operational feasibility under uncertainty. Biotechnol. Bioeng. 110(1), 206-219.

Pollock, D.P., Kutzko, J.P., Birck-Wilson, E., Williams, J.L., Echelard, Y., Meade, H.M., 1999. Transgenic milk as a method for the production of recombinant antibodies. Journal of Immunological Methods 231(1-2), 147-157.

POST, 2012. Low carbon technologies for energy-intensive industries. http://www.icheme.org/akf (accessed 26.11.12).

Puettmann, M.E., Wilson, J.B., 2005. Life cycle analysis of wood products: Cradle-to-gate LCI of residential wood building materials. Wood and Fiber Science, 37 Corrim Special Issue, 18 – 29.

Puppo, F., Murdaca, G., Ghio, M. and Indiveri, F., 2005. Emerging biologic drugs for the treatment of rheumatoid arthritis. Autoimmunity Reviews 4, 537– 54.

Rabou, L., Zwart, R., Vreudenhil, B., Bos, L., 2009. Tar in biomass producer gas, the energy research centre of the Netherlands (ECN) experience-an enduring challenge. Energy Fuels 23, 6189-6198.

Ramasamy, S.V., Titchener-Hooker, N.J., Lettieri, P., 2015. Life cycle assessment as a tool to support decision-making in the biopharmaceutical industry: Consideration and challenges. Food and Bioproducts Proc. 9, 297-305.

Rathore, A.S., Sengar, T., 2011. Achieving process intensification by scheduling and debottlenecking biotech processes. Biopharm Int. 24(2).

Rathore, A.S., Rajan, R.S. Current perspectives on stability of protein drug products during formulation, fill and finish operations. Biotechnol. Prog. 24(3), 504-514.

Rawlings, B., Pora, H., 2009. Environmental impact of single-use and reusable bioprocess systems. BioProc. Int. 7 (2), 18–25.

Raymond, M.J., Slater, C.S. and Savelski, M.J., 2010. LCA approach to the analysis of solvent waste issues in the pharmaceutical industry. Green Chem., 12(10), 1826-1834.

Rebitzer, G., Ekvall, T., Frischknecht, R., Hunkeler, D., Norris, G.,Rydberg, T., Schmidt, W.P., Suh, S., Weidema, B., Pennington,D., 2004. Life cycle assessment. Part 1: Framework, goal and scope definition, inventory analysis, and applications. Environ. Int. 30 (5), 701–720.

Reichert, J.M., 2008. Monoclonal antibodies as innovative therapeutics. Current pharmaceutical biotech 9, 423-430.

Reichert, J.M., Rosenweig, C.J., Faden, L.B. and Dewitz, M.C., 2005. Monoclonal antibody successes in the clinic. Nature biotechnology 23(9), 1073-1078.

Reilly, J.M., Jacoby, H.D., Prinn, R.G., 2003. Multi-gas contributors to global climate change: Climate Impacts and Mitigation Costs of Non-CO2 Gases. http://globalchange.mit.edu/files/document/PewCtr_MIT_Rpt_Reilly.pdf (accessed 24.03.2013).

Reinisch *et al*., 2011. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut 60(6), 780-787.

Renou, S., Givaudan, J.G., Dirassouyan, F.,Moulin, P., 2008. Landfill leachate treatment. Review and opportunity. J. of Hazardous Materials 150(3), 468-493.

Rituxan, 2017. Rituximab.
http://www.rituxan.com/ (accessed 17.12.2016).

Rodrigues, M.E., Costa, A.R., Henriques, M., Azeredo, J. and Oliveira, R., 2010. Technological progresses in monoclonal antibody production systems. Biotech. Progress, 26(2), 332-351.

Rogner, H.H., Zhou, D., Bradley, M., Crabbe, P., Edenhofer, O., Hare, B., Kuijpers, L., Yamaguchi, M., 2007. Introduction. In: Metz *et al*. (eds), Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, 95–116.

Rosano, G.L., Ceccarelli, E.A., 2014. Recombinant protein expression in *Escherichia coli*: advances and challenges. Front Microbiol. 5 (172).

Rutgeerts *et al*., 2005. Infliximab for induction and maintenance therapy for ulcerative colitis. The New Eng. J. of Medic. 353, 2462-2476.

Sandstorm, A., Benny Dolk, T.O., 2011. Life sciences companies in Sweden including a comparison with Denmark. http://www.vinnova.se/upload/EPiStorePDF/va-11-03.pdf (accessed 09.11.2014).

Scanlan, C., Shumway, J., Castano, J., Wagner, M., Waghmare, R., 2014. Challenges and strategies for the downstream purification and formulation of Fab antibody fragments. BioPharm Int. 27(1).

Schmidt, J.H., 2008. System delimitation in agricultural consequential LCA. The Int. J. of LCA 13, 350–364.

Schulze, E.D., Valentini, R., Sanz, M.J., 2002. The long way from Kyoto to Marrakesh: Implications of the Kyoto Protocol negotiations for global ecology. Global Change Biology 8(6), 505-518.

Scott, C., 2007. Single use bioreactors: A brief review of current technology. BioProcess International, 44-51.

SEPA, 2012. Landfill.
http://www.sepa.org.uk/waste/waste_regulation/landfill.aspx (accessed 17.06.2013).

Shaarai, A.H., Mahmood, N.Z., Sulaiman, A.H., 2010. Life cycle impact assessment (LCIA) using the ecological scarcity (Ecopoints) method: A potential impact analysis to potable water production. World App. Sciences J. 11(9), 1077-1088.

Shelton, K., Mckenszie, R.B., 2014. The California water crisis: policing vs. pricing? http://www.econlib.org/library/Columns/y2014/SheltonMcKenziewater.html (accessed 09.01.2015).

Shepard, D., Dunlon, M., 2007. Framing sustainable development. http://www.un.org/esa/sustdev/csd/csd15/media/backgrounder_brundtland.pdf (accessed 08.12.2011).

Shukla, A., Gottschalk, U., 2012. Single-use disposable technologies for biopharmaceutical manufacturing. Trends Biotechnol., 1–8.

Shukla, A.A., Thommes, J., 2010. Recent advances in large-scale production of monoclonal antibodies and related proteins. Trends in biotech. 28(5), 253-261.

Shukla, A.A., Hubbard, B., Tressel, T., Guhan, S. and Low, D., 2007. Downstream processing of monoclonal antibodies--application of platform approaches. J. of Chroma. B 848(1), 28-39.

Simaria, A.S., Farid, S.S., 2010. Process intensification of antibody purification processes: A comparison of MILP versus Evolutionary Algorithms. 20th European Symposium on Computer Aided Process Engineering – ESCAPE20.

Sinclair, A., 2011. Advanced manufacturing strategies continuous versus fed batch. BioPharm Services Ltd.

Sinclair, A., Leveen, L., Monge, M., Lim, J., Cox, S., 2008. The environmental impact of disposable technologies.
http://biopharmservices.com/ (accessed 27.11.12).

Smith, A., Watson, J., 2002. The renewables obligation: can it deliver? http://www.tyndall.ac.uk/sites/default/files/note04.pdf (accessed 17.06.2013).

Sommerfeld, S., Strube, J., 2005. Challenges in biotechnology production—generic processes and process optimization for monoclonal antibodies. Chem. Eng. and Processing 44, 1123–1137.

Stankiewicz, A., Moulijn, J., 2002. Process intensification. Industrial and Eng. Chemistry Research 41(8), 1920-1924.

Stewart, H., 2002. Technology assessment: making sure we get it right. Educause Centre of Applied Research Bulletin 21, 1-17.

Stonier, A., Simaria, A.S., Smith, M., Farid, S.S., 2012. Decision tool to access current and future process robustness in an antibody production facility. Biotechnol. Prog. 28(4), 1019-1028.

Stephenson, D., 2013. Monoclonal antibodies continue to drive biotech investment.
https://www.firmex.com/thedealroom/monoclonal-antibodies-continue-to-drive-biotech-investment/ (accessed on 10.12.2016)

Strebhardt, K. and Ullrich, A., 2008. Paul Ehrlich's magic bullet concept: 100 years of progress. Nature Reviews Cancer 8(6), 473-480.

Suh, S., Lenzen, M., Treloar, G.H., Hondo, H., Horvath, A., Huppes,A., Jolliet, O., Klann, U., Krewit T, W., Moriguchi, Y.,Munksgaard, J., Norris, G., 2004. System boundary selection in life-cycle inventories using hybrid approaches. Environ. Sci.Technol. 38 (3), 657–664.

Svoboda, S., 1995. Note on life cycle analysis. Environmental management: Readings and cases, 234–239.

Tagliavia, M., Nicosia, A., 2012. Regeneration and recycling of supports for biological macromolecules Purification. Current Frontiers and Perspectives in Cell Biology, Prof. Stevo Najman (Ed.), InTech, Crotia.

The Scottish Government, 2010. Scotland's higher activity radioactive waste policy environmental report.
http://www.scotland.gov.uk/Resource/Doc/298929/0093254.pdf (accessed 01.07.2013).

Thomassen, M.A., Dalgaard, R., Heijungs, R., Boer, I.D., 2008.Attributional and consequential LCA of milk production. The Int. J. of LCA 13, 339–349.

Tobiszewski, M., Marc, M., Gałuszka, A., Namiesnik, J., 2015. Green chemistry metrics with special reference to green analytical chemistry. Molecules 20, 10928-10946.

Torío, H., Schmidt, D., 2010. Framework for analysis of solar energy systems in the built environement from an exergy perspective. Renewable Energy, 35, 2689-2697.

Toro, J., Requena, I., Duarte, O., Zamorano, M., 2013. A qualitative method proposal to improve environmental impact assessment. Environmental Impact Assessment Review, 43, 9-20.

Udo De Haes, H.A., Heijungs, R., 2007. Life-cycle assessment for energy analysis and management. App. Energy 84(7-8), 817-827.

Udo De Haes, H.A., Jolliet, O., Norris, G., Saur, K., 2002. UNEP/SETAC life cycle initiative: background, aims and scope. The Int. J. of LCA 7 (4), 192–195.

UNEP, 2009. Achieving sustainable development in an age of climate change. United Nations Publications. http://www.un.org/en/development/desa/policy/cdp/cdp_publications/2009cdppolicynote.pdf (accessed 13.02.2013).

UNEP, 1996. Life cycle assessment: what is it and how to do it. United Nations Publication.

UNFCCC, 2016. Paris agreement.
https://treaties.un.org/doc/Publication/CN/2016/CN.735.2016-Eng.pdf (accessed 03.04.2017)

UNFCCC., 2008. Kyoto protocol reference manual. http://unfccc.int/resource/docs/publications/08_unfccc_kp_ref_manual.pdf (accessed 16.12.2011).

Valdes, R., Ibarra, N., Gonzalez, M., Alvarez, T., Garcya, J., Llambias, R., 2001. Hep-1 hybridoma growth and antibody production using protein-free medium in a hollow fibre reactor. Cytotechnology 35, 145-154.

Van-Reis, R., Zydney, A., 2001. Membrane separations in biotechnology. Current opinion in biotech.12(2), 208-211.

Van-Trieste, M., 2014. Ensuring a robust raw-materials supply chain. BioPharm International 27(7).

Verma, R., Boleti, E., George, A.J.T., 1998. Antibody engineering: comparison of bacterial yeast, insect and mammalian expression systems. J. of Immunological Methods **216**, 165-181.

Walsh, G., 2010. Biopharmaceutical benchmarks 2010. Nat.Biotechnol. 28(9), 917–924.

Walsh, G., 2004. Second-generation biopharmaceuticals. European J. of Pharmaceutics and Biopharmaceutics 58(2), 185-196.

Wainwright, J., Mulligan, M., 2004. Environmental modelling-Finding simplicity in complexity. John Wiley & Sons, Inc., Chichester, England.

WBCSD, 2014. Cement sustainability initiative. http://csiprogress2012.org/CSI_ProgressReport_FullReport.pdf (accessed 27.01.2015).

WCED, 1987. World commission on environment and development. Our common future, 8-9.

Weber, A., Wilde, D.D., Chaussin, S., Adams, T., Gerighausen, S., Greller, G., Fenge, C., 2014. Development and qualification of a scalable, disposable bioreactor for GMP-Compliant cell culture. Bioprocess Int. 12(8), 47-52.

Weidema, B.P., Frees, N., Nielsen, A.M., 1999. Marginal production technologies for life cycle inventories. The Int. J. of LCA 4 (1), 48–56.

Wernet, G., Conradt, S., Isenring, H.P., Jimenez-Gonzalez, C., Hungerbuhler, K., 2010. Life cycle assessment of fine chemical production: a case study of pharmaceutical synthesis. Int. J. Life Cycle Assessment 15(3), 294-303.

Wigley, T.M.L., 1998. The Kyoto Protocol: CO_2, CH_4 and climate implications. Geophysical Research Letters 25(13), 2285-2288.

Williams, K., Brooks, K. and Page, M., n.d. Biotechnology: sustainability's silver bullet. J. of Sustainability and Green Business, 1-17

Woodley, J.M., 2008. New opportunities for biocatalysis: making pharmaceutical processes greener. Trends in Biotechnology, 26(6), 321-327.

Yuan, C., Dornfeld, D., 2009. Reducing the Environmental Footprint and Economic Costs of Automotive Manufacturing through an Alternative Energy Supply. Green Manufacturing and Sustainable Manufacturing Partnership.

Zalipsky, S., 1995. Chemistry of polyethylene glycol conjugates with biologically active molecules. Advanced Drug Delivery Reviews, 16(2-3), 157-182.

Zeijl-Rozema, A., Corvers, R., Kemp, R., Martens, P., 2008. Governance for sustainable development: a framework. Sustainable Develop.16(6), 410-421.

Zhang, H., Calvo-Amodio, J., Haapala, K.R., 2013. A conceptual model for assisting sustainable manufacturing through system dynamics. J. of Manufacturing Systems 32(4), 534-549.

Zhang, J., 2010. Mammalian cell culture for biopharmaceutical production. In: Baltz Richard H, editor. Manual of Industrial Microbiology and Biotechnology. 3rd edition, 157-178.

Zhou, W., Kantardjieff, A., 2014. Mammalian cell culture for biologics. Advance Biochem. Eng. Biotechnol. 139, 1-9.

Appendices

Appendix 1: Publications

This section presents the list of papers published in the course of the PhD.

1. Ramasamy, S.V., Titchener-Hooker, N.J., Lettieri, P., 2015. Life cycle assessment as a tool to support decision-making in the biopharmaceutical industry: Consideration and challenges. Food and Bioproducts Proc. 9, 297-305.

2. Ramasamy, S.V., Titchener-Hooker, N.J., Lettieri, P., 2013. Challenges of developing decision-support tools based on life-cycle assessment (LCA) for the biopharmaceutical industry. In: *Proceedings Sardinia 2013, Fourteenth International Waste Management and Landfill Symposium, S. Margherita di Pula, Cagliari, Italy: 30 September-4 October 2013.*

Appendix 2: Avastin®(Bevacizumab) Dosage

Avastin is administered as a solution for intravenous (IV) infusion at the following dose

Table A1 *Avastin dosage (Genentech, 2015).*

Indication	Avastin Dose	Avastin Schedule	Period of Treatment
Non small cell lung cancer	25 mg/kg	Every 3 weeks	Typically around a period of a year

Appendix 3: Software Used in this Thesis

Appendix 3.1 BioSolve Software

In this section, the screenshots of the BioSolve software are presented. These figures will provide an overview of the software and its functions.

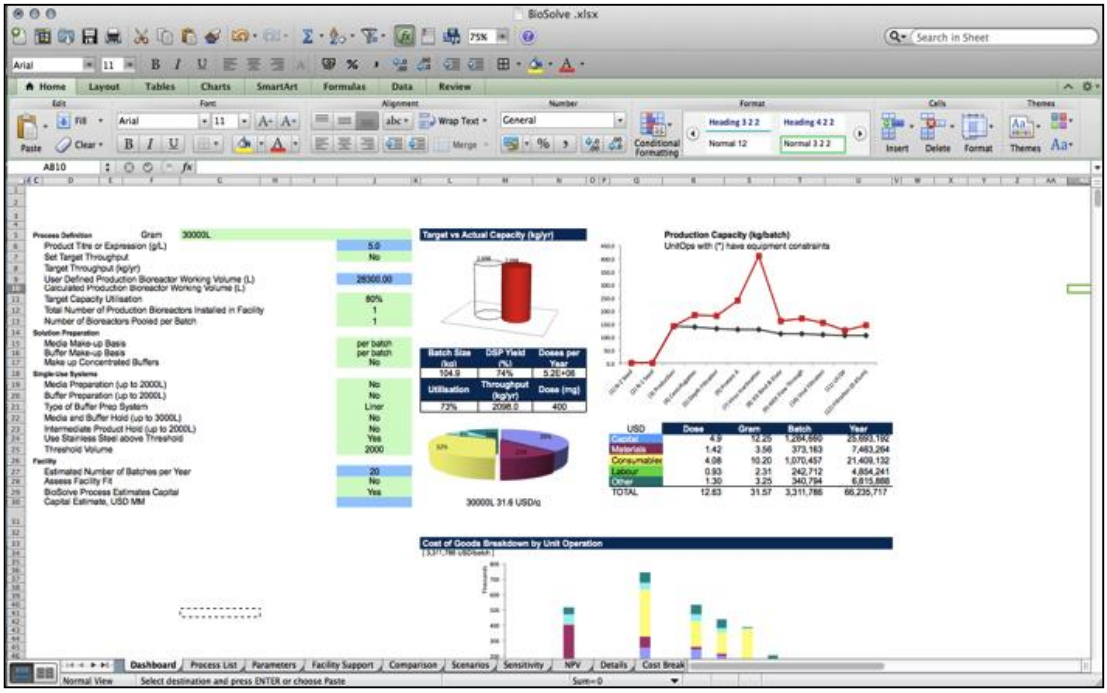
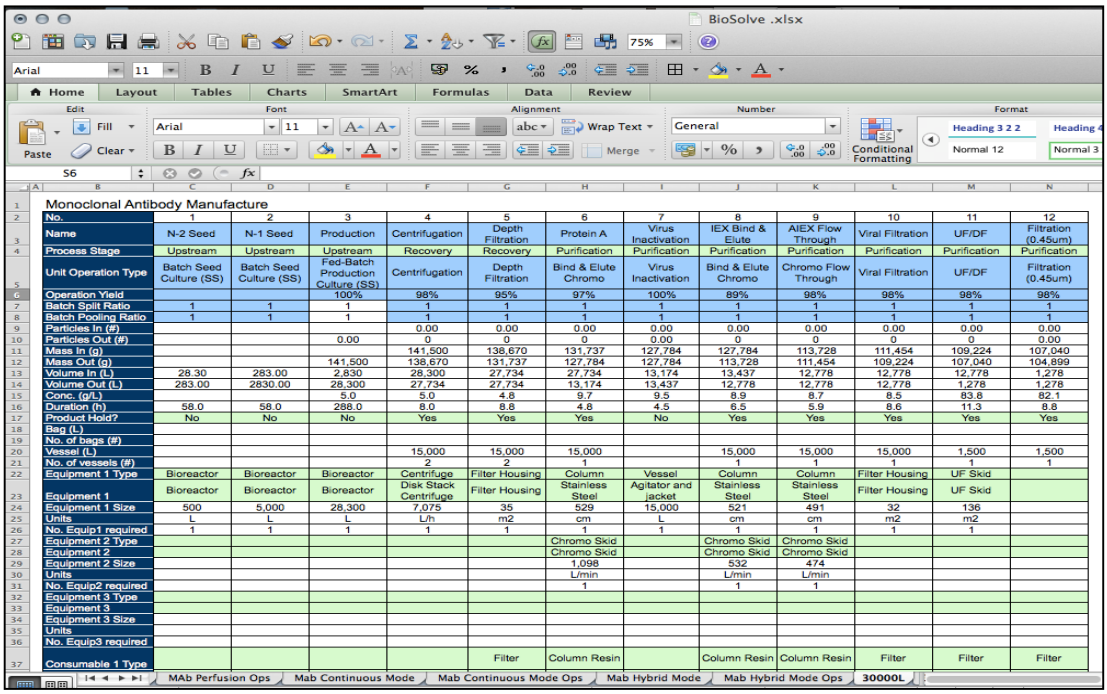


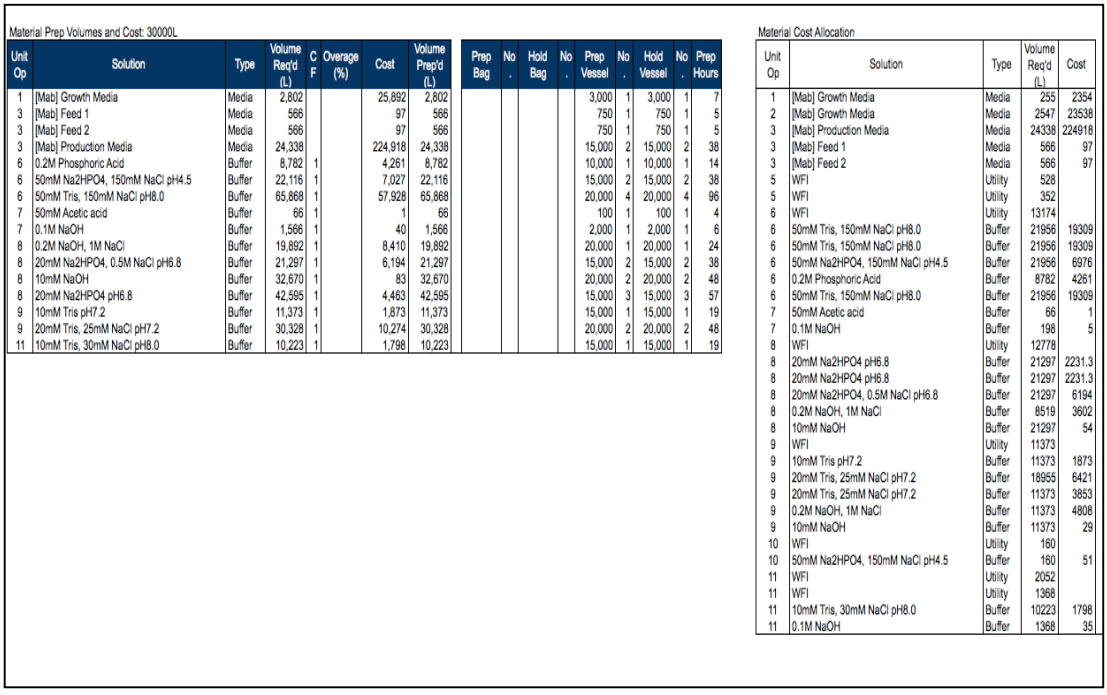
Figure A1 *Worksheet in the BioSolve software where important process parameters such as process titre and number of batches are defined.*



Monoclonal Antibody Manufacture

No.	1	2	3	4	5	6	7	8	9	10	11	12
Name	N-2 Seed	N-1 Seed	Production	Centrifugation	Depth Filtration	Protein A	Virus Inactivation	IEX Bind & Elute	AIEX Flow Through	Viral Filtration	UF/DF	Filtration (0.45um)
Process Stage	Upstream	Upstream	Upstream	Recovery	Recovery	Purification	Purification	Purification	Purification	Purification	Purification	Purification
Unit Operation Type	Batch Seed Culture (SS)	Batch Seed Culture (SS)	Fed-Batch Production Culture (SS)	Centrifugation	Depth Filtration	Bind & Elute Chromo	Virus Inactivation	Bind & Elute Chromo	Chromo Flow Through	Viral Filtration	UF/DF	Filtration (0.45um)
Operation Yield			100%	98%	95%	97%	100%	89%	98%	98%	98%	98%
Batch Split Ratio	1	1	1	1	1	1	1	1	1	1	1	1
Batch Pooling Ratio	1	1	1	1	1	1	1	1	1	1	1	1
Particles In (#)				0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Particles Out (#)			0.00	0	0	0	0.00	0	0	0	0	0.00
Mass In (g)				141,500	138,670	131,737	127,784	127,784	113,728	111,454	109,224	107,040
Mass Out (g)			141,500	138,670	131,737	127,784	127,784	113,728	111,454	109,224	107,040	104,899
Volume In (L)	28.30	283.00	2,830	28,300	27,734	27,734	13,174	13,437	12,778	12,778	12,778	1,278
Volume Out (L)	283.00	2830.00	28,300	27,734	27,734	13,174	13,437	12,778	12,778	12,778	1,278	1,278
Conc. (g/L)			5.0	5.0	4.8	9.7	9.5	8.9	8.7	8.5	83.8	82.1
Duration (h)	58.0	58.0	288.0	8.0	8.8	4.8	4.5	6.5	5.9	8.6	11.3	8.8
Product Hold?	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Bag (L)												
No. of bags (#)												
Vessel (L)				15,000	15,000	15,000		15,000	15,000	15,000	1,500	1,500
No. of vessels (#)				2	2	1		1	1	1	1	1
Equipment 1 Type	Bioreactor	Bioreactor	Bioreactor	Centrifuge	Filter Housing	Column	Vessel	Column	Column	Filter Housing	UF Skid	
Equipment 1	Bioreactor	Bioreactor	Bioreactor	Disk Stack Centrifuge	Filter Housing	Stainless Steel	Agitator and jacket	Stainless Steel	Stainless Steel	Filter Housing	UF Skid	
Equipment 1 Size	500	5,000	28,300	7,075	35	529	15,000	521	491	32	136	
Units	L	L	L	L/h	m2	cm	L	cm	cm	m2	m2	
No. Equip1 required	1	1	1	1	1	1	1	1	1	1	1	
Equipment 2 Type						Chromo Skid		Chromo Skid	Chromo Skid			
Equipment 2						Chromo Skid		Chromo Skid	Chromo Skid			
Equipment 2 Size						1,098		532	474			
Units						L/min		L/min	L/min			
No. Equip2 required						1		1	1			
Equipment 3 Type												
Equipment 3												
Equipment 3 Size												
Units												
No. Equip3 required												
Consumable 1 Type					Filter	Column Resin		Column Resin	Column Resin	Filter	Filter	Filter

Figure A2 *BioSolve worksheet where particle, mass and volume balances (i.e. feed and product mass, feed and product volume, and concentration of product for each unit operation) for a manufacturing process is carried out. In addiction, the worksheet also determines whether a vessel or a bag is used for product hold and contains key input parameters for timing and equipment and consumable sizing.*



Material Prep Volumes and Cost: 30000L

Unit Op	Solution	Type	Volume Req'd (L)	CF	Overage (%)	Cost	Volume Prep'd (L)	Prep Bag	No.	Hold Bag	No.	Prep Vessel	No.	Hold Vessel	No.	Prep Hours
1	[Mab] Growth Media	Media	2,802			25,892	2,802					3,000	1	3,000	1	7
3	[Mab] Feed 1	Media	566			97	566					750	1	750	1	5
3	[Mab] Feed 2	Media	566			97	566					750	1	750	1	5
3	[Mab] Production Media	Media	24,338			224,918	24,338					15,000	2	15,000	2	38
6	0.2M Phosphoric Acid	Buffer	8,782	1		4,261	8,782					10,000	1	10,000	1	14
6	50mM Na2HPO4, 150mM NaCl pH4.5	Buffer	22,116	1		7,027	22,116					15,000	2	15,000	2	38
6	50mM Tris, 150mM NaCl pH8.0	Buffer	65,868	1		57,928	65,868					20,000	4	20,000	4	96
7	50mM Acetic acid	Buffer	66	1		1	66					100	1	100	1	4
7	0.1M NaOH	Buffer	1,566	1		40	1,566					2,000	1	2,000	1	6
8	0.2M NaOH, 1M NaCl	Buffer	19,892	1		8,410	19,892					20,000	1	20,000	1	24
8	20mM Na2HPO4, 0.5M NaCl pH6.8	Buffer	21,297	1		6,194	21,297					15,000	2	15,000	2	38
8	10mM NaOH	Buffer	32,670	1		83	32,670					20,000	2	20,000	2	48
8	20mM Na2HPO4 pH6.8	Buffer	42,595	1		4,463	42,595					15,000	3	15,000	3	57
9	10mM Tris pH7.2	Buffer	11,373	1		1,873	11,373					15,000	1	15,000	1	19
9	20mM Tris, 25mM NaCl pH7.2	Buffer	30,328	1		10,274	30,328					20,000	2	20,000	2	48
11	10mM Tris, 30mM NaCl pH8.0	Buffer	10,223	1		1,798	10,223					15,000	1	15,000	1	19

Material Cost Allocation

Unit Op	Solution	Type	Volume Req'd (L)	Cost
1	[Mab] Growth Media	Media	255	2354
2	[Mab] Growth Media	Media	2547	23538
3	[Mab] Production Media	Media	24338	224918
3	[Mab] Feed 1	Media	566	97
3	[Mab] Feed 2	Media	566	97
5	WFI	Utility	528	
5	WFI	Utility	352	
6	WFI	Utility	13174	
6	50mM Tris, 150mM NaCl pH8.0	Buffer	21956	19309
6	50mM Tris, 150mM NaCl pH8.0	Buffer	21956	19309
6	50mM Na2HPO4, 150mM NaCl pH4.5	Buffer	21956	6976
6	0.2M Phosphoric Acid	Buffer	8782	4261
6	50mM Tris, 150mM NaCl pH8.0	Buffer	21956	19309
7	50mM Acetic acid	Buffer	66	1
7	0.1M NaOH	Buffer	198	5
8	WFI	Utility	12778	
8	20mM Na2HPO4 pH6.8	Buffer	21297	2231.3
8	20mM Na2HPO4 pH6.8	Buffer	21297	2231.3
8	20mM Na2HPO4, 0.5M NaCl pH6.8	Buffer	21297	6194
8	0.2M NaOH, 1M NaCl	Buffer	8519	3602
8	10mM NaOH	Buffer	21297	54
9	WFI	Utility	11373	
9	10mM Tris pH7.2	Buffer	11373	1873
9	20mM Tris, 25mM NaCl pH7.2	Buffer	18955	6421
9	20mM Tris, 25mM NaCl pH7.2	Buffer	11373	3853
9	0.2M NaOH, 1M NaCl	Buffer	11373	4806
9	10mM NaOH	Buffer	11373	29
10	WFI	Utility	160	
10	50mM Na2HPO4, 150mM NaCl pH4.5	Buffer	160	51
11	WFI	Utility	2052	
11	WFI	Utility	1368	
11	10mM Tris, 30mM NaCl pH8.0	Buffer	10223	1798
11	0.1M NaOH	Buffer	1368	35

Figure A3 *BioSolve worksheet for all the reagents needed per batch. The type of reagent (i.e. buffer) and the amount of reagent required are clearly specified in this worksheet.*

Consumables: 30000L

Unit Op	Type	Description	Size	Units	# Used	# Uses	Max # Uses	Cost (USD)	Resin Usage per Batch	Filter Usage per Batch	Waste (kg)	Contaminated Waste?
1	Vessel Filter	Filter for stainless	3,000	L	1	1	1	816			1.81	Contaminated
3	Vessel Filter	Filter for stainless	750	L	1	1	1	408			1.07	Contaminated
3	Vessel Filter	Filter for stainless	750	L	1	1	1	408			1.07	Contaminated
3	Vessel Filter	Filter for stainless	15,000	L	2	1	1	8,160			3.62	Contaminated
5	Filter	Depth Filter	1	m2	32	1	1	18,944		32.0	63.68	Non-Contaminated
6	Column Resin	Protein A	4,391	L	1	1	200	274,451	22.0		32.934	Non-Contaminated
6	Vessel Filter	Filter for stainless	10,000	L	1	1	1	2,720			1.81	Non-Contaminated
6	Vessel Filter	Filter for stainless	15,000	L	2	1	1	8,160			3.62	Non-Contaminated
6	Vessel Filter	Filter for stainless	20,000	L	4	1	1	21,760			7.24	Non-Contaminated
7	Vessel Filter	Filter for stainless	100	L	1	1	1	75			0.61	Non-Contaminated
7	Vessel Filter	Filter for stainless	2,000	L	1	1	1	544			1.81	Non-Contaminated
8	Column Resin	IEX	4,259	L	1	1	50	132,044	85.2		127.78	Non-Contaminated
8	Vessel Filter	Filter for stainless	20,000	L	1	1	1	5,440			1.81	Non-Contaminated
8	Vessel Filter	Filter for stainless	15,000	L	2	1	1	8,160			3.62	Non-Contaminated
8	Vessel Filter	Filter for stainless	20,000	L	2	1	1	10,880			3.62	Non-Contaminated
8	Vessel Filter	Filter for stainless	15,000	L	3	1	1	12,240			5.43	Non-Contaminated
9	Column Resin	AIEX	3,791	L	1	1	50	121,310	75.8		113.73	Non-Contaminated
9	Vessel Filter	Filter for stainless	15,000	L	1	1	1	4,080			1.81	Non-Contaminated
9	Vessel Filter	Filter for stainless	20,000	L	2	1	1	10,880			3.62	Non-Contaminated
10	Filter	Viral Filter	1	m2	32	1	1	358,400		32.0	152.96	Non-Contaminated
11	Filter	UF Filter	1	m2	120	1	10	65,280		12.0	24.72	Non-Contaminated
11	Vessel Filter	Filter for stainless	15,000	L	1	1	1	4,080			1.81	Non-Contaminated
12	Filter	Sterile Filter	1	m2	2	1	1	1,216		2.0	3.7	Non-Contaminated

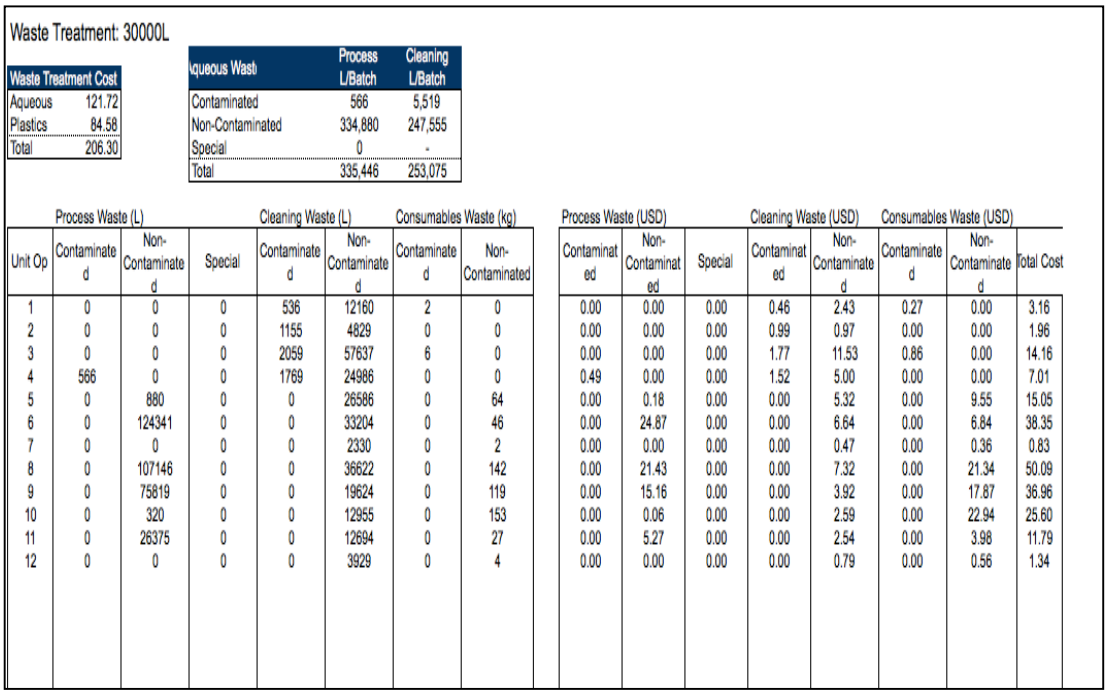
Figure A4 *BioSolve worksheet where total consumables per batch and the quantity of waste generated from the consumables component are determined. These consumables include filters for stainless steel vessels, disposable hold bags, reusable chromatography resins, process filters and any other consumables added to the process.*

Equipment: 30000L

Required Facility Equipment

Unit Op	Status	Area	Type	Sub Type	Size	Units	# Used	Factored Cost	Length (m)	Width (m)	Floor Area (m2)	Area Class
1		Upstream	Bioreactor	Bioreactor	500	L	1	381,003	3.0	2.5	7.5	D
2		Upstream	Bioreactor	Bioreactor	5,000	L	1	1,075,871	3.0	2.5	7.5	D
3		Upstream	Bioreactor	Bioreactor	28,300	L	1	1,583,510	7.2	6.0	43.7	D
4		Recovery	Centrifuge	Disk Stack Centrifuge	7,075	L/h	1	1,383,157	3.7	3.7	13.5	D
4		Recovery	Vessel	Agitator and jacket	15,000	L	2	337,840	2.5	2.5	12.5	D
5		Recovery	Vessel	Agitator and jacket	15,000	L	2	337,840	2.5	2.5	12.5	C
5		Recovery	Filter Housing	Filter Housing	35	m2	1	22,627	4.2	1.4	5.8	C
6		Purification	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
6		Purification	Column	Stainless Steel	529	cm	1	973,426	0.0	0.0	0.0	C
6		Purification	Chromo Skid	Chromo Skid	1,098	L/min	1	505,610	8.1	4.3	34.7	C
7		Purification	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
8		Purification	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
8		Purification	Column	Stainless Steel	521	cm	1	963,104	0.0	0.0	0.0	C
8		Purification	Chromo Skid	Chromo Skid	532	L/min	1	480,638	4.7	2.5	11.7	C
9		Purification	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
9		Purification	Column	Stainless Steel	491	cm	1	924,612	0.0	0.0	0.0	C
9		Purification	Chromo Skid	Chromo Skid	474	L/min	1	476,733	4.3	2.3	10.0	C
10		Purification	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
10		Purification	Filter Housing	Filter Housing	32	m2	1	21,905	4.0	1.3	5.2	C
11		Purification	UF Skid	UF Skid	136	m2	1	737,666	12.5	12.5	155.4	C
11		Purification	Vessel	Agitator and jacket	1,500	L	1	71,029	1.0	1.0	1.0	C
12		Purification	Vessel	Agitator and jacket	1,500	L	1	71,029	1.0	1.0	1.0	C
		Media Prep	Vessel	Agitator and jacket	15,000	L	1	168,920	2.5	2.5	6.3	C
		Media Prep	Vessel	Agitator and jacket	3,000	L	1	81,411	1.5	1.5	2.3	C
		Media Prep	Vessel	Agitator and jacket	750	L	1	63,778	0.9	0.9	0.8	C
		Media Hold	Vessel	Agitator and jacket	15,000	L	2	337,840	2.5	2.5	12.5	C
		Media Hold	Vessel	Agitator and jacket	3,000	L	1	81,411	1.5	1.5	2.3	C
		Media Hold	Vessel	Agitator and jacket	750	L	2	127,555	0.9	0.9	1.6	C
		Buffer Prep	Vessel	Agitator and jacket	15,000	L	3	506,760	2.5	2.5	18.8	C
		Buffer Prep	Vessel	Agitator and jacket	20,000	L	3	543,840	3.0	3.0	27.0	C
		Buffer Prep	Vessel	Agitator and jacket	10,000	L	1	131,840	2.3	2.3	5.3	C
		Buffer Prep	Vessel	Agitator and jacket	2,000	L	1	76,632	1.3	1.3	1.7	C
		Buffer Prep	Vessel	Agitator and jacket	100	L	1	48,946	0.5	0.5	0.3	C
		Buffer Hold	Vessel	Agitator and jacket	15,000	L	9	1,520,280	2.5	2.5	56.3	C

Figure A5 *BioSolve worksheet where the full list of equipment used in the manufacture is listed. The support equipment such as CIP skids, waste tanks and WFI and PW generator are also listed in this worksheet.*



Waste Treatment: 30000L

Waste Treatment Cost	
Aqueous	121.72
Plastics	84.58
Total	206.30

Aqueous Waste	Process L/Batch	Cleaning L/Batch
Contaminated	566	5,519
Non-Contaminated	334,880	247,555
Special	0	-
Total	335,446	253,075

	Process Waste (L)			Cleaning Waste (L)		Consumables Waste (kg)		Process Waste (USD)			Cleaning Waste (USD)		Consumables Waste (USD)		
Unit Op	Contaminated	Non-Contaminated	Special	Contaminated	Non-Contaminated	Contaminated	Non-Contaminated	Contaminated	Non-Contaminated	Special	Contaminated	Non-Contaminated	Contaminated	Non-Contaminated	Total Cost
1	0	0	0	536	12160	2	0	0.00	0.00	0.00	0.46	2.43	0.27	0.00	3.16
2	0	0	0	1155	4829	0	0	0.00	0.00	0.00	0.99	0.97	0.00	0.00	1.96
3	0	0	0	2059	57637	6	0	0.00	0.00	0.00	1.77	11.53	0.86	0.00	14.16
4	566	0	0	1769	24986	0	0	0.49	0.00	0.00	1.52	5.00	0.00	0.00	7.01
5	0	880	0	0	26586	0	64	0.00	0.18	0.00	0.00	5.32	0.00	9.55	15.05
6	0	124341	0	0	33204	0	46	0.00	24.87	0.00	0.00	6.64	0.00	6.84	38.35
7	0	0	0	0	2330	0	2	0.00	0.00	0.00	0.00	0.47	0.00	0.36	0.83
8	0	107146	0	0	36622	0	142	0.00	21.43	0.00	0.00	7.32	0.00	21.34	50.09
9	0	75819	0	0	19624	0	119	0.00	15.16	0.00	0.00	3.92	0.00	17.87	36.96
10	0	320	0	0	12955	0	153	0.00	0.06	0.00	0.00	2.59	0.00	22.94	25.60
11	0	26375	0	0	12694	0	27	0.00	5.27	0.00	0.00	2.54	0.00	3.98	11.79
12	0	0	0	0	3929	0	4	0.00	0.00	0.00	0.00	0.79	0.00	0.56	1.34

Figure A6 *BioSolve worksheet where all the liquid and solid waste produced per batch in the manufacture are summarized. This worksheet also provides the cost of treating the waste before disposal.*

Appendix 3.2 GaBi 6.0 Software

In this section, screenshots of the GaBi 6.0 software are presented. These figures provide an overview of the software and its functions (in this thesis, it is mainly used to obtain the LCI data).

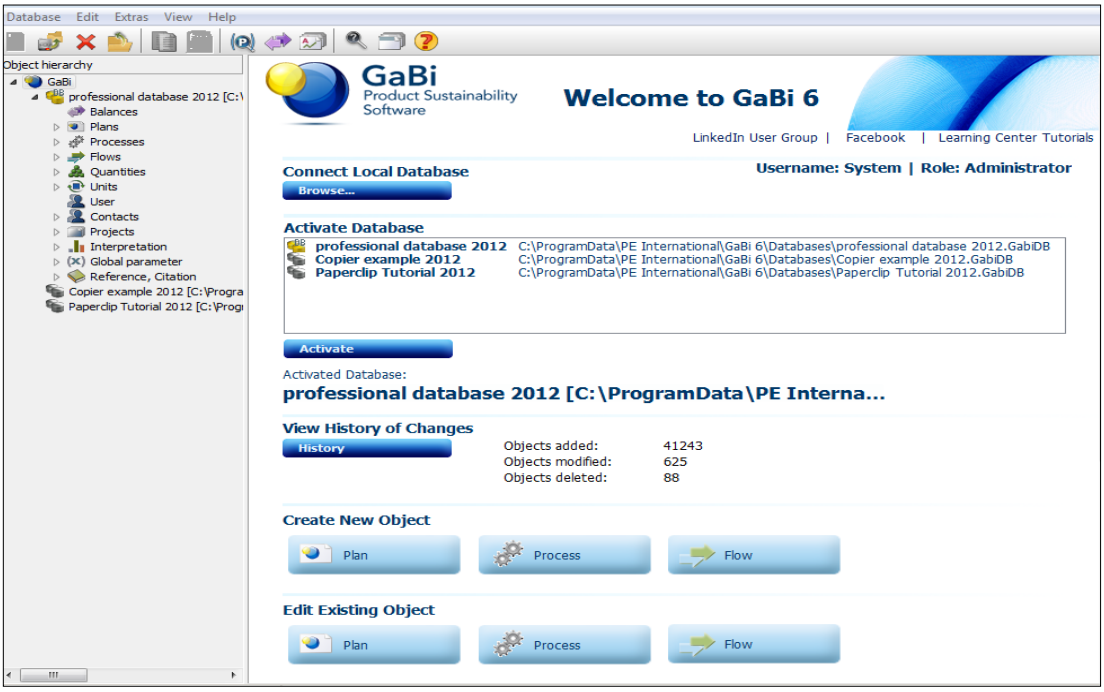


Figure A7 *Display page of the GaBi 6.0 software where the database is activated to initiate an analysis.*

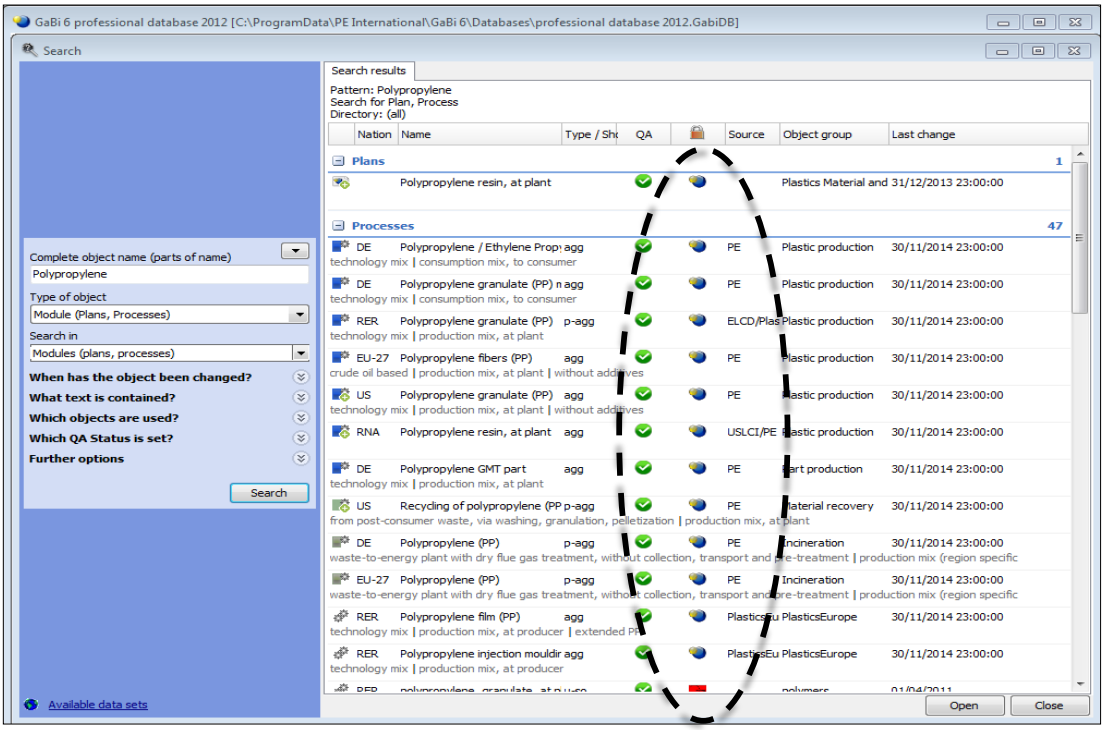


Figure A8 *This figure shows the types of LCI databases (highlighted using a circle) implemented in the software (e.g. PE, Plastics Europe, ELCD).*

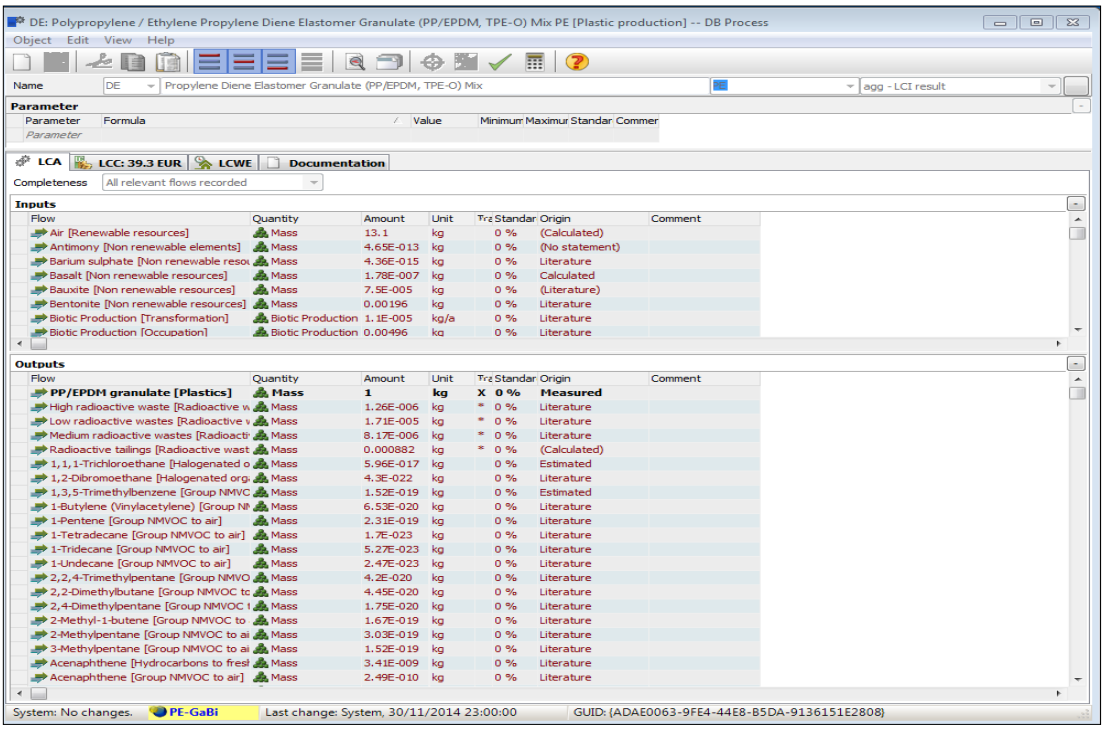


Figure A9 *This figure shows how the LCI data are presented in the software for a particular process/activity.*

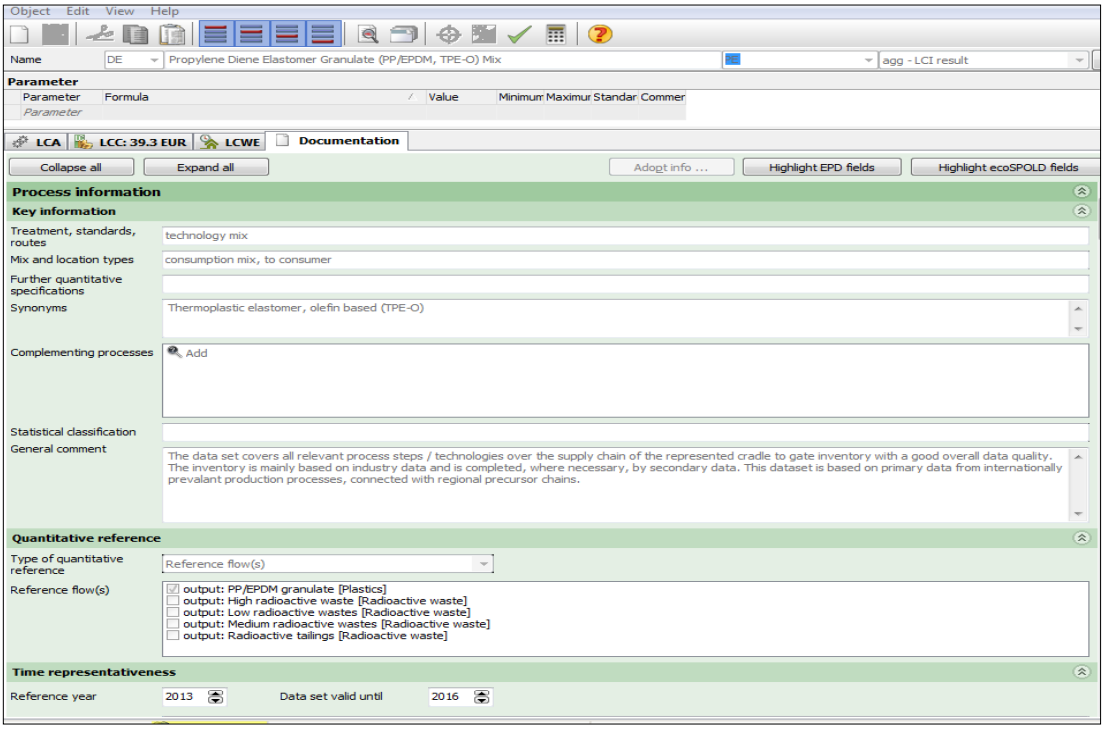


Figure A10 *This figure illustrates the descriptions provided by the software for LCI datasets.*

Appendix 4: Supplementary Data

Appendix 4.1 Mass and Volume Balances of the Traditional and Hybrid Processes at 200 L Operation Scale

Table A2 *Mass and volume balances for the traditional process.*

Traditional Process	**Mass Balance**		**Volume Balance**	
Unit Operation	Mass In (kg)	Mass Out (kg)	Volume In (L)	Volume Out (L)
N2-Seed Fermentation	-	-	-	2
N1-Seed Fermentation	-	-	2	16
Fermentation		0.77	16	155
Disc Stack Centrifuge	0.76	0.70	155	152
Depth Filtration	0.76	0.72	152	152
Protein A	0.72	0.70	152	72
Viral Inactivation	0.70	0.70	72	72
CEX	0.70	0.62	72	70
AEX	0.62	0.61	70	70
Viral Filtration	0.61	0.60	70	70
UF/DF	0.60	0.58	70	7
Sterile Filtration	0.58	0.57	7	7

Table A3 *Mass and volume balances for the hybrid process.*

Hybrid Process	**Mass Balance**		**Volume Balance**	
Unit Operation	Mass In (kg)	Mass Out (kg)	Volume In (L)	Volume Out (L)
N2-Seed Fermentation	-	-	-	2
N1-Seed Fermentation	-	-	2	17
Fermentation	-	0.85	17	171
TFF-MF	0.85	0.82	171	171
Depth Filtration	0.82	0.78	171	171
Protein A	0.78	0.76	171	78
Viral Inactivation	0.76	0.76	78	80
CEX	0.76	0.68	80	76
AEX Membrane Adsorber	0.68	0.66	76	76
Viral Filtration	0.66	0.65	76	76
UF/DF	0.65	0.63	76	8
Sterile Filtration	0.63	0.62	8	8

Appendix 4.2 The Stainless Steel Equipment, Consumables and Reagent Requirements for the Traditional and Hybrid Process Models at 200 L Production Scale.

Table A4 *List of fixed-in-place stainless steel equipment requirements for the traditional process at a 200 L production scale with 20 batches per annum (obtained from the Biosolve software).*

Uni Operation	Equipment	Size
Seed Fermentations	Seed Bioreactor	10 L
	Media Preparation Vessel	25 L
	Media Hold Vessel	25 L
	Seed Bioreactor	20 L
Fermentatiion	Fermentation	200 L
	Media Preparation Vessel	150 L
	Media Hold Vessel	150 L
	Media Preparation Vessel	25 L
	Media Hold Vessel	25 L
	Media Preparation Vessel	25 L
	Media Hold Vessel	25 L
	Product Hold Vessel	200 L
Disc Stack Centrifuge	Disc Stack Centrifuge	1900 kg
	Product Hold	150 L
Depth Filtration	Depth Filtration	56 kg
	Product Hold Vessel	150 L
Protein A Chromatography	Protein A Chromatography Column	230 kg
	Protein A Chromatography Skid	135 kg
	Product Hold Vessel	100 L
	Buffer preparation vessel	100 L
	Buffer hold vessel	100 L
	Buffer preparation vessel	150 L
	Buffer hold vessel	150 L
	Buffer Preparation Vessel	500 L
	Buffer Hold Vessel	500 L
Viral Inactivation	Viral Inactivation Vessel	100 L
	Buffer Preparation Vessel	25 L
	Buffer Hold Vessel	25 L
	Buffer Preparation Vessel	25 L
	Buffer Hold Vessel	25 L
Cation-Exchange Chromatography	Cation-Exchange Chromatography Column	230 kg
	Cation-Exchange Chromatography Skid	135 kg
	Product Hold Vessel	100 L
	Buffer Preparation Vessel	150 L
	Buffer Hold Vessel	150 L
	Buffer Preparation Vessel	150 L
	Buffer Hold Vessel	150 L
	Buffer Preparation Vessel	200 L
	Buffer Hold Vessel	200 L
	Buffer Preparation Vessel	500 L
	Buffer Hold Vessel	500 L
Anion-Exchange Chromatography	Anion-Exchange Chromatography Column	230 kg
	Anion-Exchange Chromatography Skid	135 kg
	Product Hold Vessel	100 L
	Buffer Preparation Vessel	200 L
	Buffer Hold Vessel	200 L
Viral Filtration	Viral Filtration Housing	56 kg
	Product Hold Vessel	100 L
Ultrafiltration/Diafiltration	Ultrafiltration/Diafiltration SKID	2300 kg
	Product Hold Vessel	10 L
	Buffer Preparation Vessel	100 L
	Buffer Hold Vessel	100 L
Sterile Filtration	Product Hold Vessel	10 L
Support Equipment	Waste Collection Vessel	2000 L
	Waste Collection Vessel	2000 L
	CIP Skid	450 kg
	CIP Skid	450 kg
	CIP Skid	450 kg
	CIP Skid	450 kg
	PW Skid	450 kg
	WFI Skid	450 kg
	PW Storage Vessel	4000 L
	WFI Storage Vessel	4000 L

Table A5 *List of fixed-in-place stainless steel equipment requirements for the hybrid process at a 200 L production scale with 20 production batches per annum (obtained from the Biosolve software).*

Unit Operation	Equipment	Size
Seed Fermentations	Bioreactor Support System	10 L
	Container	10 L
	Media Mixer Support System	10 L
	Bioreactor Support System	20 L
Fermentation	Bioreactor Support System	200 L
	Media Mixer Support System	10 L
	Media Mixer Support System	150 L
	Container-media hold bag	10 L
	Container-media hold bag	10 L
	Container-media hold bag	150 L
	Container-product hold bag	200 L
Tangential Flow Microfiltration (D)	Container-buffer hold bag	1000 L
	Container-product hold bag	200 L
	Buffer Mixer System	1000 L
	Tangential Flow Filtration Skid	600 kg
Depth Filtration (T)	Container-product hold bag	200 L
	Depth Filter Housing	56 kg
Protein A Chromatography (T)	Protein A Chromatography Column	230 kg
	Container-buffer hold bag	100 L
	Container-buffer hold bag	200 L
	Container-buffer hold bag	500 L
	Container-product hold bag	100 L
	Buffer Mixer System	100 L
	Buffer Mixer System	200 L
	Buffer Mixer System	500 L
	Protein A Chromatography Skid	135 kg
Viral Inactivation (D)	Container-buffer hold bag	25 L
	Container-buffer hold bag	25 L
	Buffer Mixer System	25 L
Cation-Exchange Chromatography (T)	Cation-Exchange Chromatography Column	230 kg
	Cation-Exchange Chromatography Skid	135 kg
	Container-Buffef Hold bag	200 L
	Container-Buffef Hold bag	200 L
	Container-Buffef Hold bag	200 L
	Container-Buffef Hold bag	500 L
	Container-Product Hold bag	100 L
	Buffer Mixer System	20 L
Anion-Exchange Membrane Adsorber (D)	AEX Membrane Support System	28 kg
	Container-Buffer Hold Bag	20 L
	Container-Buffer Hold Bag	20 L
	Container-Product Hold Bag	100 L
	Buffer Mixer System	20 L
	Anion-Exchange Chromatography Skid	135 kg
Viral Filtration (D)	Container-Product Hold bag	100 L
	Viral Filter Support System	6.4 kg
Ultrafiltration/Diafiltration (T)	Container-Prdoct Hold Bag	10 L
	Ultrafiltration/Diafiltration Skid	2300 kg
Sterile Filtration (D)	Container-Product Hold bag	10 L
Support System	Waste Collection Vessel	1000 L
	Waste Collection Vessel	1000 L
	CIP Skid	450 kg
	CIP Skid	450 kg
	PW Skid	450 kg
	WFI Skid	450 kg
	PW Storage Vessel	2000 L
	WFI Storage Vessel	2000 L

Table A6 *Consumables requirements for the traditional process model (production scale: 200L) per production batch.*

Unit Operation	Consumables	Size
Seed Fermentations	Media Filter	0.6 kg
Fermentation	Media Filter	0.6 kg
	Media Filter	1.1 kg
	Media Filter	1.1 kg
Depth Filtration	Depth Filter	0.4 m2
Protein A Chromatography	Affinity Resin	26 L
	Buffer Filter	0.6 kg
	Buffer Filter	1.1 kg
	Buffer Filter	1.1 kg
Viral Inactivation	Buffer Filter	0.6 kg
	Buffer Filter	0.6 kg
Cation-Exchange Chromatography	CEX Resin	25 L
	Buffer Filter	1.1 kg
	Buffer Filter	1.1 kg
	Buffer Filter	1.1 kg
	Buffer Filter	1.1 kg
Anion-Exchange Chromatography	Buffer Filter	1.1 kg
	AEX Resin	23 L
Viral Filtration	Viral Filter	0.2 m2
	Buffer Filter	1.1 kg
Ultrafiltration/Diafiltration	Buffer Filter	0.6 kg
	UF Filter	0.4 m2
Sterile Filter	Sterile Filter	0.1 m2

Table A7 *Consumables requirements for the hybrid process model (production scale: 200 L) per production batch.*

Unit Operation	Consumables	Size
Seed Fermentations	Bioreactor Bag	10 L
	Solution Mixer Bag System	20 L
	Solutionn Hold Bag	20 L
	Media Filter	0.6 kg
	Bioreactor Bag	20 L
Fermentation	Bioreactor Bag	200 L
	Solution Mixer Bag System	5 L
	Solution Hold Bag	5 L
	Solution Mixeagr BSystem	5 L
	Solution Hold Bag	5 L
	Solution Mixer Bag System	200 L
	SolutionHold Bag	200 L
	Media Filter	0.3 kg
	Media Filter	1.1 kg
	Product Hold Bag	200 L
Tangential Flow Microfiltration	Filter cassette	1 m2
	Solution Mixer Bag System	1000 L
	Solution Hold Bag	1000 L
	Buffer Filter	1.82 kg
	Product Hold Bag	200 L
Depth Filtration	Filter	0.4 kg
	Product Hold Bag	200 L
Protein A Chromatography	Column Resin	26 L
	Solution Mixer Bag System	100 L
	Solution Hold Bag	100 L
	Solution Mixer Bag System	200 L
	Solution Hold Bag	200 L
	Solution Mixer Bag System	500 L
	Solution Hold Bag	500 L
	Buffer Filter	1.1
	Buffer Filter	1.1
	Buffer Filter	1.1
	Product Hold Bag	100 L
Viral Inactivation	Solution Mixer Bag System	5 L
	Solution Hold Bag	5 L
	Solution Mixer Bag System	5 L
	Solution Hold Bag	5 L
	Buffer Filter	0.3 kg
	Buffer Filter	0.3 kg
	Solution Hold Bag	100 L
Cation-Exchange Chromatography	Column Resin	25 L
	Solution Mixer Bag System	200 L
	Solution Hold Bag	200 L
	Solution Mixer Bag System	200 L
	Solution Hold Bag	200 L
	Solution Mixer Bag System	200 L
	Solution Hold Bag	200 L
	Solution Mixer Bag System	500 L
	Solution Hold Bag	500 L
	Product Hold Bag	100 L
	Buffer Filter	1.1
	Buffer Filter	1.1
	Buffer Filter	1.1
	Buffer Filter	1.1
Anion-Exchange Membrane Adsorber	Membrane Adsorber	30 Inch
	Solution Mixer Bag System	20 L
	Solution Hold Bag	20 L
	Solution Mixer Bag System	20 L
	Solution Hold Bag	20 L
	Product Hold Bag	100 L
	Buffer Filter	0.6
	Buffer Filter	0.6
Viral Filtration	Viral Filter	0.2 m2
	Product Hold Bag	100 L
Ultrafiltration/Diafiltration	Ultrafilter	0.4m2
	Product Hold Bag	20 L
Sterile Filtration	Sterile filter	0.1 m2
	Product Hold Bag	20 L

Table A8 *Media requirements for the traditional process model (production scale: 200 L) per production batch.*

Traditional Process Model Reagents Requirements	**Per Production Batch (L)**
Media	167
Buffer	1600
Clean-in-Place (CIP):	
PW	5600
WFI	12400
Caustic	2800
Acid	2800
Steam	68
Utility	250
Total Water Requirements Per Production Batch	25690

Table A9 *Media requirements for the hybrid process model (production scale: 200 L) per production batch.*

Hybrid Process Model Reagents Requirements	**Per Production Batch (L)**
Media	170
Buffer	2095
Clean-in-Place (CIP):	
PW	156
WFI	78
Caustic	78
Acid	78
Steam	0
Utility	320
Total Water Requirements Per Production Batch	2980

Table A10 *Total reagents required per batch for a 200 L traditional process (obtained from BioSolve Software). The reagents include media, buffer, CIP agents, steam for SIP, WFI and PW.*

Unit Operation	Total Reagent (L)
N2-Seed	700
N1-Seed	1140
Fermentation	5900
Disc-Stack Centrifuge	950
Depth Filtration	960
Protein A Chromatography	2840
Viral Inactivation	1190
Cation-Exchange Chromatography	3190
Anion-Exchange Chromatography	1670
Viral Filtration	840
Ultrafiltration/Diafiltration	790
Sterile Filtration	370

Table A11 *Total reagents required per batch for a 200 L hybrid process (obtained from BioSolve Software).*

Unit Operation	Total Reagent (L)
N2-Seed	10
N1-Seed	15
Fermentation	150
Disc-Stack Centrifuge	930
Depth Filtration	140
Protein A Chromatography	850
Viral Inactivation	5
Cation-Exchange Chromatography	750
Anion-Exchange Chromatography	45
Viral Filtration	10
Ultrafiltration/Diafiltration	100
Sterile Filtration	0

Table A12 *Transport fuel consumption.*

Type of Transport	**Fuel and GHG Emissions**	**Reference**
Cargo plane	Fuel/km: 0.422 kg GHG/km: 1.35 kgCO_2-eq	GaBi, 2014; DEFRA, 2014
Cargo ship	Fuel/km:0.000395 kg GHG/km: 0.00126 kgCO_2-eq	GaBi, 2014; DEFRA 2014
Truck	Fuel/km: 0.00135 kg GHG/km: 0.00481 kgCO_2-eq	GaBi, 2014; DEFRA, 2014

Table A13 *Emissions conversion factors for electricity and fuel.*

	Emission Conversion Factor	**Reference**
UK electricity	0.45 $kgCO_2$-eq/KWh	DEFRA, 2014
Fuel (Diesel) (UK)	3.1001 $kgCO_2$-eq/kg	DEFRA, 2014

Appendix 4.3 Energy and Water Consumption and Carbon Emissions of the Scenarios Evaluated in Case-Study 1

Table A14 *Energy consumption of the scenarios.*

Scenarios	**Supply-Chain (MJ) x10^6**	**Use (MJ) x10^6**	**End-of-Life (MJ) x10^6**	**Total (MJ) x10^6**
1	1.5	4	0.3	5.7
2	1.5	4	0.3	5.7
3	1.5	4	0.3	5.7
4	2.5	2.8	0.4	5.6
5	2.5	2.8	0.4	5.6
6	2.5	2.8	0.4	5.6

Table A15 *Water consumption of the scenarios.*

Scenarios	**Supply-Chain (kg) x10^6**	**Use (kg) x10^6**	**End-of-Life (kg) x10^6**	**Total (kg) x10^6**
1	0.9	0.1	0.03	0.9
2	0.9	0.002	0.03	0.9
3	0.9	0.002	0.03	0.9
4	0.4	0.1	0.01	0.5
5	0.4	0.2	0.01	0.4
6	0.4	0.02	0.01	0.4

Table A16 *Carbon emissions of the scenarios.*

Scenarios	Supply-Chain ($kgCO_2$-eq) $x10^6$	Use ($kgCO_2$-eq) $x10^6$	End-of-Life ($kgCO_2$-eq) $x10^6$	Total ($kgCO_2$-eq) $x10^6$
1	0.3	0.5	0.06	0.8
2	0.3	0.5	0.06	0.8
3	0.3	0.5	0.06	0.8
4	0.2	0.3	-0.0006	0.6
5	0.2	0.3	-0.0006	0.6
6	0.2	0.3	-0.0006	0.6

Table A17 *The energy and water consumption and carbon emissions of all the activities evaluated by the framework for the traditional process.*

Activity	Energy (MJ)	Water (kg)	Carbon Emissions ($kgCO_2$-eq)
Reagent Preparations	12336	20469	1530
Equipment Fabrication	19246	22100	7146
Consumables Fabrication	27534	639	5020
Equipment Utilisation	6450	No water consumed	836
Liquid Waste Management	64729	No water consumed	7688
Solid Waste Management	132	640	16
Lighting and HVAC	126000	No water consumed	15590
Stainless Steel Equipment Recycling at the end of life	14735	1594	836

Table A18 *The energy and water consumption and carbon emissions of all the activities evaluated by the framework for the hybrid process.*

Activity	Energy (MJ)	Water (kg)	Carbon Emissions ($kgCO_2$-eq)
Reagent Preparations	2171	2971	270
Equipment Fabrication	11372	10966	4470
Consumables Fabrication	111922	6124	6876
Equipment Utilisation	6800	No water consumed	873
Liquid Waste Management	8437	No water consumed	1044
Solid Waste Management	302	5167	41
Lighting and HVAC	126000	No water consumed	15590
Stainless Steel Equipment Recycling at the end of life	18653	639	-29

Appendix 4.4 Landfill and incineration with energy recovery LCA diagrams

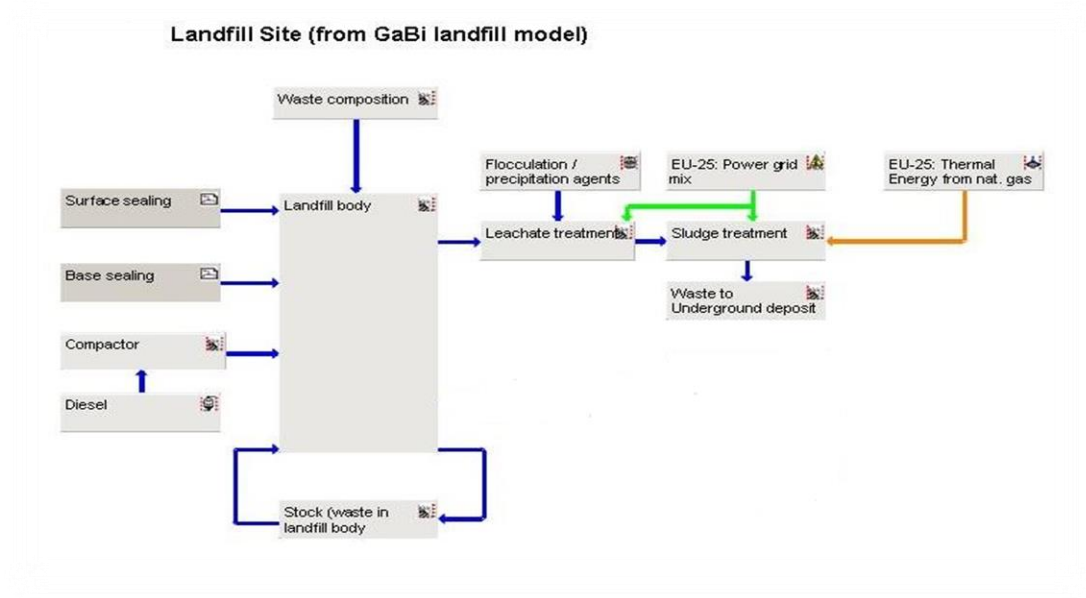


Figure A11 *Landfill model used in the thesis. This model shows all relevant process steps / technologies for the treatment of waste on a landfill.*

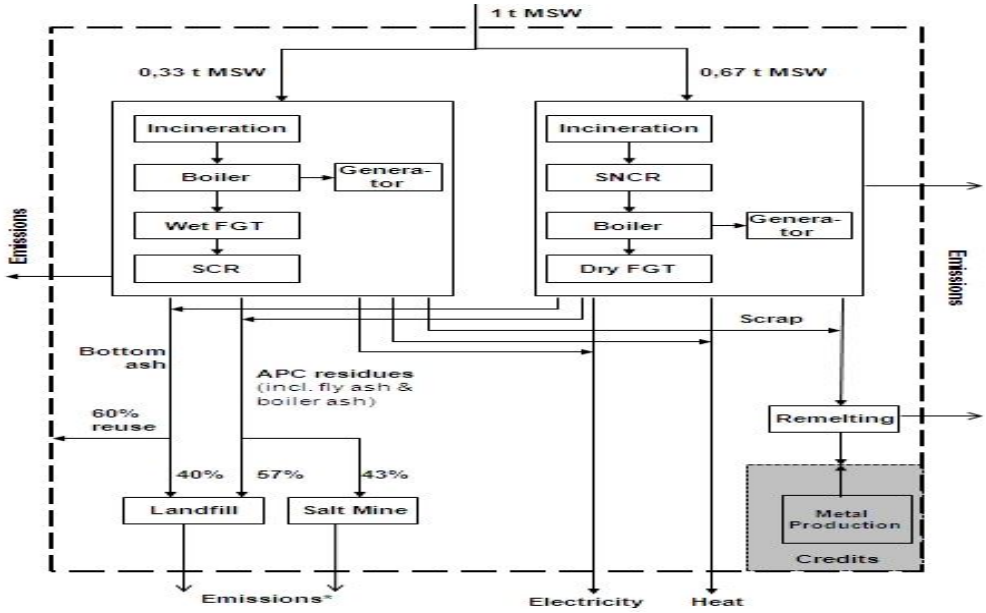


Figure A12 *Incineration with energy recovery model used in the thesis. This model shows all relevant process steps/technologies used in an average European waste-to-energy plant (WTE) for the thermal treatment of plastic waste) with typical technology used in Europe to meet the legal requirements.*

Appendix 4.5 The Stainless Steel, Consumables and Media Requirements of the Traditional and Hybrid Processes at 1000 L, 2000 L and 20, 000 L Scale of Operation (Chapter 7)

Table A19 List of fixed-in-place stainless steel equipment requirements for the traditional process at a clinical operational scale (1000 L) (obtained from the BioSolve software).

Unit Operation	Type	Size (L)
Seed Fementation	Bioreactor	20
	Vessel	100
	Vessel	100
Seed Fementation	Bioreactor	100
Fermentation	Bioreactor	1000
	Vessel	750
	Vessel	25
	Vessel	25
	Vessel	25
	Vessel	25
	Vessel	750
	Vessel	750
Disc Stack Centrifuge	Vessel	750
	Centrifuge	136 L/hr
Depth Filtration	Vessel	750
	Filter housing	1 m2
Protein A Chromatography	Column	100 cm
	Vessel	500
	Vessel	250
	Vessel	250
	Vessel	750
	Vessel	750
	Vessel	2000
	Vessel	2000
	skid	39 L/min
Viral Inactivation	Vessel	25
	Vessel	25
	Vessel	500
Cation-Excchange Chromatography	Vessel	500
	Vessel	500
	Vessel	500
	Vessel	1000
	Vessel	1000
	Vessel	1000
	Vessel	1000
	Vessel	1500
	Vessel	1500
	Skid	39 L/min
	Column	100cm
Anion-Exchange Chromatography	Vessel	500
	Vessel	750
	Vessel	750
	skid	39 L/min
Viral Filtration	Vessel	500
	Filter housing	1m2
Uf/DF	Skid	3m2
	Vessel	50
Sterile Filtration	Vessel	50

Table A20 *List of consumables requirements for the traditional process at a clinical operational scale (1000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Weight (kg)
Seed Fermentation	Vessel Filter	0.6
Seed Fermentation		0.6
Fermentation		1.1
Fermentation	Vessel Filter	1.1
Fermentation	Vessel Filter	0.6
Fermentation	Vessel Filter	1.8
Depth Filtration	Filter	4.0
Protein A	Column Resin	2.1
Protein A	Vessel Filter	1.1
Protein A	Vessel Filter	1.8
Protein A	Vessel Filter	1.8
Viral Inactivation	Vessel Filter	0.6
Viral Inactivation	Vessel Filter	0.6
CEX	Column Resin	8.2
CEX	Vessel Filter	1.8
CEX	Vessel Filter	1.8
CEX	Vessel Filter	1.8
CEX	Vessel Filter	1.8
AEX	Column Resin	7.3
AEX	Vessel Filter	1.1
AEX	Vessel Filter	1.8
Viral Filtration	Filter	9.8
UF/DF	Filter	1.1
UF/DF	Vessel Filter	1.1
Sterile Filtration	Filter	1.3

Table A21 *Media requirements for the traditional process at a clinical operational scale (1000 L) (obtained from the BioSolve software).*

Traditional Process Model Reagents Requirements	Per Production Batch (L)
Media	680
Buffer	9200
CIP Agents	30200
SIP Agents	320
Utility Requirements	3660
Total Water Requirements Per Production Batch	44060

Table A22 *List of fixed-in-place stainless steel equipment requirements for the traditional process at a clinical operational scale (2000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Size (L)
Seed Fermentation	Bioreactor	20
	Vessel	200
	Vessel	200
Seed Fermentation	Bioreactor	200
Fermentation	Bioreactor	2000
	Vessel	50
	Vessel	50
	Vessel	50
	Vessel	50
	Vessel	2000
	Vessel	2000
	Vessel	2000
Disc Stack Centrifuge	Vessel	2000
	Disc Stack Centrifuge	150 L/hr
Depth Filtration	Vessel	2000
	Filter Housing	2m2
Protein A Chromatography	Vessel	750
	Vessel	750
	Vessel	1500
	Vessel	1500
	Vessel	5000
	Vessel	5000
	Vessel	1000
	Column	150cm
	Skid	139 L/hR
Viral Inactivation	Vessel	1000
	Vessel	25
	Vessel	25
	Vessel	100
	Vessel	100
CEX	Column	150cm
	Skid	20L/min
	Vessel	1000
	Vessel	1500
	Vessel	1500
	Vessel	3000
	Vessel	3000
	Vessel	3000
	Vessel	3000
	Vessel	3000
	Vessel	3000
AEX	Vessel	1000
	Vessel	750
	Vessel	750
	Vessel	2000
	Vessel	2000
	Column	150 cm
	Skid	20L/Min
Viral Filtration	Vessel	1000
	Filter	2m2
UF/DF	Vessel	750
	Vessel	750
	Vessel	100
	UF Skid	3m2
Sterile Filtration	Vessel	100

***Table A23**. List of consumables requirements for the traditional process at a clinical operational scale (2000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Weight (kg)
Seed Fermentation	Vessel Filter	0.6
Seed Fermentation	Vessel Filter	0.6
Fermentation	Vessel Filter	0.6
	Vessel Filter	0.6
	Vessel Filter	1.1
Disc Stack Centrifuge	Vessel Filter	1.1
Depth Filtration	Filter	2.0
Protein A Chromatpgraphy	Column Resin	0.8
	Vessel Filter	1.1
	Vessel Filter	1.1
	Vessel Filter	1.1
	Vessel Filter	1.8
	Vessel Filter	1.8
Viral Inactivation	Vessel Filter	0.6
CEX	Column Resin	3.1
	Vessel Filter	1.1
	Vessel Filter	1.8
	Vessel Filter	1.8
	Vessel Filter	1.8
AEX	Column Resin	2.7
	Vessel Filter	1.1
	Vessel Filter	1.1
Viral Filtration	Filter	3.7
UF/DF	Filter	0.6
Sterile Filtration	Filter	0.6

***Table A24** Media requirements for the traditional process at a clinical operational scale (2000 L) (obtained from the BioSolve software).*

Traditional Process Model Reagents Requirements	Per Production Batch (L)
Media	1800
Buffer	17620
CIP Agents	39270
SIP Agents	750
Utility Requirements	2600
Total Water Requirements Per Production Batch	62040

Table A25 *List of fixed-in-place stainless steel equipment requirements for the hybrid process at a production scale (1000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Size
Seed Fermentation	Disposable Bioreactor Support	20 L
Seed Fermentation	Disposable Bioreactor Support	200 L
Fermentation	Disposable Bioreactor Support	1000 L
	Media Hold Container	20 L
	Media Hold Container	20 L
	Media Hold Container	200 L
Disc Stack Centrifuge	Centrifuge	128 L/hr
	Product Hold Container	1000 L
	Mixing System Support	1000 L
Depth Filtration	Filter Housing	1 m2
	Mixing System Support	1000 L
	Product Hold Container	1000 L
Protein A	Column	100 cm
	Chromo Skid	39 L/min
	Mixing System Support	1000 L
	Buffer Hold Container	500 L
	Product Hold Container	1000 L
	Buffer Hold Container	1000 L
	Buffer Hold Container	1000 L
Viral Inactivation	Mixing System Support	1000 L
	Product Hold Container	1000 L
	Buffer Hold Container	20 L
	Buffer Hold Container	200 L
CEX	Column	100 cm
	Product Hold Container	500 L
	Buffer Hold Container	1000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Chromo Skid	39 L/Min
	Mixing System Support	500 L
AEX	Column	100 cm
	Chromo Skid	39 L/min
	Mixing System Support	500
	Product Hold Container	500 L
	Buffer Hold Container	500 L
	Buffer Hold Container	2000 L
Viral Filtration	Filter Housing	2 m2
	Product Hold Container	500 L
	Mixing System Support	500
UF/DF	UF Skid	3m2
	Mixing System Support	100 L
	Product Hold Container	100 L
	Buffer Hold Container	500 L
Sterile Filtration	Mixing System Support	100 L
	Product Hold Container	100 L

Table A26 *List of consumables requirements for the hybrid process at a production scale (1000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Weight (KG)
Seed Fermentation	Bioreactor Bag	1.888
	Solution Mixer Bag System	2.731
	Vessel Filter	0.614
Seed Fermentation	Bioreactor Bag	2.731
Fermentation	Bioreactor Bag	6.965
	Solution Mixer Bag System	1.888
	Vessel Filter	0.614
	Solution Mixer Bag System	1.888
	Vessel Filter	0.614
	Solution Mixer Bag System	6.965
	Vessel Filter	1.07
Disc Stack Centrifuge	Product Mixer Bag	6.965
Depth Filtration	Filter	1.988
	Product Mixer Bag	6.965
Protein A	Column Resin	235.62
	Product Mixer Bag	6.965
	Solution Mixer Liner	3.211
	Vessel Filter	1.069
	Solution Mixer Liner	6.965
	Vessel Filter	1.808
	Solution Mixer Liner	13.93
	Vessel Filter	1.808
Viral Inactivation	Product Mixer Bag	6.965
	Solution Mixer Liner	1.888
	Vessel Filter	0.614
	Solution Mixer Liner	2.731
	Vessel Filter	0.614
CEX	Column Resin	235.62
	Product Mixer Bag	3.211
	Solution Mixer Liner	6.965
	Vessel Filter	1.07
	Solution Mixer Liner	6.965
	Vessel Filter	1.808
	Solution Mixer Liner	6.965
	Vessel Filter	1.808
	Solution Mixer Liner	6.965
	Vessel Filter	1.808
AEX	Column Resin	235.62
	Product Mixer Bag	3.211
	Solution Mixer Liner	3.211
	Vessel Filter	1.069
	Solution Mixer Liner	6.965
	Vessel Filter	1.808
Viral Filtration	Filter	9.56
	Product Mixer Bag	3.211
UF/DF	Filter	8.244
	Product Mixer Bag	2.731
	Solution Mixer Liner	3.211
	Vessel Filter	1.069
Sterile Filtration	Filter	1.853
	Product Mixer Bag	2.731

Table A27 *Media requirements for the hybrid process at a production scale (1000 L) (obtained from the BioSolve software).*

Traditional Process Model Reagents Requirements	Per Production Batch (L)
Media	640
Buffer	10300
CIP Agents	1200
SIP Agents	1560
Utility Requirements	0
Total Water Requirements Per Production Batch	13700

Table A28 *List of fixed-in-place stainless steel equipment requirements for the hybrid process at a production scale (2000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Size
Seed Fermentation	Disposable Bioreactor Support	20 L
	Media Hold Container	200 L
Seed Fermentation	Disposable Bioreactor Support	200 L
Fermentation	Disposable Bioreactor Support	2000 L
	Media Hold Container	50 L
	Media Hold Container	50 L
	Media Hold Container	2000 L
Disc Stack Centrifuge	Product Hold Container	2000 L
	Product Hold Container	2000 L
	Disc Stack Centrifuge	150 l/Hr
Depth Filtration	Product Hold Container	2000 L
	Mixing System Support	2000 l
	Filter Housing	2 m2
Protein A	Product Hold Container	2000 L
	Column	150 cm
	Buffer Hold Container	1000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Chromatography Skid	20 L/min
Viral Inactivation	Product Hold Container	2000 L
	Buffer Hold Container	20 L
	Buffer Hold Container	200 L
	Mixing System Support	200 L
	Mixing System Support	20 L
CEX	Product Hold Container	2000 L
	Column	150 cm
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Mixing System Support	2000 L
	Chromatography Skid	20 L/min
AEX	Product Hold Container	2000 L
	Column	150 cm
	Buffer Hold Container	2000 L
	Buffer Hold Container	2000 L
	Chromatography Skid	20 L/min
Viral Filtration	Product Hold Container	2000 L
	Product Hold Container	200 L
	Buffer Hold Container	2000 L
	Filter Housing	2 m2
UF/DF	UF Skid	3 m2
	Product Hold Container	200 L
Sterile Filtration	Product Hold Container	200 L

Table A29 *List of consumables requirements for the hybrid process at a production scale (2000 L) (obtained from the BioSolve software).*

Unit Op	Type	Waste (kg)
Seed Fermentation	Bioreactor Bag	1.9
	Solution Mixer Bag System	2.7
	Vessel Filter	1.1
Seed Fermentation	Bioreactor Bag	2.7
Fermentation	Bioreactor Bag	7.0
	Solution Mixer Bag System	1.9
	Vessel Filter	0.6
	Solution Mixer Bag System	1.9
	Vessel Filter	0.6
	Solution Mixer Bag System	7.0
	Vessel Filter	1.8
Disc Stack Centrifuge	Product Mixer Bag	7.0
Depth Filtration	Filter	4.0
	Product Mixer Bag	7.0
Protein A	Column Resin	461.8
	Product Mixer Bag	7.0
	Solution Mixer Liner	7.0
	Vessel Filter	1.1
	Solution Mixer Liner	7.0
	Vessel Filter	1.8
	Solution Mixer Liner	20.9
	Vessel Filter	1.8
	Product Mixer Bag	7.0
Viral Inactivation	Solution Mixer Liner	1.9
	Vessel Filter	0.6
	Solution Mixer Liner	2.7
	Vessel Filter	1.1
CEX	Column Resin	471.2
	Product Mixer Bag	7.0
	Solution Mixer Liner	7.0
	Vessel Filter	1.8
	Solution Mixer Liner	7.0
	Vessel Filter	1.8
	Solution Mixer Liner	13.9
	Vessel Filter	1.8
	Solution Mixer Liner	13.9
	Vessel Filter	1.8
AEX	Column Resin	461.8
	Product Mixer Bag	7.0
	Solution Mixer Liner	7.0
	Vessel Filter	1.8
	Solution Mixer Liner	13.9
	Vessel Filter	1.8
Viral Filtration	Filter	14.3
	Product Mixer Bag	7.0
UF/DF	Filter	16.5
	Product Mixer Bag	2.7
	Solution Mixer Liner	7.0
	Vessel Filter	1.8
Sterile Filtration	Filter	1.9
	Product Mixer Bag	2.7

Table A30 *Media requirements for the hybrid process at a production scale (2000 L) (obtained from the BioSolve software).*

Traditional Process Model Reagents Requirements	Per Production Batch (L)
Media	1800
Buffer	19600
CIP Agents	1800
SIP Agents	0
Utility Requirements	2600
Total Water Requirements Per Production Batch	25800

Table A31 *List of fixed-in-place stainless steel equipment requirements for the traditional process at a production scale (20, 000 L) (obtained from the BioSolve software).*

Unit Operation	Equipment Type	Size/Volume	Quantity
Seed Fermentation	Bioreactor	1000 L	1
	Vessel	5000 L	4
Seed Fermentation	Bioreactor	10000 L	1
Fermentation	Bioreactor	25000 L	1
	Vessel	2000 L	8
	Vessel	10000 L	2
	Vessel	20000 L	8
Disc Stack Centrifuge	Centrifuge	20000 L/Hr	1
	Vessel	20000 L	5
Depth Filtration	Filter Housing	2m2	5
	Vessel	20000 L	5
Protein A	Column	240 cm	1
	Vessel	20000 L	20
Viral Inactivation	Vessel	20000 L	3
	Vessel	500 L	2
	Vessel	1000 L	2
CEX	Column	240 cm	1
	Vessel	20000 L	16
	Vessel	10000 L	10
	Vessel	5000 L	2
AEX	Column	240 cm	1
	Vessel	20000 L	11
	Vessel	1000 L	2
Viral Filtration	Filter Housing	2 m2	5
	Vessel	20000 L	3
	Vessel	1000 L	2
UF/DF	UF Skid	5 m2	10
	Vessel	20000 L	4
	Vessel	5000 L	1
Sterile Filtration	Vessel	5000 L	1

Table A32 *List of consumables requirements for the traditional process at a production scale (20, 000 L) (obtained from the BioSolve software).*

Unit Operation	Type	Weight (kg)
Seed Fermentation	Vessel Filter	3.6
Fermentation	Vessel Filter	1.8
	Vessel Filter	1.8
	Vessel Filter	32.5
Depth Filtration	Filter	192.8
Protein A	Column Resin	24429.0
	Vessel Filter	7.2
	Vessel Filter	16.3
	Vessel Filter	30.7
Viral Inactivation	Vessel Filter	1.1
	Vessel Filter	1.8
CEX	Column Resin	14928.9
	Vessel Filter	1.8
	Vessel Filter	7.2
	Vessel Filter	12.7
	Vessel Filter	18.1
AEX	Column Resin	8143.0
	Vessel Filter	3.6
	Vessel Filter	9.0
Viral Filtration	Filter	478.0
UF/DF	Filter	16199.5
UF/DF	Vessel Filter	7.2
Sterile Filtration	Filter	22.2

Table A33 *Media requirements for the traditional process at a production scale (20, 000 L) (obtained from the BioSolve software).*

Traditional Process Model Reagents Requirements	Per Production Batch (L)
Media	79920
Buffer	666867
CIP Agents	370407
SIP Agents	27400
Utility Requirements	11670
Total Water Requirements Per Production Batch	1156264

Appendix 4.6 The energy and water consumption and carbon emissions of monoclonal antibodies manufacture in the clinical development phases and manufacture (Chapter 7)

Table A34 *Energy and water consumption and carbon emissions of monoclonal antibodies manufacture in clinical development and production (using traditional technology).*

Phases/Stages	Energy (MJ) x10^6	Water (kg) x10^6	Carbon Emissions ($KgCO_2$-eq) x10^6
I	46	19	7
II	166	51	26
III	186	58	30
Production	193	59	32

Table A35 *Energy and water consumption and carbon emissions of monoclonal antibodies manufacture in the clinical development phases.*

Phases/Stages	Energy (MJ) x10^6	Water (kg) x10^6	Carbon Emissions ($kgCO_2$-eq) x10^6
I	24	9	3
II	64	18	10
III	71	20	12

Table A36 *Energy and water consumption and carbon emissions of monoclonal antibodies manufacture when process intensification is carried out (changing the process titre from 5g/L to 10g/L).*

	Energy (MJ) x10^6	Water (kg) x10^6	Carbon Emissions ($kgCO_2$-eq) x10^6
Clinical (New)	199	64	31
Clinical (Base case)	398	128	62
Production (New)	97	17	11
Production (Base case)	193	33	23

Table A37 *Energy and water consumption and carbon emissions of monoclonal antibodies manufacture when process intensification is carried out (changing the process titre from 5g/L to 15g/L).*

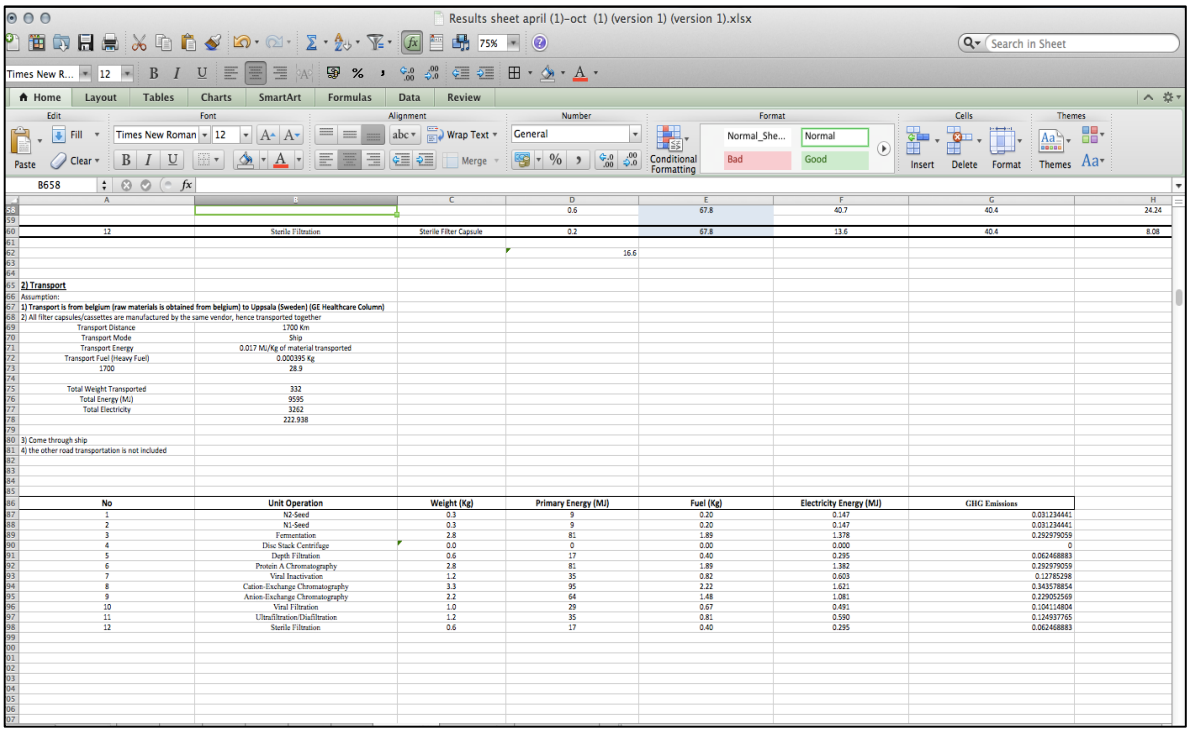
	Energy (MJ) x10^6	**Water (kg) x10^6**	**Carbon Emissions ($kgCO_2$-eq) x10^6**
Clinical (New)	125	43	21
Clinical (Base case)	375	128	62
Production (New)	64	11	8
Production (Base case)	193	33	23

Table A38 *Energy and water consumption and carbon emissions of monoclonal antibodies manufacture when process intensification is carried out (when a lower operational scale of 1000 L is employed).*

	Clinical Development	**Production**
Energy (MJ) x10^6	33	7
Water (kg) x10^6	11	3
Carbon Emissions ($kgCO_2$-eq) x10^6	5	1

Appendix 4.7 Screenshots of Excel Model

The screenshots of the framework on Excel are shown in this section.



		0.6	67.8	40.7	40.4	24.24
12	Sterile Filtration	Sterile Filter Capsule	0.2	67.8	13.6	40.4	8.08
			16.6				

2) Transport

Assumption:

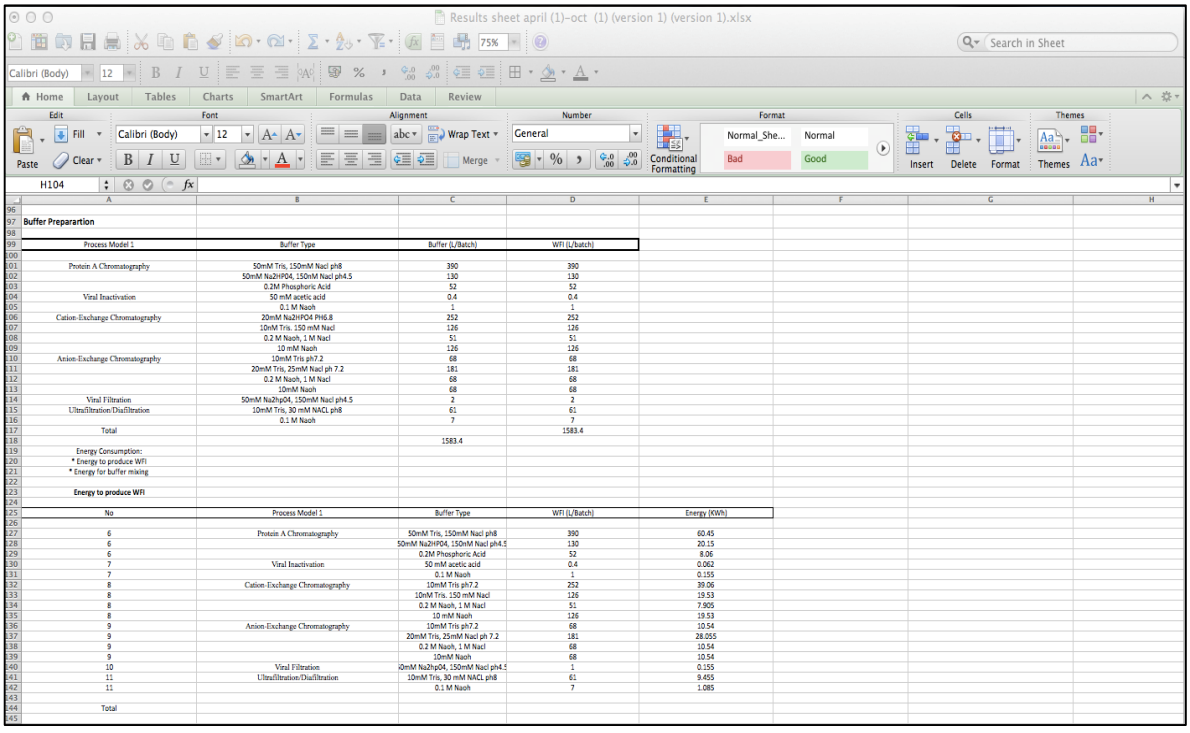
1) Transport is from belgium (raw materials is obtained from belgium) to Uppsala (Sweden) (GE Healthcare Column)
2) All filter capsules/cassettes are manufactured by the same vendor, hence transported together

Transport Distance	1700 Km
Transport Mode	Ship
Transport Energy	0.017 MJ/Kg of material transported
Transport Fuel (Heavy Fuel)	0.000395 Kg
1700	28.9
Total Weight Transported	332
Total Energy (MJ)	9595
Total Electricity	3262
	222.938

3) Come through ship
4) the other road transportation is not included

No	Unit Operation	Weight (Kg)	Primary Energy (MJ)	Fuel (Kg)	Electricity Energy (MJ)	GHG Emissions
1	N2-Seed	0.3	9	0.20	0.147	0.031234441
2	N1-Seed	0.3	9	0.20	0.147	0.031234441
3	Fermentation	2.8	81	1.89	1.378	0.292979059
4	Disc Stack Centrifuge	0.0	0	0.00	0.000	0
5	Depth Filtration	0.6	17	0.40	0.295	0.062468883
6	Protein A Chromatography	2.8	81	1.89	1.382	0.292979059
7	Viral Inactivation	1.2	35	0.82	0.603	0.12785298
8	Cation-Exchange Chromatography	3.3	95	2.22	1.621	0.343578854
9	Anion-Exchange Chromatography	2.2	64	1.48	1.081	0.229052569
10	Viral Filtration	1.0	29	0.67	0.491	0.104114804
11	Ultrafiltration/Diafiltration	1.2	35	0.81	0.590	0.124937765
12	Sterile Filtration	0.6	17	0.40	0.295	0.062468883

Figure A13 *Screenshot of the framework on Excel. Figure A13 shows the consumables transport calculation on the excel spreadsheet.*



Buffer Preparartion

Process Model 1	Buffer Type	Buffer (L/Batch)	WFI (L/batch)
Protein A Chromatography	50mM Tris, 150mM Nacl ph8	390	390
	50mM Na2HP04, 150mM Nacl ph4.5	130	130
	0.2M Phosphoric Acid	52	52
Viral Inactivation	50 mM acetic acid	0.4	0.4
	0.1 M Naoh	1	1
Cation-Exchange Chromatography	20mM Na2HPO4 PH6.8	252	252
	10mM Tris. 150 mM Nacl	126	126
	0.2 M Naoh, 1 M Nacl	51	51
	10 mM Naoh	126	126
Anion-Exchange Chromatography	10mM Tris ph7.2	68	68
	20mM Tris, 25mM Nacl ph 7.2	181	181
	0.2 M Naoh, 1 M Nacl	68	68
	10mM Naoh	68	68
Viral Filtration	50mM Na2hp04, 150mM Nacl ph4.5	2	2
Ultrafiltration/Diafiltration	10mM Tris, 30 mM NACL ph8	61	61
	0.1 M Naoh	7	7
Total			1583.4
		1583.4	

Energy Consumption:
* Energy to produce WFI
* Energy for buffer mixing

Energy to produce WFI

No	Process Model 1	Buffer Type	WFI (L/Batch)	Energy (KWh)
6	Protein A Chromatography	50mM Tris, 150mM Nacl ph8	390	60.45
6		50mM Na2HP04, 150mM Nacl ph4.5	130	20.15
6		0.2M Phosphoric Acid	52	8.06
7	Viral Inactivation	50 mM acetic acid	0.4	0.062
7		0.1 M Naoh	1	0.155
8	Cation-Exchange Chromatography	10mM Tris ph7.2	252	39.06
8		10mM Tris. 150 mM Nacl	126	19.53
8		0.2 M Naoh, 1 M Nacl	51	7.905
8		10 mM Naoh	126	19.53
9	Anion-Exchange Chromatography	10mM Tris ph7.2	68	10.54
9		20mM Tris, 25mM Nacl ph 7.2	181	28.055
9		0.2 M Naoh, 1 M Nacl	68	10.54
9		10mM Naoh	68	10.54
10	Viral Filtration	0mM Na2hp04, 150mM Nacl ph4.5	1	0.155
11	Ultrafiltration/Diafiltration	10mM Tris, 30 mM NACL ph8	61	9.455
11		0.1 M Naoh	7	1.085
Total				

Figure A14 *Screenshot of the framework on Excel. Figure A14 shows the reagent preparations (buffer) calculation on the Excel spreadsheet.*

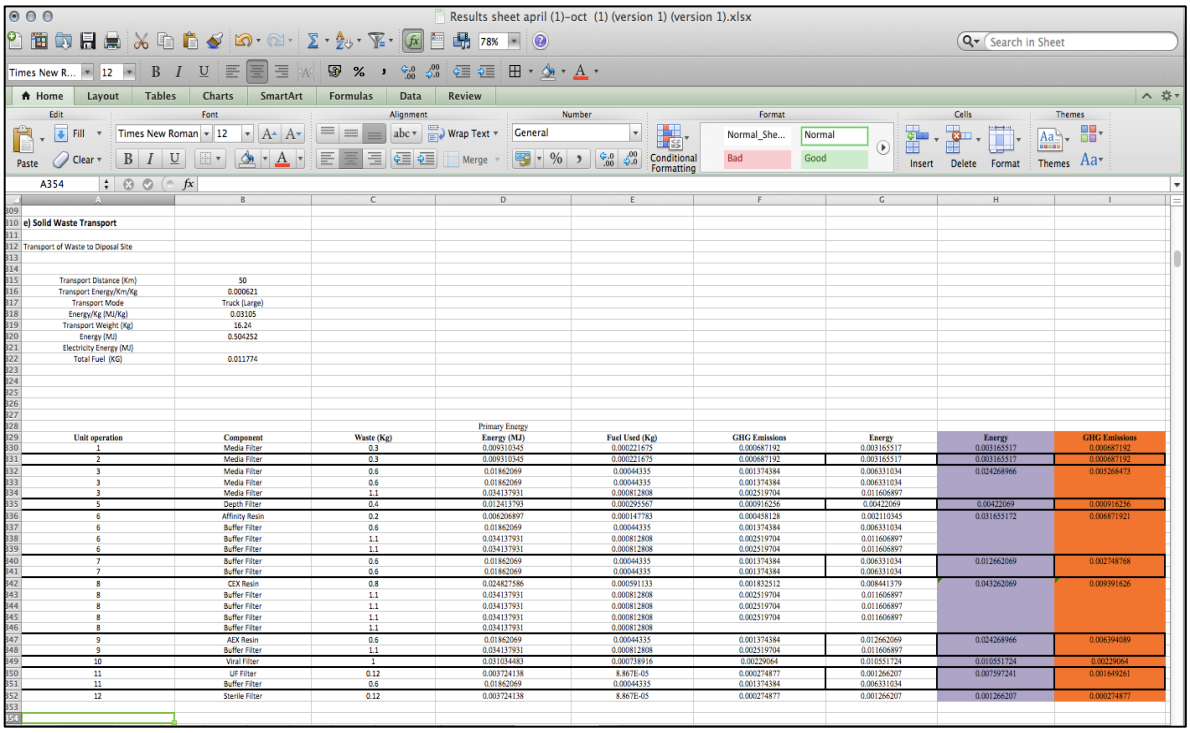


Figure A15 *Screenshot of the framework on Excel. Figure A15 shows the solid waste transport calculation on the Excel spreadsheet.*

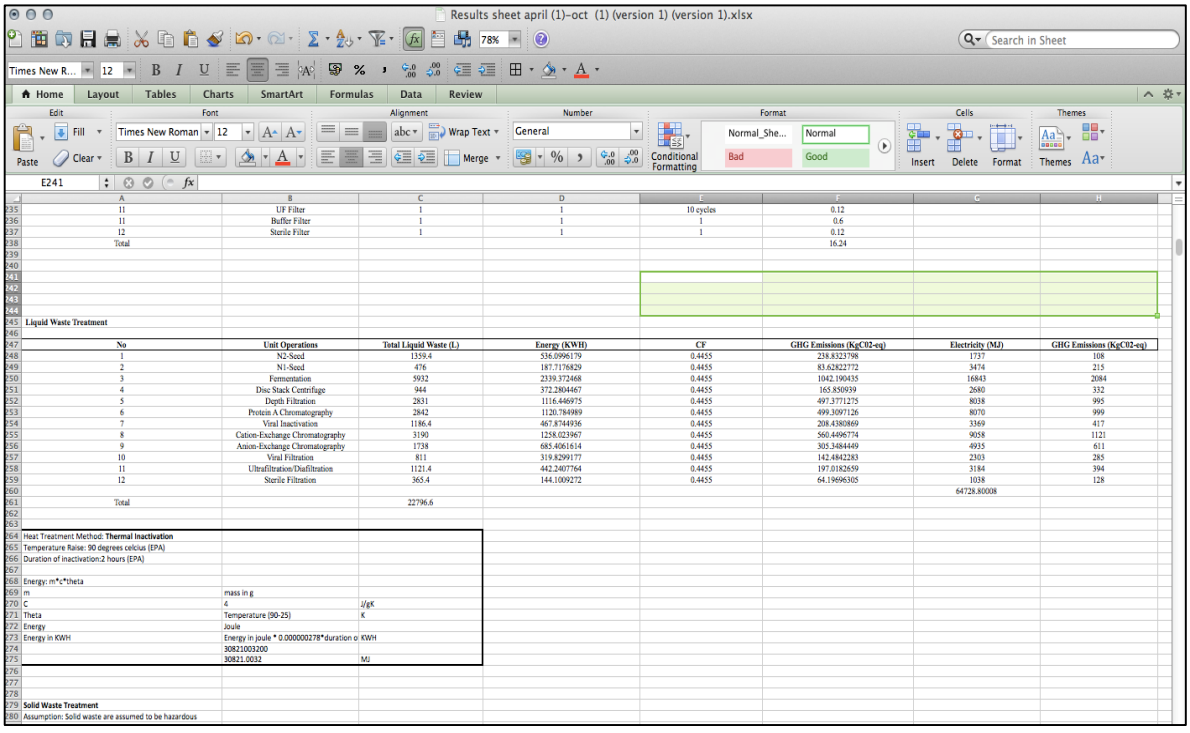


Figure A16 *Screenshot of the framework on Excel. Figure A16 shows the liquid waste treatment calculation on the Excel spreadsheet.*

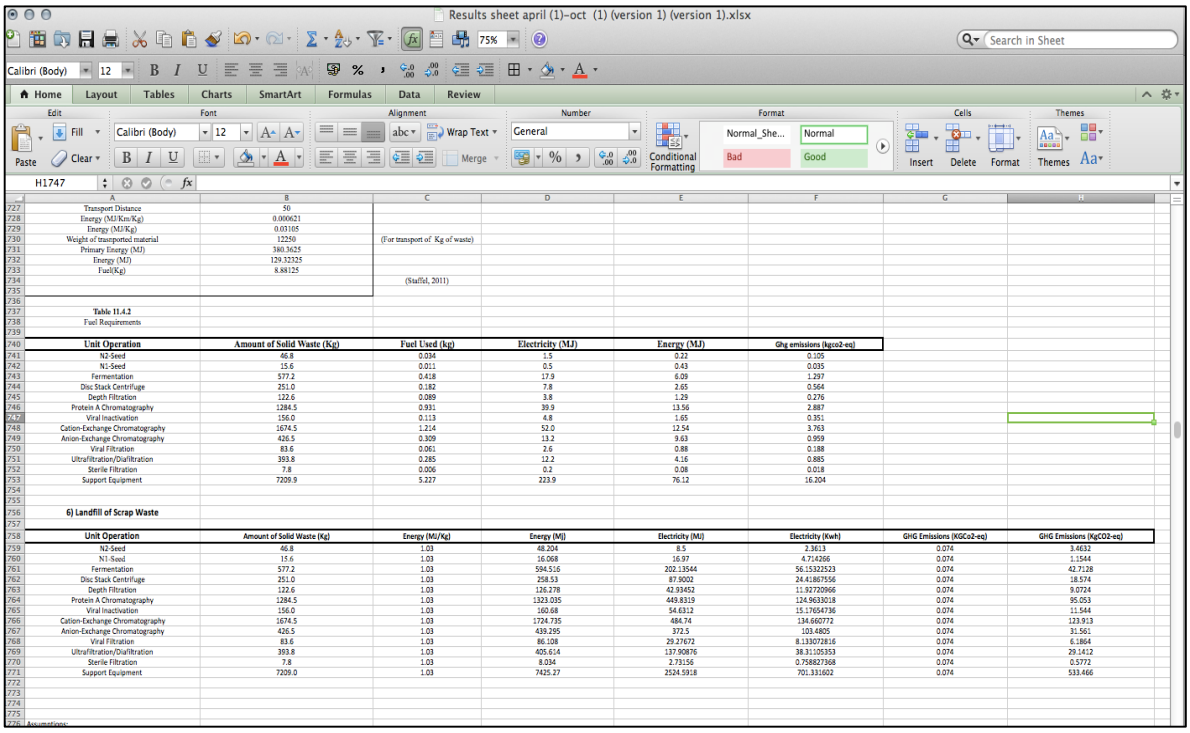


Figure A17 *Screenshot of the framework on Excel. Figure A17 shows the landfill of scrap waste (in the end of life phase) calculation on the Excel spreadsheet.*