DYNAMIC THERMODYNAMIC FLUX BALANCE ANALYSIS AND LIFE CYCLE ANALYSIS OF MICROBIAL BIOFUELS

by

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ABSTRACT

Recently, a paradigm shift from fossil fuel energy to renewable energy is observed because of the environmental awareness. Algae are abundant, carbon neutral and renewable, which make them high potential materials to be developed as a fuel source, pending economic constraints. If algae are used as a platform, a clear and precise insight to the pathway should be presented. This requires a deep understanding of gene-protein-reaction systems. Using the genome-scale metabolic networks, a better description of the cellular metabolism and strain optimization will be attained; this will help to decrease the demand for expensive in-vivo experiments.

First Phase: one major objective of this research was to maximize the production rate of algae biofuel at different process conditions with economic and environmental considerations. We did a comprehensive review on life cycle analysis of algal biodiesel. In this review, the effect of different process variables on the environmental impacts of algal biodiesel in the literature were systematically presented.

Second Phase: We integrated the biological data and thermodynamic constraints to establish a realistic metabolic phenotypic space. With the aid of public metabolic networks, the MODEL SEED database, and component contribution, we incorporated the thermodynamics and chemical reactions constraints.

Third Phase: In metabolic network modeling, many simulations carry out in “static” state whereas our interest is to predict the behavior in a “dynamical” approach and to understand how environment and intracellular interact. In addition, the metabolic phenotype of cell systems often involves high levels of nutrient uptake and excessive byproduct secretion. In silico scenarios were used to simulate diauxic growth under two different situations. The glucose and xylose as main component of lignocellulosic biomass defined in media and allow E. coli to grow on them. Then under fully aerobic condition and later of under aerobic to anaerobic transition, simulations were performed to see how our proposed dynamic thermodynamic flux balance (DT-FBA) captures cell behaviors.

List of Publications

1- **Genome-scale Simulation of Phaeodactylum Tricornutum for Biofuels Production: Impacts of CO_2 Concentration and Light Exposure**,
Ali Chamkalani, Sohrab Zendehboudi,
66th Canadian Chemical Engineering Conference, QUÉBEC CITY, QC (not provided in thesis)

2- **A Critical Review on Life Cycle Analysis (LCA) of Algae Biodiesel: Current Challenges and Future Prospects**
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LIST OF SYMBOLS, NOMENCLATURE OR ABBREVIATIONS

a	Activity of compound
AD	Anaerobic digestion
ADP	Abiotic depletion
ATP	Adenosine triphosphate
c	Metabolite concentration
CCR	Carbon catabolite repression
CHP	Combined heat and power
DT-FBA	Dynamic thermodynamic flux balance analysis
E	Ethanol concentrations
EBR	Energy balance ratio
EP	Eutrophication
EROI	Energy return on investment
F	Faraday constant
FAME	Fatty acid methyl esters
FBA	Flux Balance Analysis
FT	Fischer-Tropsch
FU	Functional unit
FVA	Flux variability analysis
G	Glucose concentrations
GHG	Greenhouse gas
GWP	Global warming potential
H	Number of transported protons
HTC	Hydrothermal carbonization

HTG	Hydrothermal gasification
HTL	Hydrothermal liquefaction
IL	Ionic liquid
IPCC	International panel on climate change
K_g	Glucose saturation constants
K_{ie}	Ethanol inhibition constant
K_{ig}	Glucose inhibition constant
K_o	Oxygen saturation constants
K_z	Xylose saturation constants
LB	Lower bound
LCA	Life cycle analysis
LCI	Life cycle impact
LCIA	Life cycle impact analysis
LHV	Lower heat value
m	Number of compounds in reaction
M	Constant
n	Stoichiometric coefficient of compound
NADPH	Nicotinamide adenine dinucleotide phosphate
NER	Net energy ratio
O	Dissolved oxygen concentrations
ODP	Ozone depletion
OP	Open pond
PBR	Photobioreactor
R	Universal gas constant
S	Stoichiometric coefficients

SBE	System boundary expansion
stFBA	Semi-thermodynamic FBA
T	Temperature
TAG	Triacylglycerol
TMFA	Thermodynamics-based metabolic flux analysis
TSS	Total solid suspension
TVA	Thermodynamic variability analysis
UB	Upper bound
V	Vector of fluxes
$v_{g,max}$,	Glucose maximum uptake rates
VKT	Vehicle kilometers travelled
$v_{o,max}$	Oxygen maximum uptake rates
$v_{z,max}$	Xylose maximum uptake rates
X	Biomass concentration
Z	Xylose concentrations
z_i	Binary variable
γ	Activity coefficients
$\Delta_r G°$	Standard Gibbs free energy of reaction
$\Delta G_f°$	Standard Gibbs energies of formation
$\Delta\psi$	Electrochemical potential
μ	Specific growth rate

CHAPTER 1

INTRODUCTION AND OVERVIEW

1 Fossil Fuels Concern

Search for alternative sources of energy that are sustainable and environmental friendly is inevitable with the rate of population growth and energy demand, and the increasing awareness of societies about their environment (Mudimu et al., 2014). Climate change, environmental damage and the depletion of fossil fuel resources are among other incentives to explore alternative fuels. The world's population is 7.6 billion in 2017 and is expected to increase to 9.8 billion in 2050 (United Nations, 2017), which will consequently increase the worldwide energy consumption. Burning conventional fossil fuels has already increased the concentration of carbon dioxide and other greenhouse gasses (GHGs) in atmosphere that has caused global warming (Faried et al., 2017). The average concentration of CO_2 in atmosphere has increased from 280 ppm in pre-industrial era (mid 1800s) to 403 ppm in 2016, which corresponds to a 40% increase (Dlugokencky and Tans, 2016; IEA Statistics, 2017; Joos and Spahni, 2008). Over the past ten years, the average CO_2 concentration has increased by a rate of 2 ppm/year along with notable increase in the concentration of other GHGs such as CH_4 and NO_x (IEA Statistics, 2017).

Now, a question arises that why greenhouse gases should be mitigated? Globally, increase in temperature and global warming, pattern change and disruption in rainfall and snow, and extreme climate events like record-breaking heavy precipitation (Donat et al., 2017; Lehmann et al., 2015; Salzmann, 2016; Westra et al., 2013) are issues driven by GHGs consequences. There is a high confidence that these phenomena are linked to climbing level of CO_2 and other GHGs in environment due to human activities, thus, any

action to reduce emission of greenhouse gas pollutions will help to debilitate the risk attributed with climate change and global warming. However, scenarios to reduce risks of climate change maybe show additional adverse side effects such as the risk associated with nuclear power and mitigation technologies of carbon capture and sequestration (CCS) (Jakob and Steckel, 2016). Similarly, water scarcity, land use change consequences, food concerns and increase prices for food crops are considerable issues should be considered (Lotze-Campen et al., 2014). On the other hand, co-benefits are achieved as results of climate change mitigation. Health benefits due to fresh air quality, energy security and import dependence reduction because of low carbon energy sources produced locally are bold incentive for making decision in energy policy (Jakob and Steckel, 2016; Nemet et al., 2010).

The gasses entering atmosphere mostly consist of CO_2 (about 72%) where the share of non- CO_2 emissions such as methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (F-gases) is 19%, 6% and 3%, respectively (Olivier et al., 2017). Total CO_2 released in environment stem from three main sources of fossil fuels, industry, and land-use change (Le Quéré et al., 2017; Peters et al., 2017).

In 2016, global total GHG emissions recorded value of 53.4 gigatonnes in CO_2 equivalent (Gt CO_2 eq) in which 49.3 Gt CO_2 eq is linked to emissions from fossil fuel and industrial processes (excluding land use) (Le Quéré et al., 2017; Olivier et al., 2017). The growth rate of greenhouse gas release into environment has been reported as 0.2% and 0.5% for 2015 and 2016 (Olivier et al., 2017).

Among these gases, total CO_2 emission (from fossil fuels, industry, and land-use change) was recorded as 41.5 billion tonnes in 2015, then decreased to 40.8 by 2.1% in 2016 (Le Quéré et al., 2017). It can be seen that during 2016, the reduction in share of CO_2 emission counterbalanced by annual growth rate of about 1% attributed to non- CO_2 greenhouse gases in global GHGs emissions (Olivier et al., 2017). Excluding the 9% weight of land use change in total CO_2 emission, fossil fuels and industry account for 91% of human-caused CO_2 emissions over the past decade (Le Quéré et al., 2017). Fossil fuel and industry accounted for 36.18 Gt CO_2 in 2016 and it will be projected to rise by 2% to level of 36.8 Gt CO_2 (Le Quéré et al., 2017). The indicators show that 4.8 tCO_2 is the

contribution of each person in global CO_2 emissions. The release CO_2 in atmosphere is either absorbed by the carbon 'sinks' literally known as oceans (~24%) and land (~30%), or retained in the atmosphere (~46%) (Le Quéré et al., 2009; Peters et al., 2017). The anthropogenic activities have led to perturbation in the global carbon cycle and have imbalanced the source and sink balance. The budget imbalance for 2016 was about 6% of total CO_2 emission equivalent to 2.2 $GtCO_2$/yr (Houghton and Nassikas, 2017; Le Quéré et al., 2017).

Fuel combustion is the big player of CO_2 emissions which account for 58% of world the share (IEA Statistics, 2017). This statistic varies greatly by each country depending on national economic structure and development as for Annex I countries it is responsible for as much as three quarters of emissions.

2 Biofuel

With growing concerns surrounding the limitation on the availability of fossil fuels, and their pollutions and influential effects on global warming, the need for a clean, sustainable and efficient chemical production platform for biofuels has received considerable interest of the society (Christenson and Sims, 2011). Plant crops, microorganism (such as algal biomass), non-food lignocellulose, can be source for biofuel production as well as wastes such as agricultural waste, animal manures, cooked oil, organic wastes, etc (Lastella et al., 2002). The use of crop-based biofuel in not appropriate for all regions since it needs arable land for cultivation besides completion with food production. Lignocellulose is the most abundant source of sugar and biomass on the earth and own about three quarters of world sugar deposit (Peralta-Yahya et al., 2012)

Nowadays, the studies for engineering microorganisms to produce energy- rich compounds such as biofuels has been verified and applicable. Biofuels produced by microorganisms have similar property to conventional oils and are able to participate in transportation market. Transport sector account for 30% of global energy consumption, 50% of oil consumption, and approximately 25% of emitted CO_2 from combustion of fossil fuels (Araújo et al., 2017). Therefore, it is a big player in energy consumption market and greenhouse gas (GHG) emissions. Advanced biofuel, as a clean alternative energy

source, are promising approach and solution for both energy concern and environmental issues as in future they can be regarded as complementary energy as well (Su et al., 2017). However, producing this fuels from live species requires metabolically engineering to enhance their yields. Synthetic biology and data driven systems biology are approaches that helps to optimally engineer metabolic pathways to maximize biomass and biofuel productions.

Carbon dioxide and carbon monoxide from industrial plants, atmosphere, can be fixed and metabolized by some microorganisms (Peralta-Yahya et al., 2012)

Biofuel can result in energy security and economic flourishing as well as mitigating the environmental burdens (DOE, 2016). However, several challenges and limitation impedes the current development and expansion of liquid biofuel commercialism such as meeting GHG emission at least 50% less than conventional transportation fuels during life cycle assessment (DOE, 2016), feedstock deficiency (Su et al., 2017), land availability (Darzins et al., 2010), and water scarcity (Arnell et al., 2011; Jakob and Steckel, 2016). Land requirement can threaten food security and biodiversity (Jakob and Steckel, 2016).

The socioeconomically remarkable production of biodiesel from microalgae has been acknowledged as the reliable replacement to dwindling reserves of petroleum diesel and as well as first and second generations of biodiesels. Algae, as aquatic biomass feedstocks, are accredited because of their distinctive characteristics (Vargas e Silva and Monteggia, 2015) such as low carbon emissions, food security, sustainability, non-competitiveness for land, high biomass production rates, and environmental bioremediation such as wastewater treatment and fixation of the atmosphere CO_2 or flue gas (Demirbas, 2010; Gouveia and Oliveira, 2009; Hu et al., 2008; Kumar et al., 2010; Scott et al., 2010; Singh et al., 2011; Suehara et al., 2005; Wang et al., 2008; Wu et al., 2012; Zeng et al., 2011; Zhu et al., 2013).

3 Introduction to Metabolic Engineering

Advance of high throughput technologies to mine data from biological processes has yielded billions of data at the gene, protein, and reaction echelons (Mahadevan and Schilling, 2003). This flux of information has triggered the development of modern

technologies to analyze and interpret the biological phenomena at larger scale but with significant depth.

3.1 Genome Scale Metabolic Reconstructions

The reconstruction of genome scale metabolic network had a revolutionary influence in microbial metabolic engineering and nowadays are regularly employed for design and analysis (de Oliveira Dal'Molin et al., 2011). This models have filled the space between genotype and phonotype in an organized and mechanistic framework.

In fact, genome-scale metabolic models are as a framework for constraint-based stoichiometric models to simulate flux distribution (Llaneras and Picó, 2008). By making some reasonable assumptions, the flux landscape of a microorganism can be investigated.

3.2 Stoichiometric Models

In early nineteen, several studies developed techniques to describe metabolic flux landscape and mechanistically elucidate the complexity of cell growth, and product secretion in microbial systems (Fell and Small, 1986; Mavrovouniotis and Stephanopoulos, 1992; Savinell and Palsson, 1992; Varma et al., 1993; Varma and Palsson, 1993, 1994). This strategy and technique has presented valuable information of how cells organized its cellular fluxes to gain optimal growth. Besides, these types of model have been used usefully for studying genetic engineering and pathway modeling. We are able to perform in silico monitoring how cellular fluxes change in response to a stress, nutrient deficient situation, and how it adapts itself to synthesize a product or degrade a compound (Pramanik and Keasling, 1997). Hopefully, prior knowledge of reaction kinetics, parameters, and enzyme mechanism is not required, and this models are based on stoichiometry of the reactions (Pramanik and Keasling, 1997).

3.3 Flux Balance Analysis (FBA)

Flux balance analysis (FBA) is the most famous approach which is based on reaction stoichiometry for studying genome scale models. The set of FBA and reconstructed networks permits us to survey cell metabolism, simulate cellular growth and flux distribution that is related to network structure and physiological constraints. Also, FBA is

very reliable framework to be upgraded and equipped with other constraints for in silico metabolic pathway design and analysis.

The essential requirement for e metabolic network reconstruction is a list of biochemical species (metabolites), intracellular reactions, and stoichiometric coefficients of metabolite in reactions (Henson and Hanly, 2014; Kauffman et al., 2003). It is assumed that the intracellular accumulation of metabolite is negligible, therefore uptake and produced flux of metabolite are same. This implies steady states of mass balance inside cell:

$$dC/dt = S.V = 0 \quad (1)$$

where c is the metabolite concentration, V is an r×1 vector of fluxes through the r reactions, and S is m×r matrix of the stoichiometric coefficients for the r reactions and m metabolites reactions in the network. Coefficient for consumed metabolite is negative and for produced one is positive.

Capacity constraints are imposed on space of fluxes. These constraints can be maximum flux for uptake reactions or physiological constraints derived from -*omic* data such as transcriptomic, metabolomics, thermodynamic, proteomic, and regulatory data.

The main outcome is defining a solution space and reduction of an n-dimensional polytope solution space into biologically feasible space and. Because for many of metabolic networks, number of unknown flux is more than metabolite, the system is underdetermined (Henson and Hanly, 2014). To find the optimal solution through space, an objective function is defined to solve the linear equations (Schuetz et al., 2007). Most common objectives are growth rate optimality to which cell use available budget to maximize growth rate

The growth rate μ is calculated as the weighted sum of the fluxes contributing to biomass formation.

The growth rate μ is a weighted summation of precursors that contribute in cell biomass generation

$$\max \ \mu = w^T v$$
$$Av = 0 \qquad (2)$$
$$v_{min} \le v \le v_{max}$$

Where v_{min} and v_{max} are lower and upper bound of the fluxes, respectively. Figure 1-1 shows a graphical explanation of how FBA attains its goal.

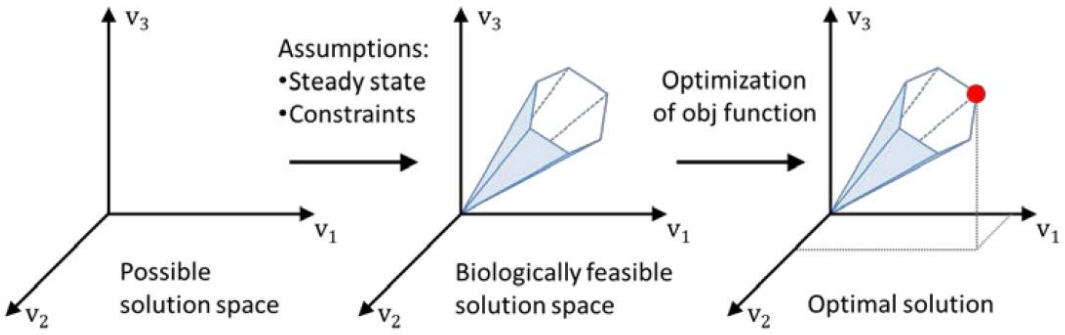

Figure 1-1: The conceptual basis of constraint-based modeling (Schilling et al., 1999)

3.4 Dynamic Flux Balance Analysis (DFBA)

Intracellular metabolism and cell growth as a complex system behave highly dynamics because of dynamic nature of fed-batch and batch cultures (Antoniewicz, 2013) . In fact, cell needs to dynamically adapt itself to changing extracellular (environment). A key assumption made for intracellular metabolism is pseudo-steady state, while the time for extracellular is longer to equilibrate with cell environment (Jouhten et al., 2012). Flux balance analysis only allows study of intracellular flux distributions but a methodology is needed to simulate cell metabolism under dynamic condition by combining extracellular to intracellular metabolism. Unstructured models are not able to portray a detailed representation of cell whereas dynamic genome scale models inherently reflect cellular dynamics and eventually lead to optimal control profile of processes (Hjersted and Henson, 2006). Macroscopic kinetic model threat cell as a black box and has been used for simple phenomena such as bacterial growth or substrate/product inhibition (Anesiadis

et al., 2013). Dynamic flux balance analysis (DFBA) modelling (Mahadevan et al., 2002) as a microscopic framework introduced to overcome the shortcoming associated with macroscopic unstructured models by providing a detailed metabolic models as well as the absence of enzyme kinetic (Hjersted and Henson, 2006; Jeong et al., 2016). Also, due to its dependability on genome scale models it can be used in larger operational range (Jeong et al., 2016).

3.5 The Application of Genome Scale Models and Thermodynamics in Metabolic Engineering

Metabolic network reconstruction is a promise to discover the unexplored metabolic capabilities of organisms which can help us in analysing organism at system level and its biological network properties, in model-driven discovery, metabolic engineering and strain design, and to predict metabolic phenotypes.

Using genome scale models (GEMs) as scaffold for metabolic engineering, we can search in silico to find essential genes or reactions whose knock-out and addition will disable or enable specific biological function. In addition, combinatory gene manipulations can be done in silico to target a biological function or design new strain with different biological phenotype.

Overwhelming amount and availability of transcriptomic, proteomic, and metabolomics data as well as GEMs platform help to utilized these multi omics data and lead to development of a significant systemic representation of metabolism. These data can be integrated to model as constraints solution space and make the model more real. Traditional metabolic networks mainly are simulated based on mass balance where as other constraints are required. A necessary constraint is thermodynamics constraints in which thermodynamically infeasible reactions are eliminated through determination of reaction reversibility and directionality, consequently, feasible solution space would be reduced. This can influence in silico gene manipulation results.

Reference

Anesiadis, N., Kobayashi, H., Cluett, W.R., Mahadevan, R., 2013. Analysis and design of a genetic circuit for dynamic metabolic engineering. ACS synthetic biology 2(8), 442-452.

Antoniewicz, M.R., 2013. Dynamic metabolic flux analysis—tools for probing transient states of metabolic networks. Current opinion in biotechnology 24(6), 973-978.

Araújo, K., Mahajan, D., Kerr, R., Silva, M.d., 2017. Global Biofuels at the Crossroads: An Overview of Technical, Policy, and Investment Complexities in the Sustainability of Biofuel Development. Agriculture 7(4), 32.

Arnell, N.W., van Vuuren, D.P., Isaac, M., 2011. The implications of climate policy for the impacts of climate change on global water resources. Global Environmental Change 21(2), 592-603.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology advances 29(6), 686-702.

Darzins, A., Pienkos, P., Edye, L., 2010. Current status and potential for algal biofuels production. A report to IEA Bioenergy Task 39.

de Oliveira Dal'Molin, C.G., Quek, L.-E., Palfreyman, R.W., Nielsen, L.K., 2011. AlgaGEM–a genome-scale metabolic reconstruction of algae based on the Chlamydomonas reinhardtii genome, BMC genomics. BioMed Central, p. S5.

Demirbas, A., 2010. Use of algae as biofuel sources. Energy conversion and management 51(12), 2738-2749.

Dlugokencky, E., Tans, P., 2016. Trends in atmospheric carbon dioxide, NOAA/ESRL.

DOE, 2016. National Algal Biofuels Technology Review (U.S. Department of Energy). . U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office.

Donat, M.G., Lowry, A.L., Alexander, L.V., O'Gorman, P.A., Maher, N., 2017. Addendum: More extreme precipitation in the world's dry and wet regions. Nature Climate Change 7(2), 154-158.

Faried, M., Samer, M., Abdelsalam, E., Yousef, R., Attia, Y., Ali, A., 2017. Biodiesel production from microalgae: Processes, technologies and recent advancements. Renewable and Sustainable Energy Reviews 79, 893-913.

Fell, D.A., Small, J.R., 1986. Fat synthesis in adipose tissue. An examination of stoichiometric constraints. Biochemical Journal 238(3), 781-786.

Gouveia, L., Oliveira, A.C., 2009. Microalgae as a raw material for biofuels production. Journal of industrial microbiology & biotechnology 36(2), 269-274.

Henson, M.A., Hanly, T.J., 2014. Dynamic flux balance analysis for synthetic microbial communities. IET systems biology 8(5), 214-229.

Hjersted, J.L., Henson, M.A., 2006. Optimization of Fed-Batch Saccharomyces cerevisiae Fermentation Using Dynamic Flux Balance Models. Biotechnology progress 22(5), 1239-1248.

Houghton, R.A., Nassikas, A.A., 2017. Negative emissions from stopping deforestation and forest degradation, globally. Global change biology.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The plant journal 54(4), 621-639.

IEA Statistics, 2017. CO_2 emissions from fuel combustion-highlights. IEA, Paris https://www.iea.org/publications/freepublications/publication/CO2EmissionsfromFuelCombustionHighlights2017.pdf.

Jakob, M., Steckel, J.C., 2016. Implications of climate change mitigation for sustainable development. Environmental Research Letters 11(10), 104010.

Jeong, D.H., Yoo, S.J., Kim, J.H., Lee, J.M., 2016. Computationally efficient dynamic simulation of cellular kinetics via explicit solution of flux balance analysis: xDFBA modelling and its biochemical process applications. Chemical Engineering Research and Design 113, 85-95.

Joos, F., Spahni, R., 2008. Rates of change in natural and anthropogenic radiative forcing over the past 20,000 years. Proceedings of the National Academy of Sciences 105(5), 1425-1430.

Jouhten, P., Wiebe, M., Penttilä, M., 2012. Dynamic flux balance analysis of the metabolism of Saccharomyces cerevisiae during the shift from fully respirative or respirofermentative metabolic states to anaerobiosis. The FEBS journal 279(18), 3338-3354.

Kauffman, K.J., Prakash, P., Edwards, J.S., 2003. Advances in flux balance analysis. Current opinion in biotechnology 14(5), 491-496.

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., Van Langenhove, H., 2010. Enhanced CO 2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in biotechnology 28(7), 371-380.

Lastella, G., Testa, C., Cornacchia, G., Notornicola, M., Voltasio, F., Sharma, V.K., 2002. Anaerobic digestion of semi-solid organic waste: biogas production and its purification. Energy conversion and management 43(1), 63-75.

Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A.C., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Jackson, R.B., Boden, T.A., Tans, P.P., Andrews, O.D., Arora, V.K., Bakker, D.C.E., Barbero, L., Becker, M., Betts, R.A., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Cosca, C.E., Cross, J., Currie, K., Gasser, T., Harris, I., Hauck, J., Haverd, V., Houghton, R.A., Hunt, C.W., Hurtt, G., Ilyina, T., Jain, A.K., Kato, E., Kautz, M., Keeling, R.F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metzl, N., Millero, F., Monteiro, P.M.S., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S.I., Nojiri, Y., Padín, X.A., Peregon, A., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B.D., Tian, H., Tilbrook, B., van der Laan-Luijkx, I.T., van der Werf, G.R., van Heuven, S., Viovy, N., Vuichard, N., Walker, A.P., Watson, A.J., Wiltshire, A.J., Zaehle, S., Zhu, D., 2017. Global Carbon Budget 2017. Earth Syst. Sci. Data Discuss. 2017, 1-79.

Le Quéré, C., Raupach, M.R., Canadell, J.G., Marland, G., Bopp, L., Ciais, P., Conway, T.J., Doney, S.C., Feely, R.A., Foster, P., 2009. Trends in the sources and sinks of carbon dioxide. Nature Geoscience 2(12), 831-836.

Lehmann, J., Coumou, D., Frieler, K., 2015. Increased record-breaking precipitation events under global warming. Climatic Change 132(4), 501-515.

Llaneras, F., Picó, J., 2008. Stoichiometric modelling of cell metabolism. Journal of bioscience and bioengineering 105(1), 1-11.

Lotze-Campen, H., Lampe, M., Kyle, P., Fujimori, S., Havlik, P., Meijl, H., Hasegawa, T., Popp, A., Schmitz, C., Tabeau, A., 2014. Impacts of increased bioenergy demand on global food markets: an AgMIP economic model intercomparison. Agricultural Economics 45(1), 103-116.

Mahadevan, R., Edwards, J.S., Doyle III, F.J., 2002. Dynamic flux balance analysis of diauxic growth in Escherichia coli. Biophysical journal 83(3), 1331-1340.

Mahadevan, R., Schilling, C., 2003. The effects of alternate optimal solutions in constraint-based genome-scale metabolic models. Metabolic engineering 5(4), 264-276.

Mavrovouniotis, M., Stephanopoulos, G., 1992. Synthesis of biochemical production routes. Computers & chemical engineering 16(6), 605-619.

Mudimu, O., Rybalka, N., Bauersachs, T., Born, J., Friedl, T., Schulz, R., 2014. Biotechnological screening of microalgal and cyanobacterial strains for biogas production and antibacterial and antifungal effects. Metabolites 4(2), 373-393.

Nemet, G.F., Holloway, T., Meier, P., 2010. Implications of incorporating air-quality co-benefits into climate change policymaking. Environmental Research Letters 5(1), 014007.

Olivier, J., Schure, K., Peters, J., 2017. TRENDS IN GLOBAL CO_2 AND TOTAL GREENHOUSE GAS EMISSIONS.

Peralta-Yahya, P.P., Zhang, F., Del Cardayre, S.B., Keasling, J.D., 2012. Microbial engineering for the production of advanced biofuels. Nature 488(7411), 320.

Peters, G.P., Le Quéré, C., Andrew, R.M., Canadell, J.G., Friedlingstein, P., Ilyina, T., Jackson, R.B., Joos, F., Korsbakken, J.I., McKinley, G.A., 2017. Towards real-time verification of CO 2 emissions. Nature Climate Change 7(12), 848.

Pramanik, J., Keasling, J., 1997. Stoichiometric model of Escherichia coli metabolism: incorporation of growth-rate dependent biomass composition and mechanistic energy requirements. Biotechnology and bioengineering 56(4), 398-421.

Salzmann, M., 2016. Global warming without global mean precipitation increase? Science advances 2(6), e1501572.

Savinell, J.M., Palsson, B.O., 1992. Network analysis of intermediary metabolism using linear optimization. I. Development of mathematical formalism. Journal of theoretical biology 154(4), 421-454.

Schilling, C.H., Schuster, S., Palsson, B.O., Heinrich, R., 1999. Metabolic pathway analysis: basic concepts and scientific applications in the post-genomic era. Biotechnology progress 15(3), 296-303.

Schuetz, R., Kuepfer, L., Sauer, U., 2007. Systematic evaluation of objective functions for predicting intracellular fluxes in Escherichia coli. Molecular systems biology 3(1), 119.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G., 2010. Biodiesel from algae: challenges and prospects. Current opinion in biotechnology 21(3), 277-286.

Singh, A., Nigam, P.S., Murphy, J.D., 2011. Renewable fuels from algae: an answer to debatable land based fuels. Bioresource technology 102(1), 10-16.

Su, Y., Song, K., Zhang, P., Su, Y., Cheng, J., Chen, X., 2017. Progress of microalgae biofuel's commercialization. Renewable and Sustainable Energy Reviews 74, 402-411.

Suehara, K.-i., Kawamoto, Y., Fujii, E., Kohda, J., Nakano, Y., Yano, T., 2005. Biological treatment of wastewater discharged from biodiesel fuel production plant with alkali-catalyzed transesterification. Journal of Bioscience and Bioengineering 100(4), 437-442.

United Nations, 2017. World Population Prospects: The 2017 Revision (Department of Economic and Social Affairs, Population Division). New York: United Nations.

Vargas e Silva, F., Monteggia, L.O., 2015. Pyrolysis of algal biomass obtained from high-rate algae ponds applied to wastewater treatment. Frontiers in Energy Research 3, 31.

Varma, A., Boesch, B.W., Palsson, B.O., 1993. Stoichiometric interpretation of Escherichia coli glucose catabolism under various oxygenation rates. Applied and environmental microbiology 59(8), 2465-2473.

Varma, A., Palsson, B.O., 1993. Metabolic capabilities of Escherichia coli: I. Synthesis of biosynthetic precursors and cofactors. Journal of theoretical biology 165(4), 477-502.

Varma, A., Palsson, B.O., 1994. Metabolic flux balancing: basic concepts, scientific and practical use. Nature Biotechnology 12(10), 994.

Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO_2 bio-mitigation using microalgae. Applied microbiology and biotechnology 79(5), 707-718.

Westra, S., Alexander, L.V., Zwiers, F.W., 2013. Global increasing trends in annual maximum daily precipitation. Journal of Climate 26(11), 3904-3918.

Wu, L.F., Chen, P.C., Huang, A.P., Lee, C.M., 2012. The feasibility of biodiesel production by microalgae using industrial wastewater. Bioresource Technology 113, 14-18.

Zeng, X., Danquah, M.K., Chen, X.D., Lu, Y., 2011. Microalgae bioengineering: from CO 2 fixation to biofuel production. Renewable and Sustainable Energy Reviews 15(6), 3252-3260.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water research 47(13), 4294-4302.

CHAPTER 2

A Critical Review on Life Cycle Analysis (LCA) of Algae Biodiesel: Current Challenges and Future Prospects

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1 ABSTRACT

Life cycle analysis of algal biodiesel production provide a quantitative measure of process sustainability and can identify opportunities to improve the process. Yet, studies in the comparing the energy and environmental advantages of algae derived biodiesel versus competing petroleum diesel using life cycle analysis (LCA) show fundamental differences in conclusions. The discrepancies are typically due to differences in type and handling of raw materials, strain composition, reactor configuration, technologies in the biodiesel production chain, system boundaries, and the baseline process for comparing the energy demand and environmental footprints.

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In this review, the advantages and disadvantages of algal biodiesel are reviewed. The impact of different algae biodiesel process variables such as algae type, process, energy and material sources, and material routes on process performance and environmental impact are systematically analyzed. Some of the variables that were studies in the life cycle analysis such as the choice of system boundaries and temporal units are subjective which make their environmental impact inconclusive in the literature studies. Common measures such as energy efficiency (ratio of produced energy to input energy) and environmental footprints (such as GHG emission per mass of biodiesel produced) were chosen in literature to quantify the impact of system variables on environment. However, important variables such as the effect of nutrients on water pollution (through eutrophication), infrastructure, transportation, and electricity sources have been overlooked in the literature. We show the origins of discrepancies in the literature, followed by methodologies to avoid these disagreements. An organized basis for proper identification of the life cycle analysis and sustainability assessment of algal biodiesel is presented in this review manuscript which will considerably help future research studies.

Keywords: Algal biodiesel, Life cycle assessment, green process, sustainability, energy efficiency, global warming, greenhouse gases, CO_2 mitigation

2 Introduction

As population growth and subsequent energy demand increases, depletion of fossil fuel resources combined with the associated environmental impacts of fossil fuel extraction and use are driving the demand for diversity in energy sources. The nonrenewable aspect of fossil fuels (oil, natural gas, and coal resources) requires expansion of the world energy supply. Given that the fossil fuel infrastructure of the world, the challenge is developing sustainable fuels in the short term that can be integrated into this infrastructure without major modifications, biofuels are one option that satisfy this criteria as predictions indicate world energy demand will still be dominated by the fossil fuels in 2040 (IEA/IRENA 2017).

In order for energy to be deemed “sustainable” the source should be secure, socially responsible, protect the environment, and promote economic growth (Omer, 2008). Renewable energy sources (hydro, geothermal, wind, solar, and biofuels) are sustainable and are part of the energy mix required to meet the growing energy demand. However, in the short term, in addition to the ability to integrate into existing fossil fuel infrastructure, selection criteria in renewable energy production method are: contribution to total energy production, technology maturity, reliability of the energy supply, greenhouse gas emission, impact on amenity, area and water requirements, energy production cost, energy transfer efficiency, contribution to economy, and social acceptance (Evans et al., 2009; Troldborg et al., 2014). The 2015 energy statistics (IEA Statistics, 2017) show a contribution of 13.4 % by renewable energy to the fuel share of world primary energy supply (including fossil fuel, renewable, and nuclear); the majority of renewable energy was provided from biofuel and waste-derived bioenergy which together reached 70.7 % of the renewable energy supply. Solid biofuels and charcoal contributed to 63.7 % of the energy from bio resources. In the global renewable energy scenario, it is the targeted goal to produce nearly half of the supply energy from renewable resources (Kralova and Sjöblom, 2010). Biofuels are the main contributors to the global renewable energy market by providing heat, power, electricity, and fuel for transportation. Although the majority of the produced bioenergy are currently solid biofuels and charcoal, the use of liquid biofuels in transportation fuel is increasing. Transport sector accounts for 30 % of the global energy consumption and 25 % of the emitted CO_2 from combusting fossil fuels (Araújo et al., 2017). In 2016, 135 billion liters of liquid biofuels (mainly ethanol and biodiesel) were supplied that is equal to about 4 % of the global road transport fuels (REN21, 2017). Ethanol biofuel and biodiesel are alternatives for gasoline and petroleum diesel, respectively. (Su et al., 2017). Lower production cost, CO_2 fixation, and sustainability advantages are among primary incentives in improving biofuel utilization (Franco et al., 2015; Randhawa et al., 2017).

A comprehensive comparison of conventional diesel, and biodiesel is available in the literature (Atabani et al., 2012; Yusuf et al., 2011). Advantages of biodiesel include renewability, better biodegradation, lubricant properties, and improved safety and lower pollutant and potential for CO_2 bio-sequestration relative to diesel, Disadvantages include

cost and lower energy content compared to petroleum diesel. In addition, biodiesels have physical properties that cause short-term and long-term problems in terms of mechanical and combustion progress/efficiency (Yusuf et al., 2011). The global annual growth rate of biodiesel between 2005 to 2011 was approximately 37 % (Perez et al., 2014). As with bioethanol, biodiesel has been used as a blend with petroleum diesel. B20 blend (20 % biodiesel and 80 % petroleum diesel) is a common mixture that does not require engine modifications (Mata et al., 2010). Biodiesels sourced from lipids are mixtures of fatty acid alkyl esters (Mata et al., 2010) obtained through chemical transesterification and/or enzymatic conversion of the lipids. In the traditional chemical transesterification the lipids are reacted with methanol or ethanol in the presence of alkaline catalysts such as KOH, or NaOH (Perez et al., 2014). Lipids with high free fatty acid levels (FFA > 5 wt %) require acid pretreatment as the FFA cause unwanted soap formation. Although methanol is more reactive and produces fatty acid methyl esters (FAME) which is more appropriate for combustion engines compared to fatty acid ethyl ester (FAEE, using ethanol), its toxicity and limited supply present a challenge (Perez et al., 2014). The lipids can account for up to 75 % of the total cost of biodiesel (Canakci and Sanli, 2008). The first generation of biodiesel feedstocks were (Pahl, 2008): rapeseed oil (59 % of the biodiesel feedstock), soybean oil (25 %), palm oil (10 %), and sunflower oil (5 %). However, these feedstocks tie into the feed vs fuel debate as they are integral to the food chain of human and animals and the produced energy (biodiesel) sacrifices food market and ecosystem biodiversity (Mata et al., 2010). As a result, alternative feedstocks such as non-edible oils, waste fried oil, animal fat, and grease are potential sources; however, are not available at adequate quantities to be considered a secure feedstocks for large scale production processes (Mata et al., 2010). Microalgae has the potential for sustainability and large scale production (Perez et al., 2014). There are over 50,000 known species of algae (Richmond, 2008) and may contain over 70 % oil (lipid) (Perez et al., 2014). The high lipid content species can achieve an oil yield which is 200 times the yield for best performing vegetable oil (Demirbas and Demirbas, 2011). Microalgae with high oil content can potentially produce greater than 12 kg biodiesel/(m2.yr) (Mata et al., 2010) while rapeseed oil (the most common feedstock) and palm oil (the best performing vegetable source) are less than 0.1 and 0.5 kg biodiesel/(m2.yr), respectively, with 120 and 20 times

land use compared to microalgae with high oil content (Mata et al., 2010). In addition to high productivity, low land usage, and food security, other advantages of algae biodiesel include potential for simultaneously wastewater treatment and CO_2 bio-sequestration, short growth cycle, and numerous value-added by-products (Demirbas, 2010; Gouveia and Oliveira, 2009; Hu et al., 2008; Kumar et al., 2010; Scott et al., 2010; Singh et al., 2011; Suehara et al., 2005; Wang et al., 2008; Wu et al., 2012; Zeng et al., 2011; Zhu et al., 2013). Despite the benefits of algae biodiesel, it is still expensive and cannot economically compete with petroleum diesel. Furthermore, glycerol is co-produced with biodiesel at a rate of 10 % of biodiesel production (Quispe et al., 2013) and there is limited market demand for glycerol. Its storage and transportation can also increase the biodiesel production cost; novel/potential applications of glycerol could enhance the economies of biodiesel (Quispe et al., 2013).

A detailed life cycle analysis (LCA) of the algae biodiesel process is required to assess its true performance. We were not able to find a comprehensive study in the literature that includes the complete process from pre-culture to final product(s). Further, published work show conflicting results due to discrepancies in the definition of system boundaries, overlooking influencing variables, and the LCA assumptions. Site location, type of strain, infrastructure, transportation, origin of electricity, water and nutrients, co-product allocation method, functional and temporal units, waste management, by-product utilization, and optimal process design and operation should be reassessed to better determine the sustainability of the algae biodiesel processes.

In this paper a review of the development of renewable and sustainable energy, and why these resources should be in energy basket of countries are presented, followed by an overview of the process of algae to biodiesel. First, we discuss the global and regional trend of energy consumption as well as the greenhouse gas (GHG) emission associated with conventional oil resources. This section presents a primary statistic of current and future environmental issues mostly subjected to fossil consumption. Then, the general overview of algae to biodiesel processes and technology will be discussed. The proposed framework of LCA is then discussed followed by a review of the energy metrics and environmental impacts used in the process analyses. Impacts associated with

transportation of the final product on the LCA analysis is also included. In this review, we gathered 31 papers that study the LCA, covering a major part of the overall algal biodiesel process in such life cycle analysis. There are numerous other studies available in the literature on algae biodiesel process, but a majority of them have focused on a specific unit and they may not give a realistic LCA for the complete process.

2.1 Energy and environment issue:

In 2015, about 13,647 million ton oil-equivalent (mtoe) energy supplied the total primary energy supply (TPES) of which, 81.5 % was solely from the fossil fuels (IEA Statistics, 2017). The dependency of TPES on the fossil fuels in 1971 and 2015 were 86 % and 82 %, respectively (IEA World Energy Balance, 2017). This is a relatively small change after 44 years. Figure 2-1 demonstrates total primary energy consumption (TPEC) share of six distinct regions in the world (Middle East, Africa, Asia Pacific, North America, South and Central America, and Europe and Eurasia) along with their fuel consumption shares in the TPEC in 2016 (British Petroleum, 2017). In Figure 2-1, the inner circle represents the 2016 energy consumption and the outer red arc denotes the contribution of each area to TPEC (in percentage and mtoe). In addition, in the outermost arc, the amount of CO_2 emission to atmosphere due to their energy consumption is plotted (British Petroleum, 2017). Based on the British Petroleum review (British Petroleum, 2017) nearly three-quarters of the global coal is consumed in Asia. Hydroelectricity is dominated by South and Central America while Europe and Eurasia are the world lead in nuclear and renewable energies. China shows reduced energy consumption due to transition to less energy-intensive industries, policies to reduce the coal consumption, and increased investment in renewable energies (IEA Statistics, 2017). As shown in Figure 2-1, Asia Pacific and Middle East together contribute to about half of the TPEC.

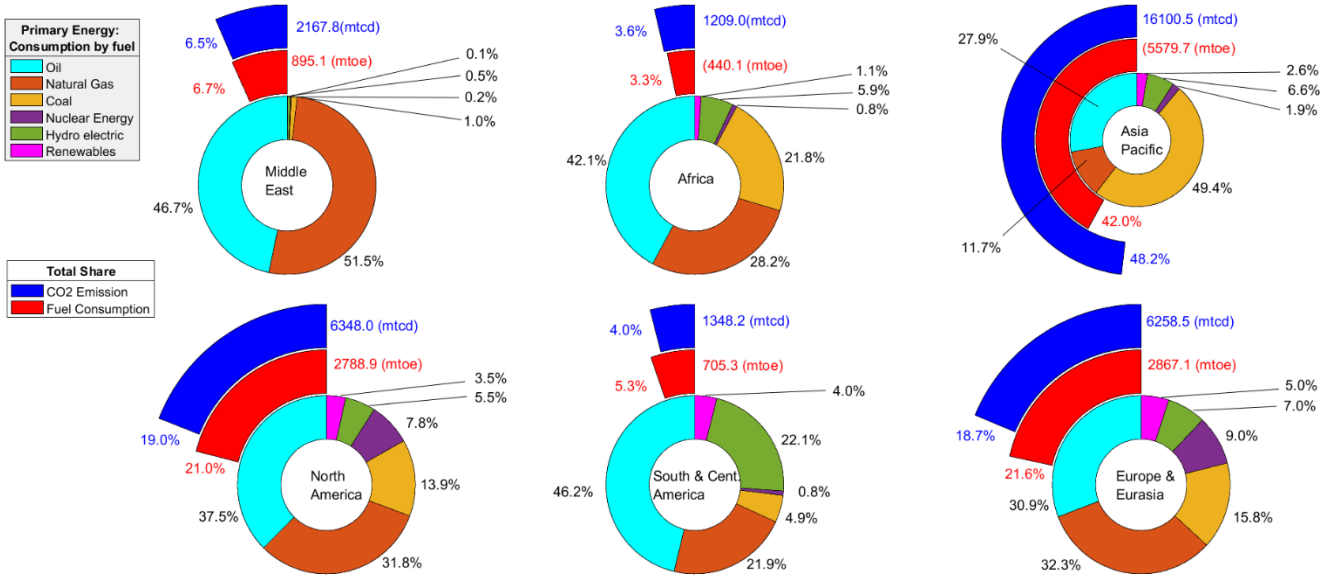


Figure 2-1: Global shares of primary energy consumption distribution by each area for 2016. The outer arcs illustrate the total CO_2 emission (blue: measured in million tonne carbon dioxide, mtcd) and equivalent fossil fuel consumption (red: measured in mtoe) in term of share and quantity by each region. Data adapted from (British Petroleum, 2017)

The major environmental impacts in energy production life cycle are global climate change, water pollution, air pollution, land use (and pollution), acid rain, ozone depletion, solid waste deposition, and radioactive pollutions (Dincer, 2000); the impacts from burning fuels (especially fossil fuels) dominate. Among all the impacts, air pollution and global warming by greenhouse gases (especially CO_2) have been more extensively studied. About 72 % of the total GHGs entering atmosphere are CO_2 and the remaining 28 % are gases such as methane (19 %), nitrous oxide (6 %), and fluorinated gases (3 %) (Olivier et al., 2017). The global CO_2 emissions (in million ton carbon dioxide, or mtcd) are plotted along with major fuel consumption in Figure 2-2, covering information in the range 1966 to 2016; it shows the strong correlation between CO_2 emission and major fuel consumption (British Petroleum, 2017). In 2016, the total global GHG emissions was 53.4 giga ton CO_2 equivalent (Gt CO_2 eq) of which 49.3 Gt CO_2 eq (over 92 %) were emitted from fossil fuels and industrial processes (Le Quéré et al., 2017; Olivier et al.,

2017). The share of CO_2 in the GHGs emitted in 2016 was 40.8 Gt CO_2 of which 36.18 Gt CO_2 were attributed to the fossil fuels and industry (Le Quéré et al., 2017). The CO_2 released in the atmosphere can be absorbed by oceans (~24 %) and land (~30 %). Fuel combustion alone accounts for 58 % of the global CO_2 emissions (IEA Statistics, 2017). The Paris Agreement (effective from 2016) has imposed scenarios for reduction of the GHG emissions to lower the global average temperature to 2 ˚C above the pre-industrial levels by 2050 (IPCC, 2015). The CO_2 emission reduction by average 3.5 % per year to a final value of 9 Gt CO_2 is required by 2050 to fulfill the Paris Agreement terms; this value is about 30 % of the current level of CO_2 in the atmosphere (IEA/IRENA 2017). The majority of CO_2 emission reduction is envisioned to be through enhancing the energy efficiency and utilizing renewable energy resources—together contributing to 75 % of the reduction. Carbon capture is expected to account for an additional 14 % CO_2 reduction (IPCC, 2015).

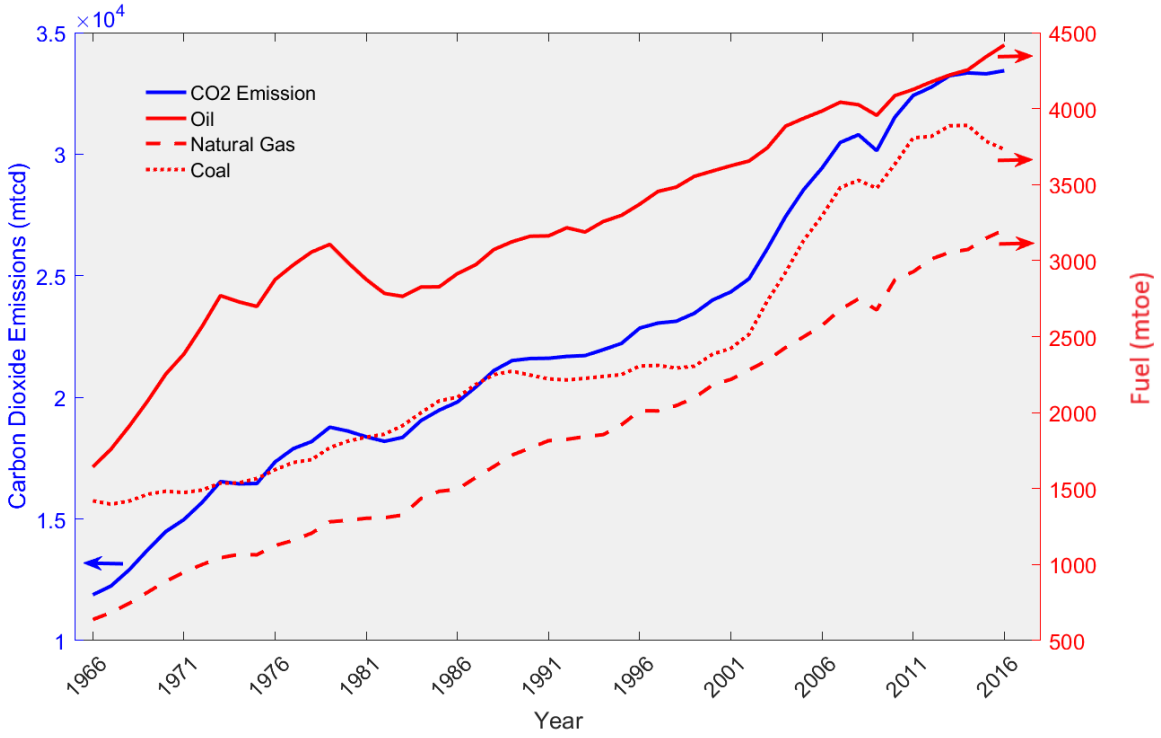


Figure 2-2: Global CO_2 emissions, measured in million tonnes carbon dioxide (mtcd), from fossil fuel since 1966 shown in primary y-axis. Fossil fuel consumption as equivalent of three major resource also expressed in mtoe in the secondary y-axis. Crude oil, shale oil, oil

sands and NGLs are also included in oil resource. Data adapted from (British Petroleum, 2017)

Biofuels are considered an alternative to the fossil fuels but as indicated above have lower energy content, and may also threat biodiversity (deforestation) and food security through decreased land and biomaterial availability and increased price (Mata et al., 2010) (Lotze-Campen et al., 2014).

3 Algae biodiesel production process overview

In most commercial applications, the algae is cultured in a bioreactor, and after growing to appropriate biomass density, the algae is harvested. The algae cells are separated from the medium through flocculation and dewatering, followed by drying. The resultant biomass can be thermochemically or chemically treated to extract the lipids. In the case of chemical conversion, lipids are extracted through an oil extraction process followed by transesterification to convert the lipid to biodiesel. If thermochemical path is selected, the biomass is directly converted to biocrude or similar product. Cultivation, harvesting, and lipid extraction from biomass are usually planned to be underwent at the same site or the extracted oil is transported to another place for biodiesel conversion process.

The remaining residue may be used as a precursor in anaerobic digestion process, as substrate to produce methane to generate bioelectricity and heat, or as a feed in a combined heat and power (CHP) system to produce electricity or heat. The CHP is a single stage (end user) system that generates electricity and converts the thermal energy to steam or hot water (Balli et al., 2008; Borbely and Kreider, 2001).

A process flow diagram for the algal biodiesel production process is shown in Figure 2-3 and includes the essential units for cultivation, processing of algal biomass, extraction of fatty acids, conversion of algal biofuel, and defatted biomass post processing are demonstrated. In fact, this chain represents the most common technologies. For, example, the dry processes which will be discussed on defatted biomass are normally used as separate route for biofuel production from biomass rather than a supplementary route for defatted biomass conversion.

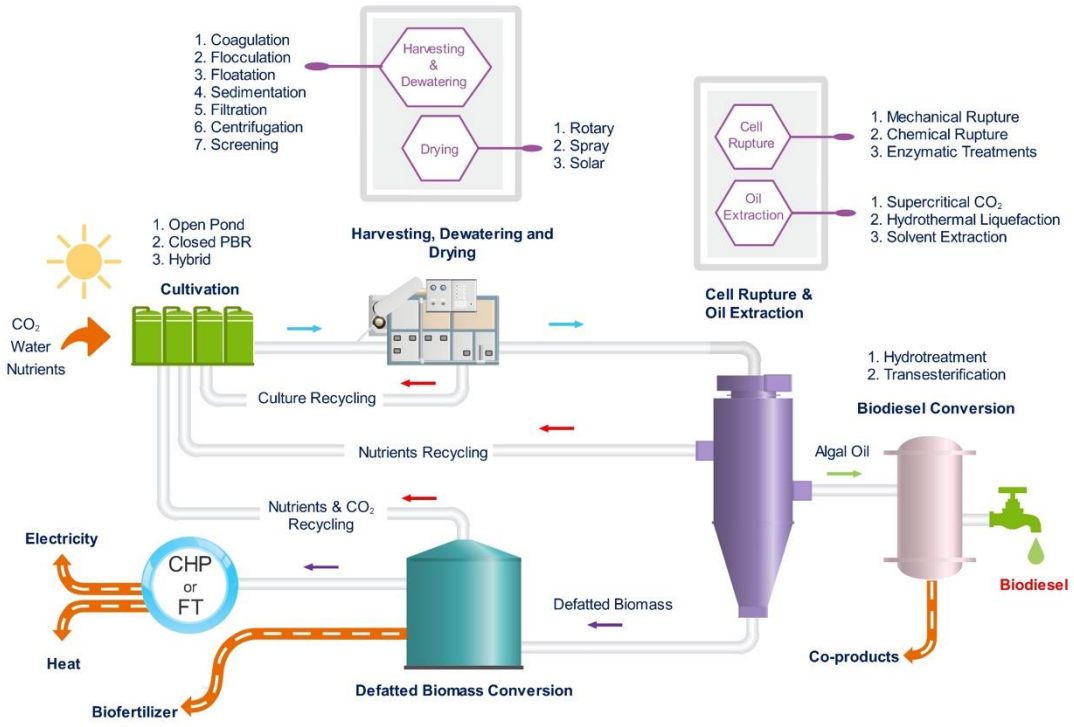


Figure 2-3: Process flowchart for production of microalgal biodiesel

3.1 Strain selection

There are two variables that greatly influence the performance of an algae biodiesel plant; the selection of algae species and the location of plant. Variables such as temperature, humidity, sunlight exposure, land type of region, natural and indigenous algae type, proximity to feedstock, availability of water resources, and nutrients requirements are a function of plant location, plant design, and production conditions (Borowitzka, 1999; Kovacevic and Wesseler, 2010; Speranza et al., 2015). The geographical distribution of the studies in this research field was reviewed in Table 2-1. We have reviewed a total of 31 studies in the context of algae to biodiesel conversion that cover the majority of processes used. Nearly half of the research studies (15 cases) were conducted in the USA.

TABLE 2-1: Scope and system boundaries and geographical location of LCA studies

Rank	Ref.	Algae Type	System Boundaries*	Functional Unit (FU)	Infrastructure	Transportation	Electricity Source	Allocation	Operation Time (day)	Time Horizon (year)	Location	Year
1	Adesanya et al.	Chlorella vulgaris	WTG	Production of 1 ton Biodiesel	Yes	Yes	Average UK National Grid	Substitution Energetic	-	205	UK	2014
2	Ajayebi et al.	Chlorella	WTW	kg biodiesel for 1 km traveled	Yes	Yes	-	-	-	-	India	2013
3	Azadi et al.	-	WTG	Production of 1 MJ Biodiesel	Yes	-	Average France National Grid + On-site renewable power generation (e.g., wind, solar)	-	-	20-30 (25)	UK	2014
4	Batan et al.	Nannochloropsis	WTP	Production of 1 MJ Biodiesel	-	Yes	Average US electricity mix Northeast electricity mix California electricity mix	Substitution Energetic Market	300	-	USA	2010
5	Campbell et al.	Dunaliella salina	WTW	Combustion of enough fuel in an articulated truck to transport one tonne of freight one kilometer (1 tonnekilometre is 0.89 MJ of diesel fuel)	-	Yes / -	-	-	-	100	Australia	2011
6	Chang et al.	thraustochytrids	WTW	Production of 1 MJ Biodiesel	-	-	-	Energetic	-	100	Australia	2015
7	Chowdhury et. al	Schizochytrium limacinum	WTG	Production of 1 ton of biodiesel	-	-	Average U.S National Grid	1. No integrate/reuse 2. No Allocation & integrate/reuse 3. Substitution & integrate/reuse	-	-	USA	2012
8	Clarens et. al	salt-tolerant algae species (e.g., Phaeodactylum sp., Tetraselmis sp., etc)	WTW	Annual vehicle kilometers traveled (VKT) per hectare-year	-	Yes	-	Energetic	-	-	USA	2011
9	Collet et. al	Nannochloropsis occulata	WTW	Combustion of 1 MJ of Biodiesel	Yes	-	Euromix	Energetic Mass	240	100	France	2014
10	Delrue et al.	-	WTW	100 km of Transportation	-	-	-	-	-	20	France	2012
11	Delrue et al.	-	-	Production of 1 MJ Biodiesel	-	-	-	-	-	20	France	2013
12	Dutta et al.	Nannochloropsis	WTW	Production of 1 MJ Biodiesel	-	-	Average US National Grid	Energetic Mass	330	10	Portugal / USA	2016
13	Gao et al.	Pleurochrysis carterae	WTG	Production of 1 MJ Biodiesel	Yes	-	-	Substitution	330	30	Australia	2013
14	Hou et al.	-	WTW	Combustion of 1 MJ Biodiesel	-	-	-	Substitution Energetic	-	-	China	2011
15	Khoo et al.	Nannochloropsis sp.	WTG	Production of 1 MJ Biodiesel	-	-	-	-	-	-	Singapore	2011

16	Lardon et al.	Chlorella vulgaris	WTW	Combustion of 1 MJ of Biofuel	Yes	Yes	European energetic mix	Energetic	-	3010	France	2009
17	Pardo-Cárdenas et al.	Chlorella sp	WTW	Production of 1 kg Biodiesel	-	Yes	-	Energetic	-	100	Colombia	2013
18	Passell et al.	Nannochloris sp. and Nannochloropsis sp.	WTW	1 MJ of fuel combusted in a CIDI vehicle	-	-	Average U.S National Grid Average German National Grid	Substitution	-	-	USA	2013
19	Pongsurapipat et al	Chlorellaceae	WTG	Production of 1 kg Biodiesel	-	-	-	Energetic	300	-	Thailand	2016
20	Quinn et al.	Nannochloropsis salina	WTW	Production of 1 MJ Biodiesel	-	Yes	-	Energetic	-	-	USA	2014
21	Resurreccion et al.	Chlorella sp. and Scenedesmus sp. as representative FW species Phaeodactylum sp. and Tetraselmis sp. as representative BSW species	WTW	20,000 vehicle kilometers traveled (VKT)	Yes	-	-	-	365	30	USA	2012
22	Sills et al.	-	WTW	Production of 1 MJ Biodiesel	Yes	-	Average US National Grid	Substitution	360	2010 5	USA	2012
23	Soh et al.	Neochloris oleoabundans Chlorella sorokini Nannochloropsis oculata Tetraselmis suecica	WTG	Production of 1 kg Biodiesel	-	-	-	-	-	-	USA	2014
24	Stephenson et al.	Chlorella vulgaris	WTG	Production of 1 ton of biodiesel	-	Yes	U.K National Grid	Substitution	-	20	UK	2010
25	Woertz et al.	-	WTW	Combustion of 1 MJ of Biofuel	-	Yes	California marginal resource mix	Energetic	300	-	USA	2014
26	Yanfen et al.	Chlorella	WTG	Production of 1 ton Biodiesel	-	-	-	Substitution	-	20	China	2012
27	Yang et al.	Chlorella vulgaris	WTG	Production of 1 kg Biodiesel	-	-	-	Substitution	-	-	USA	2011
28	Yuan et al.	Scenedesmus dimorphus	WTG	Production of 1 MJ Biodiesel	-	-	-	Substitution Economic	-	-	USA	2015
29	Zaimes and Khanna	Chlorella vulgaris	WTG	Production of 1 MJ Biodiesel	-	Yes	Data from EPA's "Power Profiler"	Substitution Energetic	-	-	USA	2013
30	Zaimes and Khanna	-	WTG	Production of 1 MJ Biodiesel	-	-	-	Substitution Energetic Market	-	-	USA	2014
31	Zhang et al.	Scenedesmus dimorphus	WTG	Production of 1 Kg Biomass	-	-	-	-	-	100	USA	2014

* **WTW:** well-to-wheel; **WTG**: well-to-gate; **WTP**: well-to-pump

The early screening criteria in assessing a microalgal biodiesel process is the selection of the strains, ideally strains that are fast growing and highly productive (Griffiths and Harrison, 2009; Rosenberg et al., 2008). Strains with a high lipid content, ability to easily

grow and survive in a given environment, and nutrient availability contribute to the success of a microalgal biodiesel process. Genetic engineering of the key enzymes in the metabolic network of fatty acids (in lipid biosynthesis) has enabled the production of lipids with higher quality and higher production rates of biodiesel (Brennan and Owende, 2010). The growth factors and harvesting procedures will also affect the final lipid productivity of the algae (Singh et al., 2011). The lipid content and growth rate are inversely proportional, or algae with higher lipid content have a lower biomass growth rate (Rösch et al., 2012). Therefore, to achieve the same productivity as a low lipid content algae, a high lipid content algae requires more nutrients (nitrogen and phosphorus) and energy input to produce the same mass of algae biomass over the same time period (Lardon et al., 2009; Rösch et al., 2012). Although an increase in the algae lipid content enhances the efficiency of both the extraction and conversion (to biodiesel) stages, the reduced growth rate will dominate, resulting in an overall decrease in biodiesel productivity (Soh et al., 2014). Therefore, a balance should be made between the lipid content and growth rate when selecting the algae strain for biodiesel (Hou et al., 2011). According to Table 2-1, among the total 31 LCA studies of the algal biodiesel process, six of them have not indicated the cultivation type. A broad range of salt-tolerant algae species was studied by Clarens et. al (Clarens et al., 2011). Given the impact strain has on the overall biodiesel production it would be a key parameter in a comprehensive LCA.

3.2 Algae cultivation unit

A major limitation in the large-scale algae cultivation is related to growing strains at large throughput because the requirement of light (Mata et al., 2010). The main cultivation modes are open-pond (OP) and photobioreactors (PBRs). The ponds are also known as ‘‘raceway’’ configuration, in which the algae, water, and nutrients are circulated by paddlewheels around a racetrack (Demirbas, 2011). The type of a bioreactor controls the biomass concentration; a maximum value of 0.5 g/L in open reactors and 5 g/L in PBRs can be achieved (Vandamme et al., 2013).

Generally, the open systems (OPs) are cheaper to construct and manage and have a longer life-time and higher production capacity, compared to the closed systems (PBRs) (Mata et al., 2010). PBRs have higher surface-to-volume ratio, higher biomass

concentration, shorter harvest time, less evaporative (losses), and also feature better controllability (through variables such as pH, temperature, mixing, and CO_2 and O_2 concentrations). Furthermore, the PBRs are less likely to be contaminated, compared to OPs (Chen et al., 2012; Chisti, 2007; Lee, 2001; Zeng et al., 2011).

Studies were conducted to compare the performance of the open and closed cultivation systems; however, there is not a strong/supporting conclusion about their performance (Resurreccion et al., 2012). In terms of energy demand, the OPs need less energy to yield one functional unit (FU) of biodiesel, compared to the closed systems (Resurreccion et al., 2012); 32 % less energy demand was estimated. The PBRs however produce more energy output per functional unit (Resurreccion et al., 2012). Economic factors such as energy return on investment and net energy ratio (which both quantify the ratio of produced energy to required energy) can assess the success of OPs and PBRs. Based on the energy return on investment, the OP cultivation systems are more desirable (Resurreccion et al., 2012) and have lower greenhouse gas (GHG) emissions, compared to the PBRs systems (Resurreccion et al., 2012; Stephenson et al., 2010). However, space and process control of the system may be a limiting factor in OPs.

A review of different cultivation conditions is given in Table 2-2. Based on the literature, the open systems have contributed to more than 70 % of the cultivation modes in the algae growth. Stephenson et al. (Stephenson et al., 2010) and Delrue et al. (Delrue et al., 2012) used hybrid systems of OP-PBR and Bag PBR was employed by Batan et al. (Batan et al., 2010). About 16 % of the algae cultivation systems reported in the literature was performed through hybrid OP-PBR.

TABLE 2-2: A review of cultivation conditions in microalgal biodiesel production

No	Ref.	Land Type	Cultivation Mode			Water Resource*				Nitrogen Deprivation	Biogenic Carbon
			OP	PBR	Hybrid	F	S	B	W		
1	Adesanya et al.	-	-	-	Hybrid	F	-	-	-	Yes	Yes
2	Ajayebi et al.	Wasteland	OP	-	-	-	S	-	-	-	-
3	Azadi et al.	-	OP	-	-	-	-	-	-	-	-
4	Batan et al.	-		BPR	-	F	-	-	-	-	-
5	Campbell et al.	Arid	OP	-	-	-	S	-	-	-	-
6	Chang et al.	-	-	-	-	-	-	-	-	-	-
7	Chowdhury et. al	-	OP	-	-	F	-	-	-	-	-
8	Clarens et. al	Marginal	OP	-	-	-	-	B	W	-	-
9	Collet et. al	Shrub	OP	-	-	F	S	-	-	Yes	Yes
10	Delrue et al.	-	-		Hybrid	-	-	-	W	-	-
11	Delrue et al.	-	-	-	Hybrid	-	-	-	W	-	-
12	Dutta et al.	-	OP	-	-	-	-	-	-	-	-
13	Gao et al.	Farm	OP	-	-	-	S	-	-	-	-
14	Hou et al.	-	OP	-	-	F	-	-	-	-	-
15	Khoo et al.	-	-	-	Hybrid	F	S	-	-	-	-
16	Lardon et al.	-	OP	-	-	F				Yes	Yes
17	Pardo-Cárdenas et	Coastal	OP	-	-	-	S	-	-	-	-
18	Passell et al.	-	OP	-	-	F	S	-	-	-	-
19	Pongsurapipat et al		OP	-	-	-	-	-	W	-	-
20	Quinn et al.		OP	-	-	-	S	-	-	-	-
21	Resurreccion et al.	-	OP	BPR	-	F	-	B	W	-	-
22	Sills et al.	Coastal	-	-	Hybrid	-	S	-	-	-	-
23	Soh et al.	-	OP	-	-	F	S	-	-	Yes	Yes
24	Stephenson et al.	Degraded	OP	BPR	-	F	-	-	-	Yes	Yes
25	Woertz et al.	-	OP	-	-	-	-	-	W	-	-
26	Yanfen et al.	-	OP	-	-	-	-	B	-	Yes	Yes
27	Yang et al.	-	OP	-	-	F	S	-	W	-	-
28	Yuan et al.	Desert	OP	-	-	-	-	B	-	Yes	Yes
29	Zaimes and Khanna	-	OP	-	-	F	-	-	-	-	-
30	Zaimes and Khanna	-	OP	-	-	F	-	-	-	-	-
31	Zhang et al.	-	OP	-	-	F	-	-	-	-	-

* F=Fresh water, S=Sea or saline water, B=Brackish or groundwater, and W=Wastewater

3.3 Harvesting unit

Harvesting is done using a variety of methods such as coagulation and floatation, flocculation, sedimentation, immobilization, filtration, centrifugation, sedimentation, and ultrasound aggregation. Recent reviews of the advantages and disadvantages of

harvesting methods are available in the literature (Barros et al., 2015; Gupta et al., 2017). Flocculation is extensively employed as it has been shown to reduce the cost and energy requirement of the harvesting, and creates a more concentrated algae medium (Brentner et al., 2011) (Pienkos and Darzins, 2009). In simple gravity sedimentation, the aggregated microalgae cells can be separated easily during flocculation (Vandamme et al., 2013).

Flocculation with the addition of inorganic (Tenney et al., 1969) and organic (König et al., 2014) chemicals is one strategy. Autoflocculation can be achieved by increasing the pH (pH > 9) by adding chemicals that consume the dissolved carbon dioxide in solution (Knuckey et al., 2006). Bio-flocculation is a process where the added micro-organisms (algae, bacteria, and fungi) accelerate the flocculation of suspended algae (Salim et al., 2011). Bio-chemical flocculation using a combination of polymers and proteins (as flocculation enhancing agent) has also been used (Gerde et al., 2013; Muñoz et al., 2015). Flocculation by emitting ultrasound waves (Bussemaker and Zhang, 2013) is another version of the conventional flocculation harvesting. Electrolytic flocculation or electroflocculation (Schlesinger et al., 2012) has been used where an electrostatic field is applied to charge the algae molecules and to facilitate its separation from the solution medium without adding chemicals. Electrodes such as iron or aluminum are conventionally utilized to accelerate the coagulation process (Pearsall et al., 2011). The optimal selection of harvesting method depends on various variables such as cell type, cell density, cell size, downstream process specifications, and the end-value of the product (Brennan and Owende, 2010).

The biomass needs to be concentrated before the oil extraction stage to reduce the processing, oil extraction, and biodiesel conversion costs (Uduman et al., 2010). High biomass density also reduces the water footprint in the process (Schlesinger et al., 2012). Dewatering is one of the most energy demanding harvesting steps requiring approximately 20 % to 40 % of the total energy input (Brentner et al., 2011; Mo et al., 2015). Centrifugation, sedimentation, and flotation use density differences between the cells and the cultivation medium as the driving force while filtration and screening (as particle-constrained systems) use size of permeate as the driving force Differences in the

physical, chemical and electrical properties of the microalgae cells and liquid medium govern the separation efficiency (Pahl et al., 2013).

In the initial harvesting phase (known as bulk harvesting), the weight percentage of biomass is in the range 2 % to 7 % of total suspended solids (TSS). In comparison to other harvesting methods, centrifugation provides several advantages such as the biomass which is recovered through centrifugation has no flocculants or chemicals and exhibits a higher cumulative recovery, recovery rate, and biomass concentration (Gerardo et al., 2015). Harvesting by flotation results in algae particles floating near surface and skimming, rise of the cells be improved by adding surfactants or coagulants (Gerardo et al., 2015). Centrifugation, while high efficiency separation systems are also energy intensive. Filtration-based harvesting techniques have lower energy demands and environmental footprints (Chowdhury et al., 2012; Uduman et al., 2010). Zaimes and Khanna (Zaimes and Khanna, 2013) compared chamber filter press with centrifugation. The chamber filter press reduces the energy consumption by 2.4 % to 21.4 % of the total produced bioenergy, leading to reduction of GHG emissions by 1.7 to 15.0 (g CO_2 eq/MJ biomass). A belt filter press is also less energy intensive than a centrifugation (Sills et al., 2012) however its effectiveness for small algal cells is uncertain (Grima et al., 2003; Sills et al., 2012; Wiley et al., 2011).

3.4 Drying unit

Drying of the algae slurry allow minimizes mass transfer resistances for solvent extraction and decreasing the size of the extraction units (Htet et al., 2013). Various technologies to dry biomass which include different processes, including thermal and solar, utilizing the heat provided from the defatted biomass post-processing stage. The drying of water from the algae cake in the biomass processing unit requires more energy than the dewatering process in the harvesting stage, it is desirable to reduce the water content in the harvesting stage (Milledge and Heaven, 2013; Yoo et al., 2012). Drying is one of the most important processes in the biomass processing unit with its high energy demand, imposing a significant challenge (and additional cost) on the microalgae biodiesel production (Aziz et al., 2014; Lardon et al., 2009; Passell et al., 2013). Microalgae drying consumes about 59.3 % (Yanfen et al., 2012) to 85 % (Aziz et al., 2014) of the total energy

demand. Rotary drying, spray drying, solar heat drying, cross-flow drying, vacuum shelf drying, flashing drying, incinerator drying, and toroidal drying typical methods employed for drying (Show et al., 2015).

The harvested algae is dried using an industrial boiler fed by natural gas (Zaimes et al., 2013; Zaimes and Khanna, 2013). The quantity of combusted natural gas is responsible for the high energy demand and GHG emissions (Delrue et al., 2012). The primary energy needed for drying varies from 73 % to 87 % of total produced bioenergy. The life cycle GHG emissions are estimated to be in the range 37.55 to 44.62 (g CO_2 eq /MJ biomass), depending on the choice of harvesting method prior to drying (Zaimes et al., 2013; Zaimes and Khanna, 2013).

Using the heat from flue gas of a nearby power plant to concentrate the algal slurry is another drying option (Chowdhury et al., 2012; Pokoo-Aikins et al., 2010; Xu et al., 2011). Depending on the lipid content, this heat can dry slurry to produce biodiesel at 64–111 t/d (Chowdhury et al., 2012). The biogas generated through the anaerobic digestion process can be utilized as a source of energy in the dying unit; this may reduce the net energy demand for the drying process by about 84 % (Yanfen et al., 2012).

Solar assisted drying was found to increase the solid content of the algae slurry from 20 % to 31 %, which is favorable in the gasification process (Azadi et al., 2014). They proposed that solar energy could reduce the GHG from 109 (g CO_2 eq/MJ biomass) to 59 (g CO_2 eq/MJ biomass) in the coupled gasification-CHP process and from 124 (g CO_2 eq/MJ biomass) to 87 (g CO_2 eq/MJ biomass) in the coupled gasification-Fischer-Tropsch process. The energy balance ratio (EBR, ratio of energy input by fossil fuel to total energy output) reduces from 1.48 MJf/MJ biomass to 0.76 MJf/MJ biomass in the gasification–CHP process, and from 1.81 MJf/MJ biomass to 1.24 MJf/MJ biomass in the gasification–Fischer Tropsch process.

3.5 Oil extraction unit

In the first stage, the cells in the algal cake are lysed to enhance the extraction of lipids and fatty acids. In the extraction stage, the lipids and fatty acids are extracted and

separated. The defatted algal biomass will also be separated for further processing to achieve value-added bio-products.

3.5.1 Cell disruption

Without the cell lysing/disruption, the contact of extraction solvent and the lipid in the algae will be difficult. In general, any improvement in the oil extraction stage will significantly influence the viability of algal biodiesel production. Algae cell disruption can be achieved through physical, chemical, or enzymamtic processes (Andrich et al., 2006; de Boer et al., 2012; Lacaze et al., 2007; Lardon et al., 2009; Pernet and Tremblay, 2003). Each method has its own disadvantage(s) which require careful investigations, especially for the large-scale applications. For example, enzymes processes have slow reaction rates; chemical methods typically use toxic chemicals, and the mechanical disruption approaches are energy intensive (Williams and Laurens, 2010).

Physical methods include pressing and homogenization (Mercer and Armenta, 2011); bead beating (Doucha and Lívanský, 2008); ultrasonication (Lee et al., 2010); microwave radiation (Balasubramanian et al., 2011); osmotic shock (Mercer and Armenta, 2011); and electroporation (Ghasemi Naghdi et al., 2016; Halim et al., 2011; Jeon et al., 2013; Joannes et al., 2015; Khoo et al., 2011; Kim et al., 2013; Schenk et al., 2008). The use of enzyme treatment in the cell disruption stage is found to decrease the energy input (from fossil fuel) and consequently, to lower global warming potential (GWP) when compared to the disruption by homogenization in both OP and BPR cultivation systems (Adesanya et al., 2014; Stephenson et al., 2010). Among the three methods of cell rupture, the physical method is usually used in the extraction of lipids from algae biomass; the chemical method is used for other biomass but not for the algae to the best of our knowledge. Although physical methods are conventionally used in the cell disruption, the type of physical method has not been mentioned in most of the relevant literature works. Comparison between studies shows that only 12 out of 31 studies practiced cell rupture as a way to facilitate access to the cell content. Only Adesanya et al. (Adesanya et al., 2014) compared the enzyme treatment of cell disruption with the physical method (homogenization).

3.5.2 Oil extraction

In this unit, oil (lipids) will be extracted from algae biomass. Lipids can be divided into eight classes: 1) fatty acyls, 2) glycerolipids, 3) glycerophospholipids (*so-called phospholipids*), 4) sphingolipids, 5) saccharolipids, 6) polyketides, 7) sterol lipids and 8) prenol lipids (Fahy et al., 2011). Figure 2-4 shows these classes and their subgroups. A tag attached to each group represents the polarity (or neutrality). Depending on the type and quantity of each of these lipid types, different compositions of the algae lipids are possible (Guschina and Harwood, 2006) with different chemical and physical properties such as polarity, viscosity, solubility, and cellular location (Dong et al., 2016). These properties will affect the performance of cell disruption and lipid extraction processes. In the eukaryotic cells (i.e., cells whose nucleus and other organelles are bounded with membranes like animal, plant cells and majority of algae), the main constituents of the cell membrane are phospholipids, glycolipids (a class within sphingolipids lipids), and sterols (Léonard et al., 2017); the phospholipids, glycolipids are polar while the sterols are neutral. About 75% of total membrane lipids are composed of phospholipids while glycolipids are the strategic components of the cell surface (Ridgway and McLeod, 2015). Fatty acyls (free fatty acids (FFAs)), glycerolipids (mono-, di-, and triglycerides), and membrane-associated lipids such as phospholipids, sphingolipids, and glycolipids are fatty acid-containing lipids that are key precursors for biodiesel (Callahan et al., 2015). Among these lipids, the glycerolipids are highly preferred and the most favorite/ significant glycerolipids are triacylglycerides (TAGs) for biodiesel production because their simple structure allows them to be easily converted to biodiesel (Callahan et al., 2015).

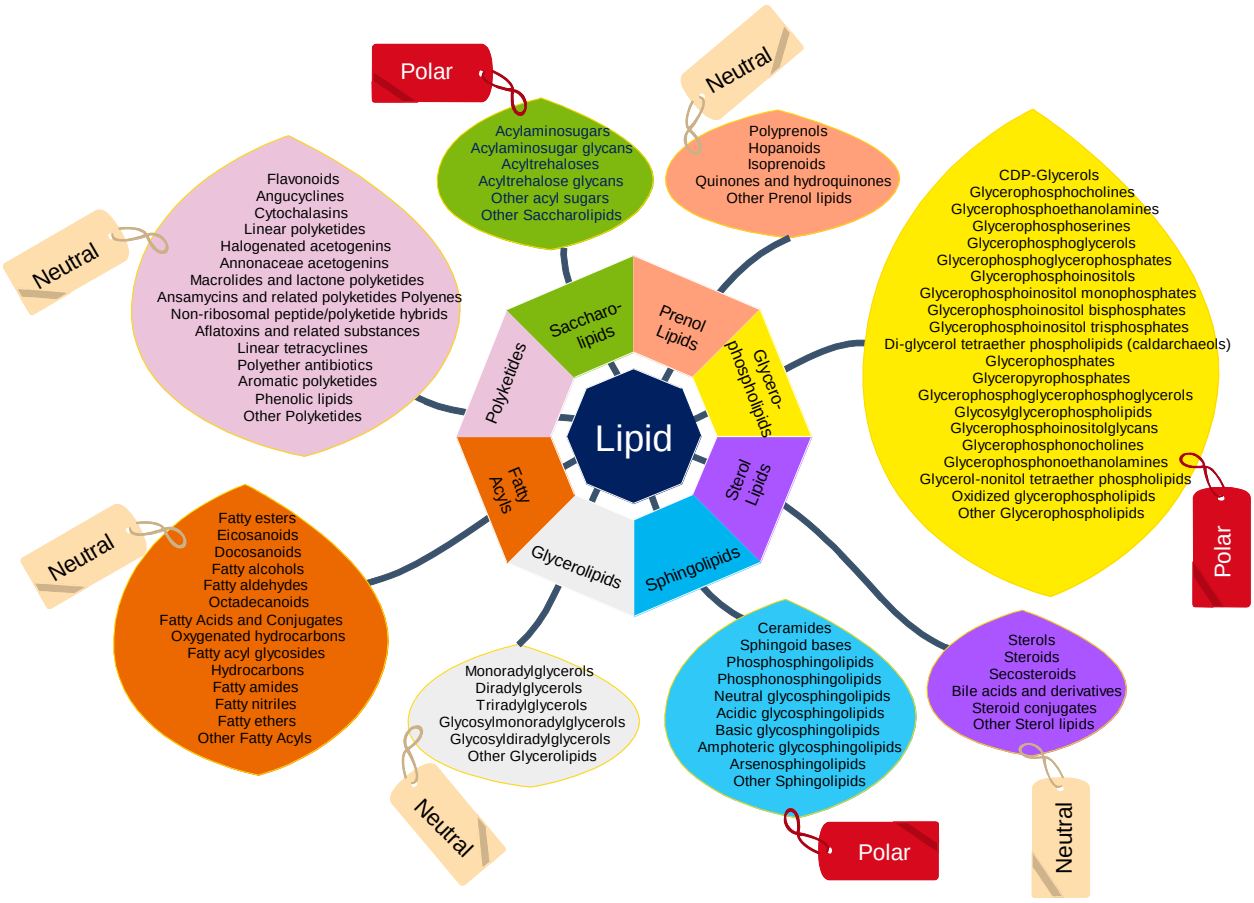


Figure 2-4: The eight classes of lipids as well as a tag shows the polarity or neutrality of each lipid.

Polarity is an important physical property in lipid isolation and conversion to biodiesel (Callahan et al., 2015). The neutral lipids have a low tendency to dissolve in water (are hydrophobic), and a high affinity to dissolve in nonpolar solvent, forming small oil droplets in an aqueous environment (Dong et al., 2016). Polar lipids however, show high solubility in water (are hydrophilic), and can exist in both aqueous and organic phases. Therefore, to extract nonpolar (or neutral) lipids from an aqueous solution, nonpolar solvents (e.g. hexane, chloroform) should be employed due to higher solubility of neutral lipids in nonpolar solvents, enhanced mass transfer, less difficulties with emulsion formation and enhanced solvent retrieval (Dong et al., 2016). Similarly, for polar lipids, polar solvent should be used in the extraction stage. In the conventional biodiesel production using

nonpolar solvents (e.g. hexane), polar lipids are considered as contaminant due to their emulsification properties. Also, free fatty acids have displayed a higher resistance to the presence of water than the triglycerides during biodiesel production from lipids (Cheng et al., 2014).

Under stressed growing condition (i.e., nutrient deprivation or high light intensities), algaes accumulate energy in the form of energy-dense neutral lipids (triacylglycerides or TAG), while under non-stressed cultivation operating conditions, phospholipids will be accumulating in the lipid (Wijffels and Barbosa, 2010).

Generally, the optimal design of the lipid extraction stage should not be limited to a specific lipid content (Pragya et al., 2013), lipid composition, lipid type. It needs to consider the minimization of co-extracted non-lipid contaminants, selective solvents toward favorable lipid fractions (Halim et al., 2011), environmental and energy demand aspects of solvents, and large scalability of extraction unit.

There are three main methods to extract lipids (mainly triglycerides) from algae biomass: *1*- solvent extraction, *2*- supercritical carbon dioxide extraction, and *3*- hydrothermal liquefaction (HTL). Some of the above processes require further drying of the biomass, others can be done under “wet” conditions. (Batan et al., 2010; Collet et al., 2014; Frank et al., 2011; Lardon et al., 2009; Liu et al., 2012; Quinn et al., 2014). At large scales, the energy consumptions of the dry and wet extraction processes can contribute up to 90 % and 79 % of the energy demand of overall algae to biodiesel process, respectively (Lardon et al., 2009). Wet extraction under deprived nitrogen conditions can reduce the energy requirement, considerably. Using deprived-nitrogen algae cultivation and wet lipid extraction scenario reduced all of the studied energy and environmental impacts except for the photochemical oxidation (Lardon et al., 2009).

Solvent extraction is the most common process. In the solvent extraction process, solvents such as polar and non-polar organic solvents, ionic liquids (ILs), accelerated solvent extraction and solvents with switchable polarity are typically utilized (Chen et al., 2012; Cooney et al., 2009; Ghasemi Naghdi et al., 2016; Kim et al., 2012; Pragya et al., 2013; Richter et al., 1996; Samorì et al., 2013; Young et al., 2010). Traditional solvents include hexane, mixture of chloroform and methanol, benzene, and ether. Although the

lipid extraction by chloroform is favored due to its flexibility and performance, large scale lipid extraction using chloroform is prohibited due to environmental concerns (Ranjith Kumar et al., 2015). Hexane has advantages (over chloroform) such as lower toxicity, minimum solubility in the non-lipid components (contaminations), and a higher selectivity for the neutral lipid fractions (Halim et al., 2011). However, the presence of water and cell membrane lipids (polar lipids) make the conventional hexane solvent extraction more challenging with many algaes and fat-soluble pigments because of the additional complexity in fatty acids extraction and biodiesel purification (Islam et al., 2014). Also, using organic solvents raises health and safety concerns, being wasteful, and time consuming (Santana et al., 2012). It has been shown that using solvent mixtures containing a polar and a non-polar solvent (for example, mixture of chloroform (non-polar), methanol (polar) and water) remarkably increases the lipid extraction efficiency (Li et al., 2014).

Supercritical carbon dioxide (sc-CO_2) can also be employed in extraction. CO_2 is chemically inert, nontoxic, environmentally-friendly, and inexpensive with favorite critical properties (i.e., moderate critical temperature of 31.1 °C and pressure of 72.9 ATM). The supercritical condition reduces the extraction time through enhancing miscibility and mass transfer. Moreover, the extraction is conducted at a high selectivity for lipids which make sc-CO_2 a viable option for the lipid extraction stage (Aguirre et al., 2013; Makareviciene et al., 2013; Santana et al., 2012; Taher et al., 2014b; Zeng et al., 2014). During the extraction process, CO_2 is depressurized which can be easily separated from the extracted phase which is practically solvent-free and non-toxic (Crampon et al., 2013). Lipid extraction from algae is usually conducted at temperatures between 40 °C to 80 °C and pressures of 20 MPa to 38 MPa (Cheung, 1999; Cooney et al., 2009; Santana et al., 2012). The time taken to achieve a specified lipid yield is about 5.6 times of that for sc-CO_2 lipid extraction (Halim et al., 2011). Due to low polarity, it is less effective in extracting polar lipids (mostly membrane lipids) (Herrero et al., 2006; Islam et al., 2014). In the literature, sc-CO_2 lipid extraction is applied on both wet and dried algae (Santana et al., 2012; Soh and Zimmerman, 2011; Taher et al., 2014a).

The major disadvantages of the sc-CO_2 lipid extraction are: high cost of infrastructure and operation, high energy demand (related to high pressure), safety issues with high pressure, pre-treatment step requirement, and low dielectric constant (causes problems in extracting polar analytes) (Beckman, 2004; Díaz-Reinoso et al., 2006; Halim et al., 2011; Lucas et al., 2001).

Quinn et al. (Quinn et al., 2014) used sc-CO_2 in the lipid extraction stage with dried algae as the only study among 31 reports that discuss the LCA for the majority of algae biodiesel process; they concluded that the process lacks energy and environmental benefits.

HTL has been used for the lipid extraction at lab scale (Delrue et al., 2013; Pongsurapipat et al., 2016) (more descriptions on HTL in Section 3.6.1).

Compared to dry and wet solvent extraction, hydrothermal liquefaction has shown less fossil energy requirement (Delrue et al., 2013; Frank et al., 2013; Sills et al., 2012). Despite better extraction efficiency, HTL produces more GHG emissions than the dry and wet solvent extraction (Delrue et al., 2013; Frank et al., 2013).

According to the explored researches, three studies Sills et al. (Sills et al., 2012), Pongsurapipat et al.(Pongsurapipat et al., 2016), and Delrue et al. (Delrue et al., 2013) chose hydrothermal liquefaction, 30 studies used the traditional solvent extraction method. Among 31 studies, the only researchers who designed process based on a dry supercritical CO_2 extraction process are Quinn et al. (Quinn et al., 2014) because of complications subjected to high water concentrations.

3.6 Lipid to biodiesel conversion unit

The two most common processes to convert the lipids to biodiesel transesterification and hydrotreatment are described below. A summary of possible methods for biodiesel conversion is shown in Figure 2-5.

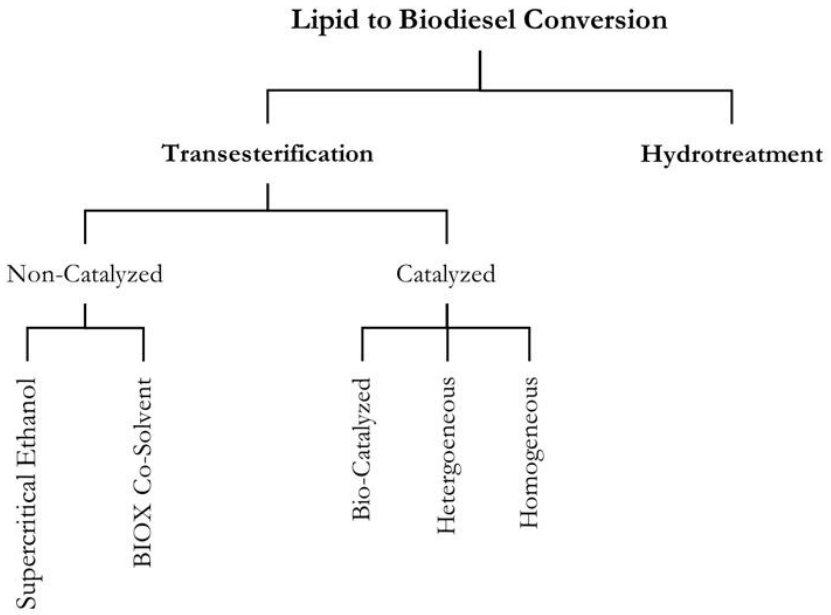


Figure 2-5: A summary of possible processes for conversion of algae lipid to algae biodiesel

3.6.1 Transesterification

In the presence of a catalyst, triacylglycerol reacts with methanol to produce fatty acid methyl esters (FAME); this reaction is known as transesterification (Zeng et al., 2011). FAME is the main product (biodiesel) and glycerol is a co-product. The transesterification reaction between the triglycerides and alcohol is slow without using catalyst. Abbaszaadeh et al. (Abbaszaadeh et al., 2012) have reported researches that used non-catalytic conversion of lipids to algae biodiesel.

A homogeneous catalyzed, heterogeneous catalyzed, or biocatalyst can be used to enhance the transesterification. Homogeneous catalyzed transesterification reactions can be performed by acidic or basic catalysts. Basic catalysts can be alkaline metal alkoxides, hydroxides, and sodium or potassium carbonates while acidic catalysts include sulfuric acid, hydrochloric acid, and sulfonic acid. Heterogeneous acidic and basic solid catalysts include basic zeolites, alkaline earth metal oxides, and hydrotalcites (MgO, CaO,) and acidic based catalysts such as Nafion-NR50, sulfated zirconia, and tungstated zirconia. Biocatalysts have also been used in the production of algae biodiesel such as

naturally occurring lipases and commercial enzymes such as Novozyme (Gog et al., 2012). These enzymatic biocatalysts are classified into two classes of extracellular lipases and intracellular lipases.

Non-catalytic biodiesel production: Supercritical methanol and BIOX co-solvent processes are among the non-catalytic methods for the production of biodiesel. In the supercritical alcohol process, the transesterification process is conducted at high pressure and temperature (Kiwjaroun et al., 2009; Tan et al., 2009). Supercritical methanol may be used for biodiesel conversion without forming two phases. This process has unique features; it does not require catalysts, requires a simpler process for the purification of products, has a shorter reaction time, and is more environmentally-friendly (Demibras, 2008; Kusdiana and Saka, 2004). The BIOX co-solvent process is another non-catalyzed transesterification process. BIOX transesterification is a two-step process. In the first step, about 10% of the free fatty acids are reacted through transesterification. The second step uses base-catalyzed transesterification of triglycerides to produce methyl esters, using methanol. As the solubility of methanol in triglycerides is low, the reaction rate is very slow and a co-solvent (tetrahydrofuran, THF) is conventionally used to solubilize the alcohol (Boocock et al., 1998; Demibras, 2008, 2009; Sarin, 2012). The advantages of BIOX reaction process are: being a continuous and fast process with reaction time of 5 to 10 min, requiring no other feedstocks, using a recoverable co-solvent that is inert in a single-pass reaction at room conditions with no catalyst residue in either glycerol or biodiesel phases (Boocock et al., 1998; Demibras, 2008, 2009; Math et al., 2010; Sarin, 2012; Van Gerpen et al., 2004).

3.6.2 Hydrotreatment

Hydrotreatment is an alternative process for the conversion of triglycerides to biodiesel. At moderate temperature and high pressure, TAGs react with hydrogen using metal-supported catalyst to form biodiesel (Serrano-Ruiz et al., 2012). The lipid feedstocks are fed to the hydrotreatment reactor where oxygen, nitrogen, and other heteroatoms are removed (Chang et al., 2015; Yang et al., 2016). The final product is also known as “green diesel” and has a composition similar to the petroleum diesel. A comparison of the reaction conditions for algae lipid conversion by transesterification and hydrotreatment is

shown in Figure 2-6 (Serrano-Ruiz et al., 2012). Differences between the applied catalysts, pressure and temperature, by-products, and the chemical structure of products for transesterification and hydrotreatment are clearly illustrated in Figure 2-6.

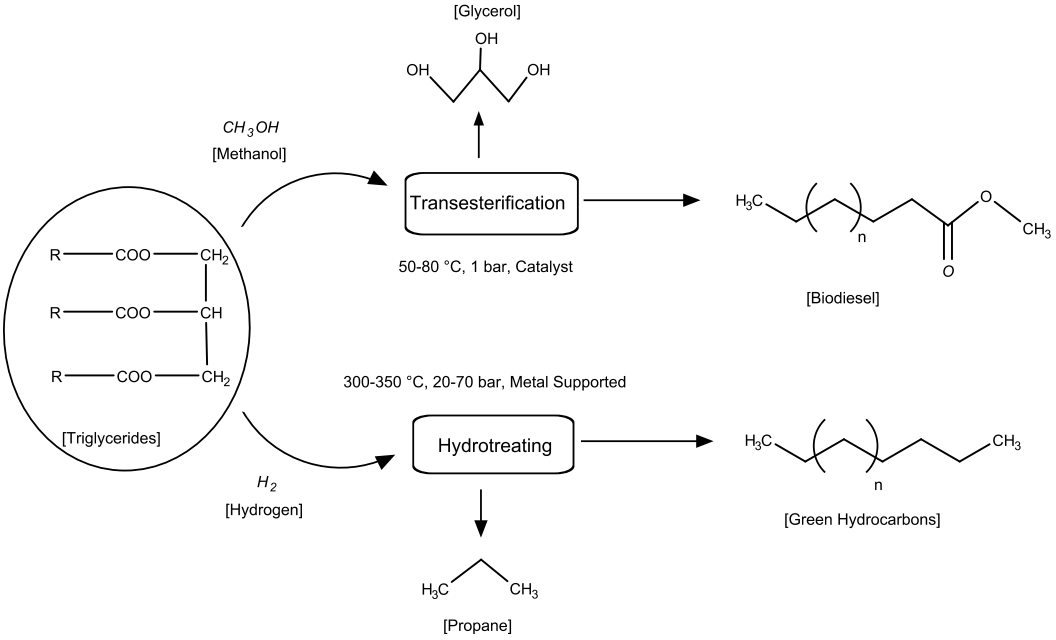


Figure 2-6: Comparison of reaction pathways and operating conditions for transesterification and hydrotreating (Adopted from Serrano-Ruiz et al., 2016)

The selection of the lipid to biodiesel process on the overall life-cycle energy requirement is small compared to the other steps (Delrue et al., 2012). In terms of reaction conversion, cost and associated environmental impacts, the hydrotreatment process performs slightly better than the transesterification process because of higher net energy return (NER, to be discussed in chapter 6), lower production cost and lower GHG gas emission. Delrue et al. (Delrue et al., 2012) found a 7 % higher net energy ratio (NER), 3 % lower production cost, and 8 % lower GHG footprint for the hydrotreatment, compared to transesterification. Zaimes and Khanna, (Zaimes et al., 2013) also concluded that the energy return on investment for fossil fuel demand ($EROI_{fossil}$, to be discussed in chapter 6) and GHG emissions are lower for the hydrotreatment process. Sills et al. (Sills et al., 2012) raised the concern with the future market for glycerol as a byproduct. Furthermore, they

envisioned the production of H_2 from renewable sources in the future, which could be potentially integrated with the hydrotreatment reaction pathway to reduce the energy demand from non-renewable resources. A new strategy is investigated by Delrue et al. (Delrue et al., 2013) to produce oil and alkanes using direct secretion of molecules. This technique bypasses the energy intensive units including harvesting and extraction processes.

Table 2-3 compares the ranges of temperature, pressure, residence time, yield, and the main product for different lipid to biodiesel conversion processes.

TABLE 2-3: Comparison of the processes for converting lipids to biodiesel.

Process			Reaction Temperature	Reaction Pressure	Residence time	Yield	Product
Transesterification	Catalyzed	Homogeneous	30–65 °C	0.1 MPa	0.5–4 h	Normal to high	Methyl ester
		Heterogeneous	30–200 °C	0.1–5 MPa	0.5–3 h	Normal	Methyl ester
		Bio-catalyzed	35–40 °C	0.1 MPa	1–8 h	Low to high	Methyl ester
	Non-catalyzed	Sc-Methanol	>239.4 °C	>8.09 MPa	3-15 min	High	Methyl ester
		BIOX	25 °C	0.1 MPa	5-10 min	High	Methyl ester
Hydrotreatment			125-405 °C	2-13.6 MPa	3 min to 4 h	Low to Normal	Bio-oil

3.7 Defatted biomass conversion unit

The algae biomass residue (defatted biomass) from the lipid extraction unit can be processed to produce other forms of bioenergy (Zaimes et al., 2013) using different processes as shown in Figure 2-7.

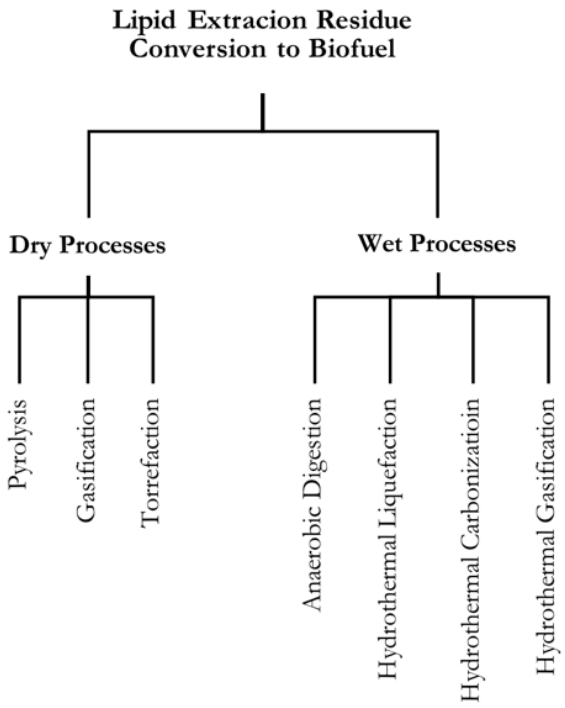


Figure 2-7: Possible processes to convert algae biomass (from lipid extraction residue) to biofuel

The defatted biomass contributes to about 75 % to 80 % of the total mass of the algal biomass. Thus, it can be regarded as a potential feed to produce bioenergy if the process contributes positively to the overall life cycle, and sustainability of the algal biodiesel production (Clarens et al., 2011; Gao et al., 2013). It is necessary to include the energy content of the defatted biomass in assessing the biodiesel process. The conversion processes are divided into wet processes (including anaerobic digestion and hydrothermal methods), and dry processes (such as torrefaction, pyrolysis, and gasification) (Barreiro et al., 2013). Technical, economic, and environmental parameters are the critical criteria in decision making (Azadi et al., 2014). For example, Gao et al. (Gao et al., 2013) found that about 16 % of the energy stored in the microalgae can be recovered in the form of biodiesel while about 83 % of the energy stored in the defatted biomass slurry can be extracted as bio-oil and biogas through hydrothermal processes.

In the literature a number of options to manage the residual biomass have been compared including (Azadi et al., 2014; Gao et al., 2013; Yuan et al., 2015): no utilization of the

residual biomass ; on-site anaerobic digestion (AD) of the wet biomass slurry; on-site hydrothermal liquefaction (HTL) of wet biomass slurry; on-site hydrothermal gasification (HTG) of wet biomass slurry; on-site gasification of dry biomass slurry; and on-site direct combustion of dry biomass slurry.

There is an additional benefit of defatted biomass slurry for use as animal feed or bio fertilizer (such as soil amendment).

3.7.1 Wet processing of defatted biomass

Anaerobic digestion (AD): AD is a suitable process for a defatted biomass with high moisture content (80 % to 90 %) (ABDULLAH et al., 2014). The microalgae contains significant amounts of nitrogen and phosphate; the AD process can be used to recover the nitrogen and phosphorus for use as a fertilizer (Keymer et al., 2013; Sialve et al., 2009; Zamalloa et al., 2011). In the AD process, the organic nitrogen and phosphorus compounds are partially remineralized to produce a liquid phase that contains ammonium and phosphate ions.

Methane is also produced as a biogas which can be utilized on-site to generate heat, electricity or both. The amount of volatile solids converted to the biogas during the AD (or biodegradability) is an important parameter in the algae biodiesel process sustainability (Bohutskyi et al., 2014). Ajayebi et al. (Ajayebi et al., 2013) showed that about 52 % of the electricity requirements of the process can be supplied by the generated methane (as co-product). The maximum theoretical methane yield (based on algal composition) can vary from 0.47 to 0.8 L of methane/g volatile solids (L CH_4/g VS) (Sialve et al., 2009; Yuan et al., 2015). In practice, the methane yield varies from 0.10 to 0.60 L CH_4/g VS in the AD process (Bohutskyi et al., 2014; De Schamphelaire and Verstraete, 2009; Gunaseelan, 1997; Keymer et al., 2013; Ras et al., 2011; Ward et al., 2014; Yuan et al., 2015). Møller et al. (Møller et al., 2009) estimated that about 3 % of the total CH_4 produced can be considered as fugitive emissions (Zaimes et al., 2013). The produced biogas in AD can be fed to a combined heat and power (CHP) unit. It can also be combusted to obtain heat or electricity (Chang et al., 2015; Delrue et al., 2013; Gao et al., 2013; Stephenson et al., 2010; Woertz et al., 2014; Yuan et al., 2015; Zaimes et al., 2013). The biogas combusted

in the CHP produces CO_2 which can be fed into the culture medium to satisfy the CO_2 demand in the algae biomass production (Woertz et al., 2014; Zhang et al., 2014).

The solid portion of the biomass after AD process can transported for animal feed or/and biofertilizer applications (Zaimes et al., 2013). The biomass slurry can provide up to ~61 % of the N and ~52 % of the nutrient demand in the cultivation process through recycling the nutrients from AD unit to the culture medium (Gao et al., 2013). The nitrogen and the phosphorus contents of the liquid digestate varies between 30 % to 60 % of the recycled liquid (Delrue et al., 2012; Ras et al., 2011). It is estimated that about 89 % of the nutrients are recovered and recycled for the algae cultivation step.

Hydrothermal liquefaction (HTL): is conducted at mild temperatures (280 °C to 370 °C), and high pressures (10 MPa to 25 MPa). The wet biomass reacts to produce a liquid bio-oil as the main product, and three coproducts of gas, aqueous, and solid phases. Similar to the AD, a key feature of the HTL process is its capacity to recycle the nutrients such as P and N constituents to the cultivation unit (Biller et al., 2012; Elliott et al., 2015; Frank et al., 2013). The main sources of nitrogen in the liquid HTL product are the organic N-rich compounds and ammonia (Alba et al., 2013; Minowa and Sawayama, 1999). The gas co-product contains 84 % to 96 % CO_2 and therefore cannot be used in the boiler (Delrue et al., 2013). The produced biocrude oil has a wide range of LHV (lower heating value), encompassing 32 to 44 MJ/kg biocrude-oil (Elliott, 2007; Qu et al., 2003; Tzanetis et al., 2017). The final biocrude may not be suitable for direct utilization; it may gain commercial value after hydrotreatment (Gollakota et al., 2017). The produced biocrude and gas together contain about 90 % of the energy content in the algal feedstock (Valdez et al., 2012).

In the HTL process, the bio-oil and biochar are combined with glycerol to obtain a bio-slurry fuel that can be utilized to generate electricity in the coal-based power plants (Abdullah et al., 2010; Abdullah and Wu, 2011; Gao et al., 2013; Wu et al., 2010; Yu and Wu, 2010). This produced electricity is about 48 % extra to the process demand (Gao et al., 2013).

Hydrothermal carbonization (HTC): HTC is conducted at a temperature near 200 °C and a pressure below 2 MPa, in water (Heilmann et al., 2010). The reaction is exothermic

and spontaneous which converts the defatted biomass (wet) to an energy-dense solid, called hydrochar with energy content between 21-30 (MJ/kg hydrochar) (Broch et al., 2013). The process produces liquid and gas phases as by-products which retains more than half of the feedstock mass (30-45 % mass yield) (Broch et al., 2013; Heilmann et al., 2011).

Hydrothermal gasification (HTG): The HTG process is carried out at high temperatures (400 °C to 700 °C) and high pressures (25 MPa to 30 MPa), in supercritical water (temperature above 374 °C, pressure above 22.1 MPa) (Kruse, 2009). The HTG low end-product char (between 18.5 and 20 MJ/kg Dry Matter) value has discouraged researchers to conduct further modifications and processing because the HTG process is harsh and demands high temperature and pressure while the end-product has a low energy value, and requires high energy and capital costs (Barreiro et al., 2013).

Among different hydrothermal conversion routes, the HTL process has attracted industrial interest because of its flexibility to be incorporated in the existing petroleum refining infrastructure (Elliott et al., 2015; Liu et al., 2013). The combustion of bio-oil (obtained from HTL) produces high NOx emissions which is an environmental concern for the HTL process to be taken into consideration in the biofuel production processes (Costa and De Morais, 2011).

3.7.2 Dry processes on defatted biomass

Torrefaction: This process is conducted at a temperature from 200 °C to 300 °C, atmospheric pressure, under oxygen free conditions (Van der Stelt et al., 2011). Through partial degradation of the dry biomass, char (solid) substance is produced as the main product. The torrefied biomass has a higher heating value (HHV) of 17.6-24.7 (MJ/kg char) whereas the HHV of algal biomass residue after oil extraction is measured to be 16.91 (MJ/kg dry biomass) (Chen et al., 2015). Compared to the biomass feedstock, the torrefied product has a lower oxygen-to-carbon and hydrogen-to-carbon ratios, and its heating value falls between that of the wood and coal.

Pyrolysis: This process is performed at a temperatures between 225 °C to 600 °C (depending on pyrolysis type), atmospheric pressure, under oxygen free conditions (Dhyani and Bhaskar, 2017). The reaction products are bio-oil, charcoal, and gaseous fraction from the dry biomass. Based on the reactor temperature and residence time, the pyrolysis process can be classified into flash, fast, mild, and slow categories. The yield of the products is a function of the pyrolysis type used. According to (Basu, 2013; Diebold et al., 1997), the produced bio-oil has a lower LHV (13-18 MJ/kg wet basis) with respect to its parent biomass (19.5-21 MJ/kg dry basis). The energy contents (i.e., LHV) of produced char and gas are 32 MJ/kg and 11 MJ/Nm^3, respectively (Basu, 2013; Diebold and Bridgwater, 1997).

Gasification: This particular process needs to be carried out at an elevated temperature (higher than 700 °C), and atmospheric pressure (Kumar et al., 2009). In gasification, syngas is the key product along with small amounts of tar and char as co-products. Syngas or synthesis gas is a blend of CO, H_2, CO_2, CH_4 with small traces of other elements (Sikarwar et al., 2017). Gasification is conducted in the presence of gasifying agents such as air, oxygen (O_2), steam (H_2O) or carbon dioxide (CO_2) (Sikarwar et al., 2016). The syngas HHV using different gasifying agents are reported in the literature as 21.79 to 24.38 MJ/Nm^3 with O_2, 19.97 to 22.25 MJ/ Nm^3 with steam, 21.15 to 22.91 MJ/ Nm^3 with O_2/steam, and 18.29 to 21.05 MJ/ Nm^3 with CO_2 (Ebadi and Hisoriev, 2017).

In general, the conventional dry thermochemical processes for defatted biomass conversion are not as attractive as the wet processes, mainly because of their environmental impacts and poor economical perspectives, duet to their high energy demand (for high pressure and temperature) as well as lower energy content of the products compared to wet processes of defatted biomass (Amin, 2009). For these reasons, the studies on dry defatted biomass conversion processes are limited.

Table 2-4 presents a comparison for different defatted biomass conversion processes, including the temperature and pressure ranges, yields, residence times, and their by-products.

TABLE 2-4: A summary of processes of defatted biomass conversion.

	Process	Reaction Temperature	Reaction Pressure	Residence time	Yield	By-products
Wet	Anaerobic digestion	20-60 °C	0.1 MPa	wet: 60-95 days dry: 9-45 days	-	***S****: residue ***L***: containing organic N and P ***G***: CH_4: ~60%,CO_2: ~30%, NH_3
	Hydrothermal liquefaction	280-370 °C	10-25 MPa	3−5 min	Normal to high	***S***: char ***L***: containing organic N, S, P ***G***: CO_2: ~84-96%
	Hydrothermal carbonization	~ 200 °C	<2 MPa	<30 min	Low to normal	***A***: ~45-50 wt% (high-value chemicals and nutrient) ***G***: ~1 wt%
	Hydrothermal gasification	400-700 °C	25-30 MPa	<50 h	High	***L***: residual water containing inorganic elements ***G***: H_2: 46%, CH_4: 19%, CO_2: 29%
Dry	Torrefaction	200-300 °C	0.1 MPa	15–60 min	Normal to high	***S***: ash ***L***: condensable volatile organic compounds comprising water, organics, lipids ***G***: noncondensable gases like CO_2, CO, CH_4
	Pyrolysis	225-600 °C	0.1 MPa	1-3600 s	Low to normal	***S***: char ***L***: heavier hydrocarbons, and water ***G***: noncondensable gases like CO_2 ,CO, CH_4, H_2
	Gasification	>700 °C	0.1 MPa	1-40 s	Low to normal	***S***: char ***L***: tar

* ***S***: Solid phase

** ***L***: Liquid phase

*** ***G***: Gaseous phase

3.7.3 Comparison of defatted biomass processes

Co-generation of the defatted biomass in a CHP plant was compared to AD by (Zaimes et al., 2013). The CHP pathway achieves higher energy return on investment ($EROI_{fossil}$) compared to the AD process because of its lower downstream processing and higher efficiency (in the CHP plant). Clarens et al. (Clarens et al., 2011) also reported higher efficiency in the direct combustion, compared to the AD process. They concluded that the AD is energetically unfavorable for both cases: with or/and without extraction of algae

lipids. Zaimes and Khanna (Zaimes et al., 2013) questioned the potential of the AD for the commercial utilization in the microalgal biodiesel production because of high capital costs and long payback period. In addition, its commercial scale utilization is challenged by the access to freshwater resources and saline water. They found that the inhibition of methanogensis (in AD) at high salt concentrations occurs which may happen with salt-water or saline medium, as well (Lakaniemi et al., 2013; Zaimes et al., 2013). The direct combustion of dry biomass produces heat and power; however, it fails to recycle the nutrients (Yuan et al., 2015).

Gao et al. (Gao et al., 2013) compared the HTL and AD for at high moisture weight content feedstock (~85 %), using variables/factors such as overall carbon footprint, and energy and nutrient demand in the microalgal biodiesel production. Both technologies can be used to produce heat and electricity and recover nutrients remaining in the defatted biomass. The energy input to achieve 1 MJ biodiesel is reduced from 4.3 MJ to 1.3 MJ and to 0.7 MJ using the AD and HTL on the defatted biomass, respectively, compared to the baseline without defatted biomass conversion. The overall GHG emissions are about 80 g CO_2 (eq)/MJ for AD and 33 g CO_2 (eq)/MJ biodiesel for HTL, respectively. In 2014, the life cycle, GHG emissions and energy yield of the HTL and AD were modelled by Zhang et al. (Zhang et al., 2014). The HTL achieves slightly higher energy production per kg of defatted biomass (10.55 MJ/kg in HTL and 9.87 MJ/kg in AD); but the AD process results in more reduction in the GHG, and it can also recycle the nutrients.

In the HTL process, about 53.8 % of the carbon in the defatted biomass reacts to produce bio-oil and about 17.1 % is converted to CH_4. In the AD process, however, about 31.4% of the carbon biomass is converted to CH_4 that can be combusted to produce electricity and heat (Gao et al., 2013). Also, in the HTL process, less N is recovered because about 23 % of nitrogen is left in the bio-oil. The HTL process benefits from a faster reaction time, compared to the AD. Delrue et al. (Delrue et al., 2013) compared the AD and HTL for upgrading of the defatted biomass. They found the energy demand of the AD to be lower, with approximately 28 % lower GHG emissions. However, higher biodiesel yield was obtained in the HTL which reduced the water consumption by 15 % (Delrue et al., 2013).

Delrue et al. (Delrue et al., 2012) compared AD and gasification processes . They showed that the AD is a better alternative for the algal residues conversion process in terms of higher net energy ratio (NER) and lower GHG emissions, compared to gasification. Azadi et al. (Azadi et al., 2014) investigated technologies for processing of the defatted biomass residues. For wet residue, HTG and AD were chosen. To assess the effectiveness of different dry residue-processing technologies, gasification–power generation and gasification–Fischer–Tropsch routes were studied. In the wet pathways, hydrothermal gasification achieved lower GHG footprint (41 g CO_2 (eq)/MJ biomass), compared to AD (86 g CO_2 (eq)/MJ biomass). Methane production was proposed to contribute to generation of more heat and electricity. The solid slurry from the dry oil extraction stage can be gasified to generate syngas to be utilized in a Fischer–Tropsch (FT) reactor to produce diesel fuel, or to be burnt in a CHP, leading to generation of heat and electricity (Azadi et al., 2014). Gasification-CHP outperformed the gasification–FT from the perspective of emission reduction and energy yield increase.

In Table 2-5, a summary of different operating conditions and process outputs in the upgrading of defatted biomass and includes different process pathways for defatted biomass conversion, co-products, recycled materials, and lower heating value (LHV or net heating value). The energy content of biodiesel is an important characteristic that affects the overall environmental impact of the process. Sorguven and Özilgen reported energy content of 38 to 42 MJ/kg (Sorguven and Özilgen, 2010); Delrue et al. (Delrue et al., 2013) reported 15 to 22 MJ/kg biodiesel. The highest reported value in the energy content provided by Pongsurapipat et al (Pongsurapipat et al., 2016) at 41 MJ per kg biodiesel.

TABLE 2-5: A summary of studies on defatted biomass conversion processes in algae to biodiesel process

No.	Ref.	Defatted biomass conversion method*	Coproducts**	Recovery/recycle***	LHV (MJ/kg dry biomass)
1	Adesanya et al.	AD	BE, G	n, s	
2	Ajayebi et al.	AD, CHP	BE, BF, H	w, c, n	
3	Azadi et al.	AD, GAS, HTG, CHP, DC, FT	G (burnt to gas	w, n, s	
4	Batan et al.	-	OK, G	w, s	-
5	Campbell et al.	AD	BE		
6	Chang et al.	AD	H (AD)	s	
7	Chowdhury et. al.	AD	H	w, n	
8	Clarens et. al	AD, DC	BE, H, BF, G	w, c, n, s	36.9-38.5
9	Collet et. Al	-	OK, G	w	23.2
10	Delrue et al.	AD	H	w, n	38-45
11	Delrue et al.	AD, HTL	H	w, n	15-22
12	Dutta et al.	AD	G	w, s	
13	Gao et al.	AD, HTL, HTG,CHP	BE, H, G	w, c, n, s	37.2
14	Hou et al.	-	G	w, n, s	
15	Khoo et al.	-	-	s	40
16	Lardon et al.	-	G, OK	w, s	37.8
17	Pardo-Cárdenas et	-	OK, G	w, s	41
18	Passell et al.	-	OK	w, n, s	28
19	Pongsurapipat et	-	AR, SR, G	w	
20	Quinn et al.	AD	AF, BE, BF, H (AD)	w, n, s	
21	Resurreccion et	AD	BE, BF, G	w, c, n, s	37.7
22	Sills et al.	AD, CHP	G, AF, BE, H (AD)	w, n, s	
23	Soh et al.	AD	AF, BE, BF, H	w, n, s	38.5
24	Stephenson et al.	AD	G, H	w, n, s	37.2
25	Woertz et al.	AD, CHP	BE, H, G	w, n, s	
26	Yanfen et al.	AD	H (AD), G	w, s	37.2
27	Yang et al.	-		w, n	
28	Yuan et al.	AD, CHP	G, H (AD, CHP), BE, BF	w, c, n, s	
29	Zaimes and Khanna	AD, CHP	G, BE, BF, H (CHP), C_3H_8	w, n	44
30	Zaimes and Khanna	AD, CHP	G, AF, H, BE, BF	n	37.6
31	Zhang et al.	AD, HTL, CHP	H (AD, HTL), BE, BF, G	w, c, n, s	

* AD : Anaerobic Digestion; HTG: hydrothermal liquefaction; GAS :Gasification; HTG: Hydrotherlmal Gasification; CHP: Combined heat and power; DC: Direct combustion; FT: Fischer–Tropsch

** AF: Animal Feed; AR: Aqueous Residue; BE: Bio Electricity; BF: Biofertilizer, G: Glycerol; H: Heat; OK: Oil-Cake; SR: Solid Residue

*** c: Carbon Dioxide Recycling; n.: Nutrient Recovery; s: Solvent Recovery; w: Water Recycling

The production of co-products in the biodiesel production plant in general improves the environmental impact of the overall process (Cherubini et al., 2009). In an algal biodiesel production plant, several co-products are produced, including oil cake and glycerol, each having significant energy content (Collet et al., 2014). About 84 % of the total output energy exist in these de-oiled co-products. The process sustainability is negatively affected if the co-products are not utilized (Gao et al., 2013). Different approaches have been adapted to use the co-products as energy, nutrient, and fertilizer. Energy in the form of electricity, heat or both can be produced from the combustion of biogas, bio-oil, and bio-slurry. Nitrogen or phosphorous fertilizers can be recycled from these co-products; the oil cake (after AD or HTL) can be utilized as the animal nutrition or/and soil fertilizer, and the glycerol can be used on-site or it can be sold as a by-product. Glycerol might be also used in the production of biodiesel and docosahexaenoic acid (Chowdhury et al., 2012); it can be conversion on-site to produce gas (Azadi et al., 2014; Gao et al., 2013).

4 Supplementary input materials to algae cultivation unit

An algae cultivation unit requires light, carbon, water, and fertilizer for efficient biomass growth. The optimal operation of the algae cultivation unit is significantly affected by these supplementary materials.

4.1 Carbon and carbon dioxide sources

Inorganic carbon from CO_2, organic carbon from wastewater, and glycerol are potential sources for carbon supplements to the algal medium (Chang et al., 2015; Woertz et al., 2014). The level of CO_2 in the atmosphere is not enough for large-scale cultivation of algae. The flue gas is a cheap alternative source of concentrated CO_2 that can be used. The flue gas contains up to 20 % CO_2 (Brennan and Owende, 2010). The CO_2 input flow rate is generally calculated based on cell requirements, CO_2 input concentration, and rate of CO_2 mass transfer to algae medium (Delrue et al., 2013; Delrue et al., 2012). Based on the chemical composition of algae, the theoretical demand of CO_2 to produce 1 kg of biomass is estimated about 1.83 kg (Yuan et al., 2015). Putt et al. (Putt et al., 2011) estimated CO_2 mass transfer efficiency (to the algae medium) is approximately 83 %, implying that there is about 17 % CO_2 loss to atmosphere. Delrue et al. forecasted an

adsorption efficiency to be 50 % (Delrue et al., 2012). A similar prediction of about 42.6 % for CO_2 absorption efficiency from flue gas was made by Yanfen et al. (Yanfen et al., 2012).

Several sources are possible for the CO_2 to be fed to the algae cultivation unit as follows such as indicated above the flue gas from a nearby power facility could be injected (sparged) into an algae cultivation pond (or reactor). This reduces costs for CO_2 production, but the algae cultivation unit has to be located within the proximity of power plant. The presence of other compounds in the flue gas may adversely affect the algae growth (Cho et al., 2011; Lv et al., 2010; Zaimes and Khanna, 2013). Pure CO_2 from MEA scrubbing is another alternative. This option is energy intensive, because of the quantity of stream which is required in the MEA processes (Zaimes et al., 2013; Zaimes and Khanna, 2013). Commercial grade CO_2 is also available in the form of liquefied CO_2 that can be delivered by trucks (Campbell et al., 2011) but likely prohibitively expensive.

Biogenic carbon is the carbon whose source is from biomass (Christensen et al., 2009; Stichnothe and Azapagic, 2009). In the algal biodiesel production, CO_2 is captured form the atmosphere and eventually emitted when the produced biofuel is consumed as an energy source. The biofuel production is therefore categorized as carbon neutral (e.g., no carbon footprint) and less attention has been paid to the net GHG emissions (van der Voet et al., 2010). However, it has been shown recently that the process is not carbon neutral (DeCicco et al., 2016). The biogenic carbon is excluded from the LCA rather than adding first and then subtracting. The exclusion of biogenic carbon produces the same result if no byproduct allocation is assumed. However, there would be a gap between results if byproducts allocation is considered (Luo et al., 2009) in that the LCA results will be affected by the method to assess the biogenic CO_2 . Currently in the LCA, the impact assessment often overlooks the biogenic CO_2 emissions, based on an assumption that similar amounts of CO_2 were captured and emitted, yielding a net zero emission (Chang et al., 2015; Hischier et al., 2010; Levasseur et al., 2010) (Table 2-2). To avoid such inconsistencies, it is suggested that the CO_2 capture and emission should be included for each process in the LCA (Rabl et al., 2007). For instance, in the process of biomass to

biodiesel conversion (where biomass is burned in power plants), CO_2 capture by biomass cultivation and CO_2 emission by burning in the power plant should be considered.

4.2 Water sources

The algal biofuel production in open and closed systems demands a large quantity of water (Dominguez-Faus et al., 2009; Gerbens-Leenes et al., 2009; Louw et al., 2016). The surface and groundwater resources are also significantly influenced by the algae cultivation unit (Yuan et al., 2015). Compared to the conventional feedstock-based oils (first and second generation of biofuels), the biodiesel which is from algae has less water usage if cultivated using seawater or wastewater (Groom et al., 2008; Yang et al., 2011). Regardless of the water source in the algae cultivation, fresh water will be always required to compensate for possible losses during the process and to control and adjust the medium concentration (Harto et al., 2010). The use of wastewater for algae cultivation medium has a remarkable effect on energy and material demand, environmental footprints, and economy of process (Delrue et al., 2012; Park et al., 2011; Pittman et al., 2011). The use of sea water or wastewater reduces the fresh water demands by about 90 %; except for the phosphate nutrient demand, the sea water or wastewater can satisfy the rest of nutrients need (Yang et al., 2011). Kligerman and Bouwer reported a 21.4 % increase in the biodiesel production when local wastewater from different Brazilian municipalities was used in the algae cultivation unit (Kligerman and Bouwer, 2015). Brackish or saline water outperforms the fresh water in terms of energy efficiency, greenhouse gas emissions, and process economy both in the OPs and PBRs (Resurreccion et al., 2012).

4.3 Fertilizer sources

In an algae-to-biodiesel process, the nutrient requirements can contribute to about 26 % of the total energy demand, and approximately 22 % of the total GHG emissions (Lardon et al., 2009; Stephenson et al., 2010; Yuan et al., 2015). Collet et al. (Collet et al., 2014) showed that electricity and fertilizer productions are the main contributors to the life cycle impact assessment (LCIA), together accounting for about 50 % of the total impacts. To

decrease the fertilizer and energy consumptions in algae to biodiesel processes, three methods were suggested (Louw et al., 2016): 1) recycling wastewater, 2) N-fixing the organisms, and 3) recovering the nutrients remained in the defatted biomass. Although the wastewater is considered as a cheap source of nutrients, the variability in its composition challenges a robust design of wastewater recycling. Furthermore, the concentration of some of the nutrients in the wastewater may not be sufficient to provide the optimal algal growth (Louw et al., 2016). In the case of seawater or wastewater, a 94 % reduction in the net nitrogen demand was reported. For other nutrients such as potassium, magnesium, and sulfur, the wastewater was found to completely satisfy the algae cultivation demand (Yang et al., 2011). Recycling wastewater in the hybrid algae cultivation systems (OP-PBR) can supply nearly 3 % to 30 % of the nitrogen and phosphorus feedstock, with an average of about 7% (Delrue et al., 2012). Using urea as a fertilizer (instead of ammonium nitrate) can result in a 32 % -reduction in the GHG emissions and an increase by 1.2 % in the Cumulative Energy Demand (CED) (Collet et al., 2014).

Coupling wastewater as a nutrient supply would decrease the demand for commercial fertilizer and consequently lower the environmental burden. Table 2-6 shows the main source for nitrogen and phosphorous is commercial fertilizer. According to Table 2-6, only seven studies (from the 31 LCA studies of algae biodiesel) have investigated the use of nitrogen-deprivation condition. Proximity to sustainable and economic nutrient resources should match with microalgae cultivation systems to guarantee the effective production of algal biodiesel.

TABLE 2-6: A summary of nutrients and their sources that are used in the algae cultivation stage in literature

No	Ref.	CO_2 Source	Phosphorous and Nitrogen Sources	N-deprived
1	Adesanya et al.	flue gas	ammonium nitrate, triple super phosphate	Yes
2	Ajayebi et al.	flue gas	urea, diammonium phosphate	-
3	Azadi et al.	flue gas	ammonia, single superphosphate	-
4	Batan et al.	virgin	urea, fertilizer, wastewater	-
5	Campbell et al.	virgin, flue gas, liquefied CO_2	urea, NPKS	-
6	Chang et al.	-	glycerol	-
7	Chowdhury et. Al	flue gas	ammonium nitrate, triple superphosphate	-

No	Ref.	CO_2 Source	Phosphorous and Nitrogen Sources	N-deprived
8	Clarens et. Al	Virgin (Pure), flue gas, CO_2 capture	ammonium phosphate, urea, wastewater	-
9	Collet et. Al	flue gas	ammonium nitrate	Yes
10	Delrue et al.	flue gas	ammonium diphosphate, anhydrous ammonia, wastewater	-
11	Delrue et al.	flue gas	wastewater, ammonium diphosphate, anhydrous ammonia	-
12	Dutta et al.	flue gas	ammonia, diammonium phosphate	-
13	Gao et al.	flue gas	ammonium sulfate, triple superphosphate	-
14	Hou et al.	-	N, P_2O_5	-
15	Khoo et al.	flue gas	$NaNO_3$, NaH_2PO_4, $FeCl_3.6H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $NaMoO_4.2H_2O$, seawater	-
16	Lardon et al.	-	calcium nitrate, superphosphate potassium, magnesium phosphate	Yes
17	Pardo-Cárdenas et al.	flue gas	urea	-
18	Passell et al.	flue gas	nitrogen fertilizer, phosphorus fertilizer	-
19	Pongsurapipat et al	flue gas	wastewater, N-fertilizer, P_2O_5	-
20	Quinn et al.	flue gas	urea, ammonium hydrogen phosphate	-
21	Resurreccion et al.	virgin	ammonium phosphate, urea, wastewater	-
22	Sills et al.	flue gas	ammonium nitrate, super triple phosphate	-
23	Soh et al.	virgin	nitric acid, Glycerol + Na2HPO4, FeSO4 + (NH4)2SO4, calcium chloride, magnesium sulfate, KOH + phosphoric acid	Yes
24	Stephenson et al.	flue gas	ammonium nitrate, triple superphosphate	Yes
25	Woertz et al.	flue gas	fertilizers, wastewater	-
26	Yanfen et al.	flue gas	urea, P_2O5, K_2O	Yes
27	Yang et al.	-	fertilizer, wastewater	-
28	Yuan et al.	flue gas	urea, monopotassium phosphate	Yes
29	Zaimes and Khanna	flue gas, virgin	synthetic urea, potassium chloride, superphosphate	-
30	Zaimes and Khanna	virgin, flue gas	urea, superphosphate, potassium Chloride	-
31	Zhang et al.	-	urea, triple superphosphate	-

Under N-deprived conditions, the algae produces more lipid, compared to a normal growth condition (Hu et al., 2008; Rösch et al., 2012). The nitrogen demand for the production of 1 kg triacylglycerol from algae is estimated to be about 0.36 kg nitrogen for an algae which has about 20 % TAG by weight (Peccia et al., 2013). The N-deprivation strategy has shown an increase in the lipid accumulation of various algae, which is related to the interaction between carbon, nitrogen, and phosphorus (Fields et al., 2014; Hu et al., 2008). The N-deprivation conditions (in the algae growth stage) can activate other pathways such as fatty acid synthesis to accumulate TAG (Msanne et al., 2012;

Valenzuela et al., 2013). Adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) are generated in the photosynthesis and electron transport chain, which are consumed (oxidized) during the cellular growth in the form of adenosine diphosphate (ADP) and NADP+. Under normal conditions, an equilibrium is established between the production and consumption. In the N-deprived environment, the reaction growth is slow, thus, the ADP and NADP+ production rate will also be low, resulting in the depleted pool of ADP and NADP+. To compensate for this shortage, fatty acids use NADPH and ATP to produce NADP+; the fatty acids are dominantly deposited in the form of lipid (TAG) (Brown et al., 2009). Thus, in the N-deprived conditions, the biomass growth is often inhibited, the lipid yield increase in the algae, and a major fraction of carbon is delivered in lipids (Shen et al., 2009). Any disturbance to the nitrogen source will affect the lipid content and consequently, the biodiesel productivity (Rodolfi et al., 2009). Two advantages of high TAG concentration over low lipid yield are facility of lipid extraction and higher C/N ratio (Ras et al., 2011; Rodolfi et al., 2009). Yuan et al. (Yuan et al., 2015) concluded that the biomass residue utilization is an important factor in decision making when choosing between low and normal nitrogen-based cultivation systems. If defatted biomass is not utilized, deprived condition is a proper choice as it produces biomass with a higher lipid content that increases the energy yield of the produced biodiesel. However, if de-oiled residue is employed for nutrient recycling and energy recovery, the proper option would be normal nitrogen condition. The reason is related to high energy recovery from AD which fulfills a part of the energy and nutrient demand. Quinn et al. (Quinn et al., 2014) presented a similar result where high TAG content caused an enhancement of 20 % in the net energy ratio. They inferred that the attained outcome is because of low mass which enters the AD, and consecutively, leads to lower generated waste heat and electricity. Chowdhury et al. (Chowdhury et al., 2012) showed that by increasing the lipid weight content from 40 % to 70 %, the energy demand decreases substantially; however, the simulation results showed less impact by the lipid content in an integrated AD fermentation process.

Figure 2-8 shows distribution of lipid content versus productivity (g/m^2. d) from published studies. It shows the large variability in the productivity at the same lipid content. Some information is also missing in the research to address both metrics. The horizontal axis

with green filled circle is for research studies in which productivity was reported; but no further information about lipid content was given. Blue points on the vertical axis indicate where lipid yield data is shown without reporting the productivity. This wide diversity of published data is one of the major discrepancy between existing LCAs.

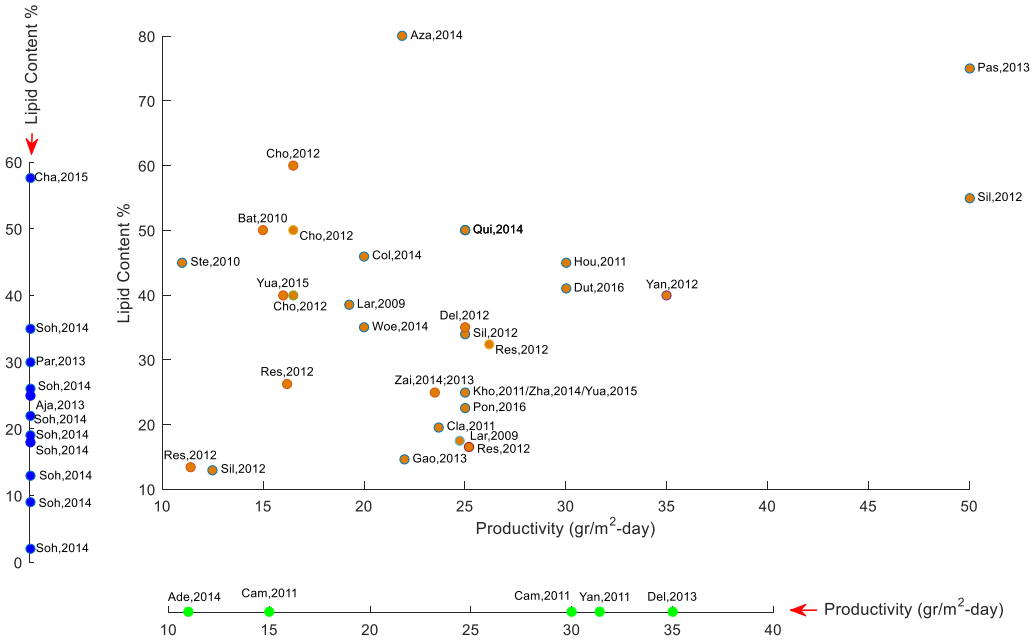


Figure 2-8: The effect of lipid content on productivity in the literature.

4.4 Recycling material in algae biodiesel production process

For sustainability of microalgal biodiesel production, it is critical to recycle the nutrients such as phosphate, nitrogen and carbon dioxide (Chisti, 2013), water, and other chemicals such as solvent used in the lipid extraction stage. Further description is provided below.

After the lipid extraction stage, the residue slurry can be used in the anaerobic digestion (AD) process to produce CH_4 which can be burnt to produce heat, power, and CO_2. Ammonia and phosphates can also be recovered from the remaining liquid stream (Cai et al., 2013; Sheets et al., 2014; Sialve et al., 2009; Ward et al., 2014). The combusted methane (from non-lipid constituents of the algae) produces CO_2 that can be used in the

cultivation unit. The HTL process has been proven successful in converting about 80 % of the energy stored in the residue slurry to bio-oil, in addition to producing a liquid stream which is rich in nitrogen and phosphate that can be recycled (Valdez et al., 2012). Chowdhury et al. (Chowdhury et al., 2012) claimed that about 65 % of the nutrients can be recovered and recycled after an AD process. Approximately 61 % of the N and 52 % of the P can be recycled from the liquid medium which is obtained after an AD. In the catalytic hydrothermal gasification, these values are estimated at about 36 % for N and 54 % for P (Gao et al., 2013). The nutrient recycling potentials as high as 70 % and 89 % were reported in the literature for N and P, respectively (Alcántara et al., 2013; Rösch et al., 2012; Yuan et al., 2015). Through recycling both water and nutrients after an AD, about 66.2 % of N and 89.7 % of P nutrients can be supplied (Yuan et al., 2015). These results were attained based on a lipid extraction efficiency of 73.6 %, and a methane yield of 310 to 340 mL/g-VS. The nutrient recovery factor in the HTL and AD processes are 26.0 g N and 6.8 g P (for HTL) and 40.7 g N and 3.8 g P (for AD) for 1 kg (dry weight) of the algae cultivated in an open raceway pond (Zhang et al., 2014).

Recycling CO_2 from flue gas can also improve the results of LCA (Resurreccion et al., 2012). Although some researchers found the effect of recycled CO_2 on the LCA to be insignificant (Gao et al., 2013), others concluded that a 40 % decrease in carbon demand occurs by recycling CO_2 from biogas combustion (Yuan et al., 2015). The CO_2 recycling in algae to biodiesel production process has been considered in several research works (Yuan et al., 2015); (Ajayebi et al., 2013); (Gao et al., 2013); (Resurreccion et al., 2012); (Clarens et al., 2011).

Based on current technologies, the utilization of fresh water to produce 1 L of diesel varies between 3.15 L to 3650 L of water, depending on which pathway and technologies have been implemented (National Research Council, 2012). Stephenson et al. (Stephenson et al., 2010) hypothesized that reusing the recycled water is not desirable due to the presence of contamination or inhibitors such that they sent the used water to the wastewater treatment unit. According to Stephenson et al.'s study (Stephenson et al., 2010), without water recycling, the net water demand per ton biodiesel produced for the OP systems is about 1,750 m^3 which is much higher than the value of 335 m^3 for the PBR

systems. The water which is obtained in the flocculation and dewatering units can be recovered and recycled back to the cultivation reactor to reduce the overall water footprint. This step is crucial for a sustainable algae cultivation process at large scales (Biller et al., 2012). Using the water from harvesting stage also lowers the nutrient use and harnessing fertilizer fate, in favor of a more sustainable process with reduced environmental footprints and fossil fuel consumption (Hou et al., 2011). Recirculating the harvest medium decreases the water requirement by 84 % and the required nutrients by 55 % (Yang et al., 2011). Yuan et al. (Yuan et al., 2015) also reported a similar reduction of about 89 % for the freshwater demand by recycling the harvest medium. Without water recycling, the water footprint of algal biodiesel (volume water per ton biodiesel produced) will increase to 3,726 m^3 (Yang et al., 2011). Yang et al. (Yang et al., 2011) reparted that in OP system, about 25.9 % of the water will be lost by evaporation, thus, around 2,760 kg water/kg biodiesel will be discharged. If all of the harvest water is recycled to the ponds, the water footprint will decrease to 591 kg water/kg biodiesel (Yang et al., 2011). Chowdhury et al. simulated the water footprint for three different scenarios: 1) no-reuse of nutrients, 2) reusing nutrients without allocation, and 3) reusing nutrients with the allocation (Chowdhury et al., 2012). In the first case, water demand to produce 1 ton algae biodiesel varied from 99 to 142 m^3, depending on the lipid content of the algae. The lower and higher values were obtained for the algae with 40 % and 70 % lipid contents, respectively. For the second case, the water demand to produce 1 ton algae biodiesel changed between 98 m^3 (for 40 % lipid) to 85 m^3 (for % lipid). In the third case (reusing nutrients with-allocation), the water demand to produce 1 ton algae biodiesel changed from 46 m^3 (for 40 % lipid) to 70 m^3 (for 70 % lipid). The effect of the algae lipid content on the water footprint is obviously crucial in the algae to biodiesel processes.

5 General pathway comparison of studies

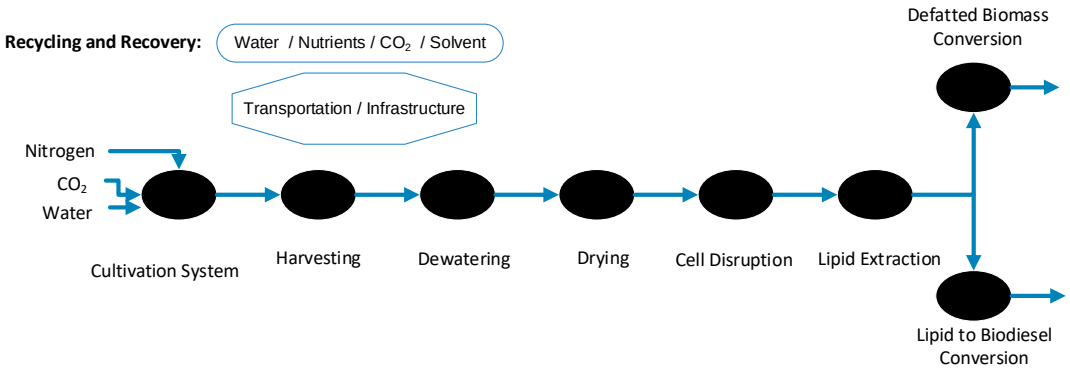
Figure 2-9 summarizes proposed pathways for the production of algal biodiesel in the 31 experimental studies. In the upper part of the figure, there is two templates which the upper one is the block and color coded representation of the process. The lower one is a more descriptive demonstration of Azadi et al., (2014). The blocks represent the eight

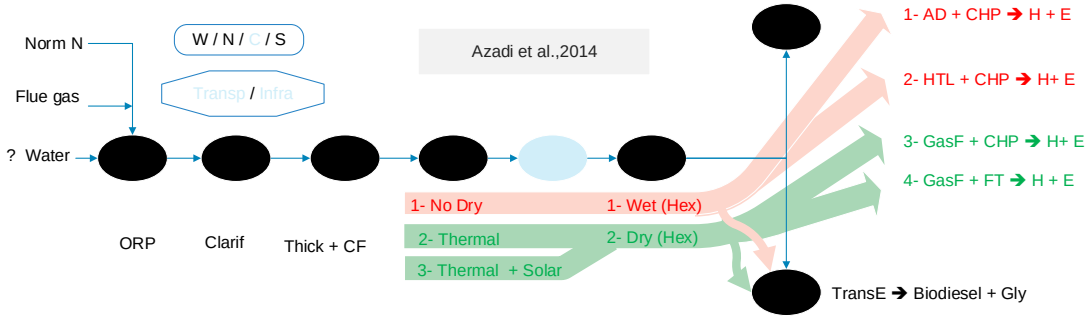
units in the overall process, including: 1) cultivation, 2) harvesting, 3) dewatering, 4) drying, 5) cell disruption, 6) lipid extraction, 7) defatted biomass conversion and 8) lipid to biodiesel conversion. The blocks-and-line template contains black ovals which represent processing units, and rounded rectangle for recycling water (W), nutrient (N), CO_2 (C), and solvent (S). For the abbreviations, the reader may refer to the footnote of this figure. If any of these four components are recycled into the process, they are shown with black color, otherwise they are shown in pale blue as an inactive part of the overall process. On this figure, a hexagon block exists below the recycle box which shows whether or not transportation and infrastructure have been included in the life cycle analysis, if included, they are shown with black color, otherwise shown in pale blue as inactive part of the overall LCA. To explain other points on the figure, we discuss two selected studies:

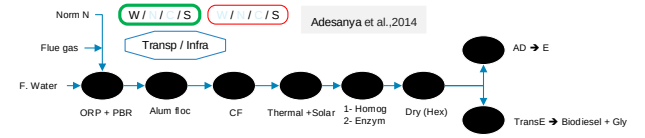
Azadi et al., 2014: The cultivation system is an open race pond (ORP) bioreactor in which, normal nitrogen condition (Norm N), flue gas (as a source for CO_2), and unknown water type (? Water) are used. The harvesting unit for algae biomass uses clarifier agent (Clarif). A combination of two dewatering technologies is included in this study; thickener (Thick) and centrifugation (CF). In the drying three options are considered; no drying (NO Dry, shown in red), thermal heating (Thermal, shown in green), and combination of solar drying and thermal heating (Solar + Thermal, shown in green). In this specific study, there is no cell disruption stage, so this block is presented in pale blue, showing it as inactive. In the lipid extraction unit, two alternatives are studies which are wet hexane extraction (Wet (Hex), shown in red) and dry hexane extraction (Dry (Hex), shown in green). The lipid to biodiesel conversion unit uses transesterification (TransE) to produce biodiesel and glycerol (Gly). Four options are studied for the defatted biomass conversion, including *1*- combination of anaerobic digestion and CHP (AD+CHP), *2*- combination of hydrothermal liquefaction and CHP (HTL+CHP), *3*- combination of gasification and CHP (GasF+CHP), *4*- combination of gasification and fischer tropsch (GasF+FT). The product of these four options are electricity (E) and heat (H).

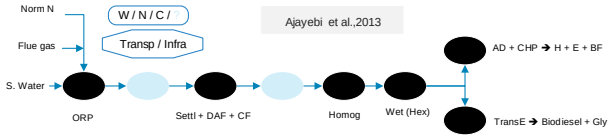
In this study, the authors have investigated the effect of recycling water, nutrients and solvent to the cultivation media on the LCA (shown in black) whereas CO_2 recycling was

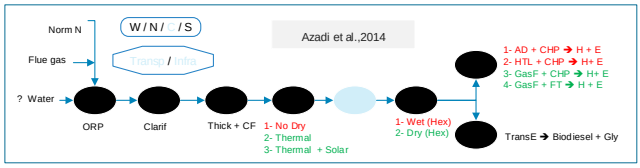
not explored (in pale blue). The effect of both transportation and infrastructure is not considered in the LCA. For clarity, units in black color, are used in common in both the red and green pathways. Red and green color are representative of two categories of pathways each of which has its specific units and no-unit share with another category. Here, each category has two pathways, therefore, generally this study explores four pathways. For example, red flow diagrams contain only wet hexane extraction (Wet (Hex)) followed by anaerobic digestion or hydrothermal liquefaction to produce heat (H) and electricity (E). On the other hand, green color unit embedded in green flow diagrams utilize dry hexane extraction (Dry (Hex)) preceded by thermal or thermal-solar drying to be prepared for gasification with either CHP or FT to produce heat (H) and electricity (E). Both categories (i.e. colors) include open race pond cultivation (OPR) system, clarifier harvesting (Clarif), thickening and centrifugation (Thick+CF), and transesterification (TransE).

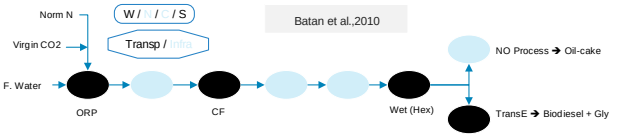

Recycling and Recovery:
Water / Nutrients / CO_2 / Solvent
Transportation / Infrastructure
Nitrogen
CO_2
Water
Cultivation System
Harvesting
Dewatering
Drying
Cell Disruption
Lipid Extraction
Defatted Biomass Conversion
Lipid to Biodiesel Conversion

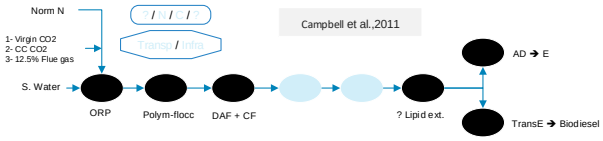

Norm N
Flue gas
? Water
W / N / C / S
Transp / Infra
Azadi et al.,2014
ORP
Clarif
Thick + CF
1- No Dry
2- Thermal
3- Thermal + Solar
1- Wet (Hex)
2- Dry (Hex)
1- AD + CHP → H + E
2- HTL + CHP → H+ E
3- GasF + CHP → H+ E
4- GasF + FT → H + E
TransE → Biodiesel + Gly

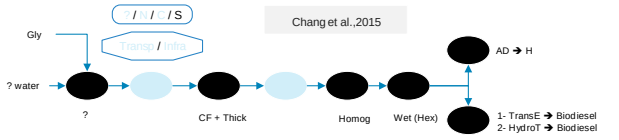

Norm N
Flue gas
F. Water
W / N / C / S
Transp / Infra
Adesanya et al.,2014
ORP + PBR
Alum floc
CF
Thermal +Solar
1- Homog
2- Enzym
Dry (Hex)
AD → E
TransE → Biodiesel + Gly

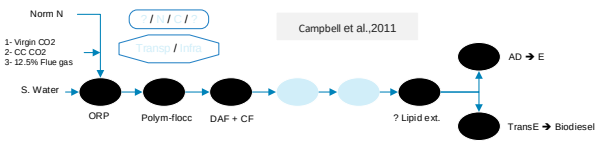

Norm N
Flue gas
S. Water
W / N / C /
Transp / Infra
Ajayebi et al.,2013
ORP
Settl + DAF + CF
Homog
Wet (Hex)
AD + CHP → H + E + BF
TransE → Biodiesel + Gly


Norm N
Flue gas
? Water
W / N / C / S
Transp / Infra
Azadi et al.,2014
ORP
Clarif
Thick + CF
1- No Dry
2- Thermal
3- Thermal + Solar
1- Wet (Hex)
2- Dry (Hex)
1- AD + CHP → H + E
2- HTL + CHP → H+ E
3- GasF + CHP → H+ E
4- GasF + FT → H + E
TransE → Biodiesel + Gly

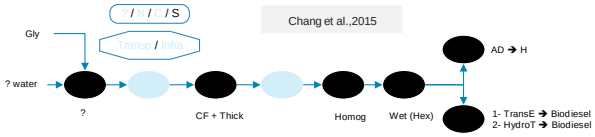

Norm N
Virgin CO2
F. Water
W / N / C / S
Transp / Infra
Batan et al.,2010
ORP
CF
Wet (Hex)
NO Process → Oil-cake
TransE → Biodiesel + Gly


Norm N
1- Virgin CO2
2- CC CO2
3- 12.5% Flue gas
S. Water
W / N / C / S
Transp / Infra
Campbell et al.,2011
ORP
Polym-flocc
DAF + CF
? Lipid ext.
AD → E
TransE → Biodiesel

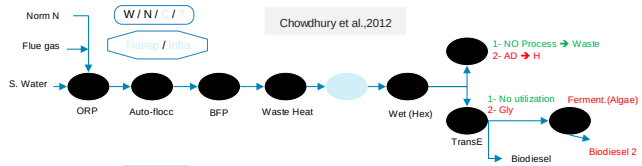

Gly
? water
W / N / C / S
Transp / Infra
Chang et al.,2015
?
CF + Thick
Homog
Wet (Hex)
AD → H
1- TransE → Biodiesel
2- HydroT → Biodiesel



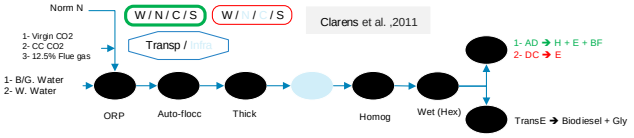
Campbell et al.,2011



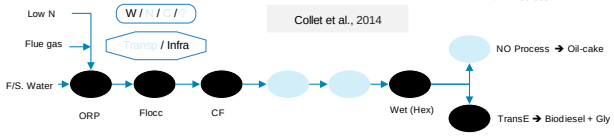
Chang et al.,2015



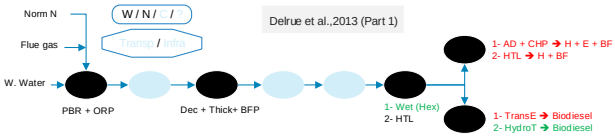
Chowdhury et al.,2012



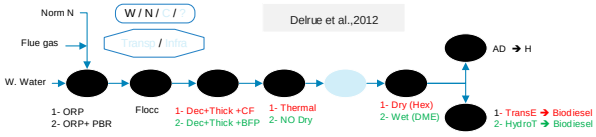
Clarens et al. ,2011



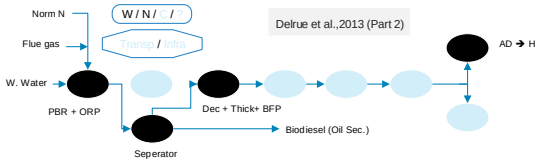
Collet et al., 2014



Delrue et al.,2013 (Part 1)



Delrue et al.,2012



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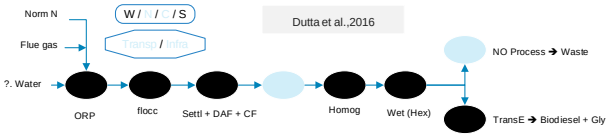
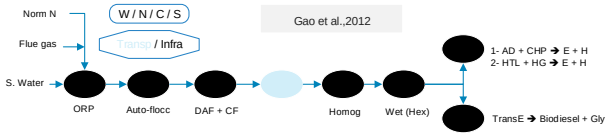
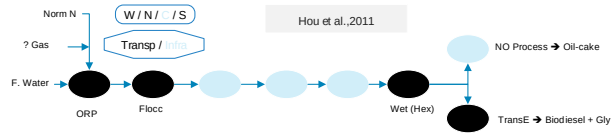


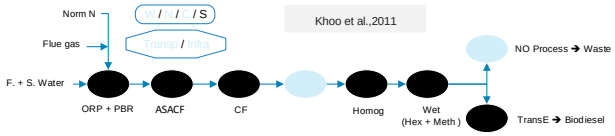
Dutta et al.,2016



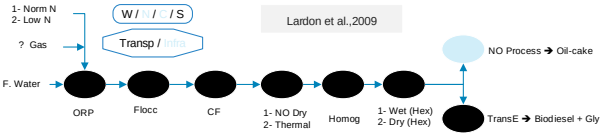
Gao et al.,2012



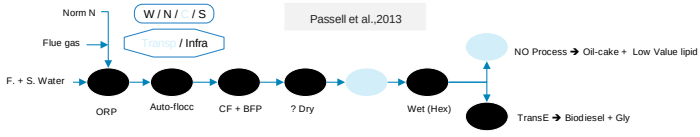
Hou et al.,2011



Khoo et al.,2011



Lardon et al.,2009



Passell et al.,2013

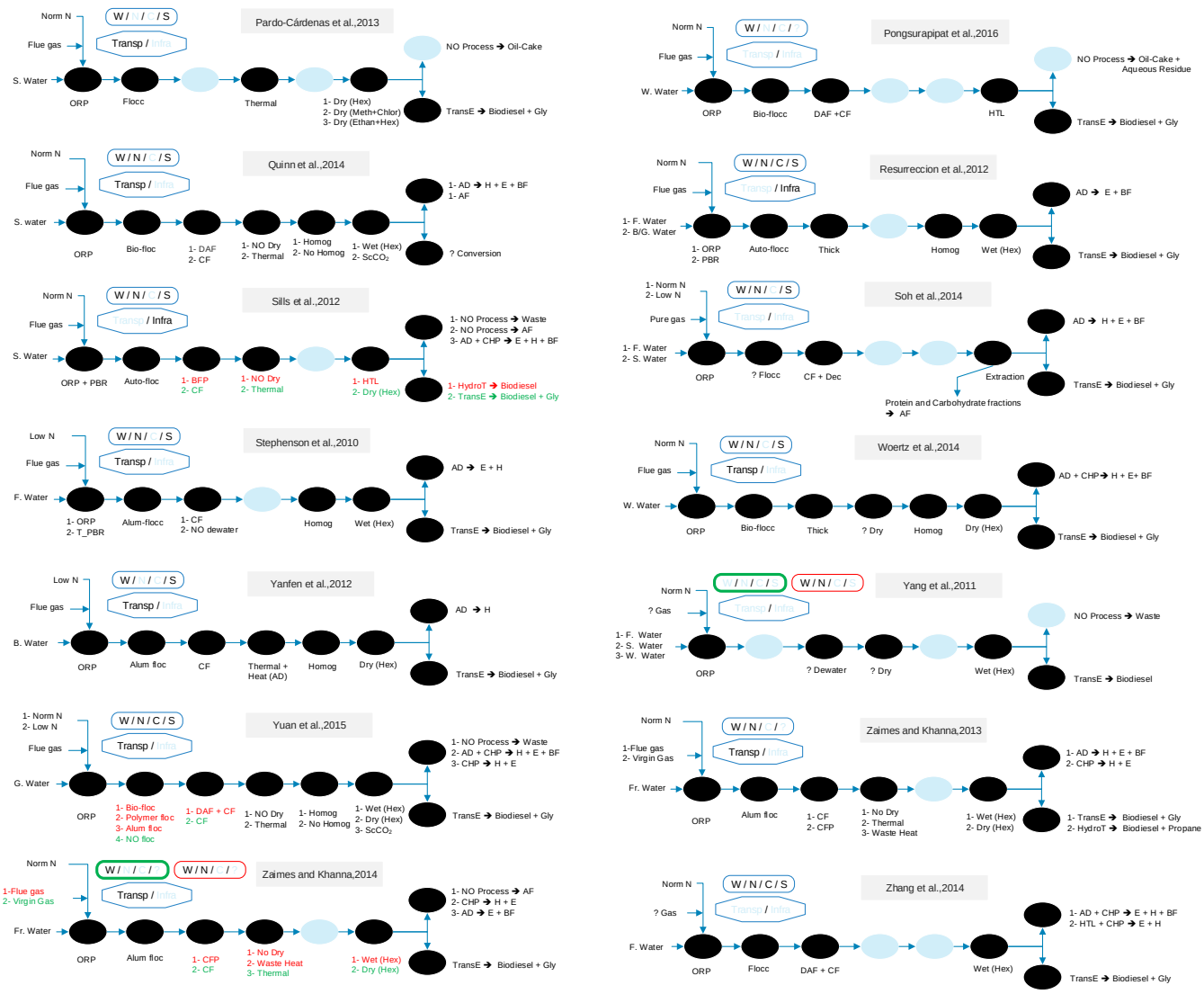


Figure 2-9: A summary of process pathways used for the conversion of algae to biodiesel in literature

Abbreviation: {**?**: insufficient data, **AD**: Anaerobic Digestion; **AF**: Animal Feed; **Alum-flocc**: Aluminum Flocculation; **ASACF:** Air Sparging Assisted Coagulation Flocculation; **B/G water**: Brackish/ground Water; **BF**: Biofertilizer; **BFP**: Belt Filter Press; **Bio-flocc**: Bioflocculation; **CC**: Carbon Capture; **CF**: Centrifugation; **CFP**: Chamber Filter Press; **CHP**: Combined Heat and Power; **DAF**: Dissolved Air Flotation; **Dec**: Decantation; **Dry (Hex)**: Dry Hexane Extraction; **Dry (Ethan-Hex)**: Dry Ethanol-Hexane Extraction; **Dry (Meth-Chlor)**: Dry Methanol-Chloroform Extraction; **E**: Electricity; **Enzym**: Enzyme

Treatment; **F. Water**: Fresh Water; **Ferment**: Fermentation; **Flocc**: Flocculation; **FT**: Fischer–Tropsch; **GasF**: Gasification; **Gly**: Glycerol; **H**: Heat; **HG**: Hydrothermal Gasification; **Homog**: Halogenation; **HTL**: Hydrothermal Liquefaction; **HydroT**: Hydrotreatment; **Norm N**: Normal Nitrogen; **ORP**: Open Raceway Pond; **PBR**; Photobioreactor; **S. Water**: Saline Water; **ScCO$_2$**: Supercritical CO_2 Extraction; **Settl**: Settlement; **Thermal**: Thermal Drying; **Thick**: Thickening; **TransE**: Transesterification; **W. Water**: Waste Water; **Wet (Hex)**: Wet Hexane Extraction; **W**: Water Recycling; **N**: Nutrient Recovering; **C**: CO_2 Recycling; **S**: Solvent Recovering; **Transp**: Transportation; **Infra**: Infrastructure}.

6 Life cycle assessment (LCA)

Life cycle assessment (LCA) is a method to quantify the environmental impacts by a product during its life from raw material extraction to its production, utilization, recycling and ultimate discard of the wastes (Adesanya et al., 2014). This systematic approach produces information on the environmental footprints of the product chain (Pant et al., 2011). It can be used as a decision-making tool to obtain a better design with minimal environmental impact (Lam et al., 2009). The LCA identifies the environmental impacts over the life cycle of the system, through assessing the net material and energy inputs and outputs (Bribián et al., 2011; ISO, 2006). As there is no large-scale biodiesel production plant from the algae feedstock, the real data for such an assessment is not possible yet (Sander and Murthy, 2010). Due to this shortcoming, researchers use mathematical models that can enable the process evaluation at an industrial-scale production rate of biodiesel (Collet et al., 2015). The simulation results and conclusions which have been made for LCA in the literature are however controversial so that different raw materials, process configurations, and operating conditions are suggested. Process variables such as algae strain type, technologies for the cultivation, harvesting, biomass processing, lipid conversion, system boundary rigidity, and by-product allocation have remained debatable in the literature (Collet et al., 2015; Sander and Murthy, 2010; Wang, 2005). A database containing the components of input and output in the life cycle assessment is called life cycle inventory (LCI) which is defined within the system

boundaries by listing the input and output requirements for each stage of the biodiesel production process (Davis et al., 2009). Discrepancies in the LCI list results in the considerable variation and differences in the LCA evaluations.

6.1 Functional units in LCA

A functional unit is a measure of the function of the system to be studied by the inputs-output relations in the LCA (Davis et al., 2009). The selection of the functional units influences the LCA results (Adesanya et al., 2014; Cherubini and Strømman, 2011). There is not an agreement/procedure observed in the literature regarding the selection of the functional units by different analysts. This drawback has resulted in various LCA outcomes. In the open sources, three classes of functional units are proposed for the LCA of the microalgal biodiesel production process, including: 1) service-oriented functional units, 2) energy-oriented functional units, and 3) material-oriented functional units. The service-oriented functional unit is defined as transport distances such as annual Vehicle Kilometers Traveled (VKT) per hectare-year, Vehicle Kilometers Traveled (VKT), km of transportation, kg biodiesel per 1 km traveled, and combustion of sufficient fuel (in t or, metric ton) in an articulated truck to transport one ton of freight for one kilometer (1 t . km is equivalent to 0.89 MJ of diesel fuel). The energy-oriented functional unit is a specific amount of energy that exists in the biodiesel in the form of chemical energy such as production (or combustion) of 1 MJ biodiesel. The mass-oriented functional unit is a specific extent of mass of product which is produced in the overall process of algae to biodiesel such as production of 1 ton of biodiesel, 1 kg of biodiesel or production of 1 kg biomass.

6.2 System boundaries in LCA

Investigation of the system boundaries can help to assess the impacts of biodiesel production or to recognize some parts of the chain that may possibly have potentials for upgrading. The use of different system boundaries is arguably the main reason for inconsistency in the life cycle evaluation of the microalgal biodiesel process in the literature (Davis et al., 2009). System boundaries vary not only by start and end points in the process chain for biofuel production but also by space and time. These variations may cause a dramatic effect on energy and environmental burdens. A well-to-gate LCA

is a general expression used in the biodiesel production process to show a class of LCA, covering the process from resources extraction to the factory gate. The transportation of biofuel to the fuel station will be extended to well-to-pump LCA. A well-to-wheel LCA incorporates the feedstock production, fuel processing, fuel delivery, transportation, and finally combustion in a vehicle. A cradle-to-grave (well-to wheel) system boundary involves not only the biodiesel, but also the vehicle and the transportation route. In terms of LCA, this is a case that covers the entire aspects of process and is preferred for the environmental analysis (van der Voet et al., 2010). Whatever the process number is going up, the data requirement increases. This is an important issue that should be considered. With respect to the system boundaries (Table 2-1), they are usually accompanied by a specific functional unit. Among 31 cases reported in the literature, 15 reports utilized well-to-wheel variant to assess all phases of the life cycle. Well-to-gate impact includes 14 events, whereas 1 study (Delrue et al., 2013) did not mention any life cycle system boundaries.

6.3 Temporal units in LCA

The lack of temporal data, meaning time horizon and operational time during year, is a vital shortcoming in the LCA (Levasseur et al., 2010). The Intergovernmental Panel on Climate Change (IPCC) has restricted the amount of GHG release (carbon footprints) to decrease the level of GHG emissions during the life cycle of a product but has failed to envision its timing (Kendall, 2012). This may result in a poor assessment of the short time global warming consequences. Determining a time-frame for the climate change effects can improve the assessments (Kendall and Price, 2012; Levasseur et al., 2012; Levasseur et al., 2010; Levasseur et al., 2013; O'Hare et al., 2009; Pinsonnault et al., 2014). In addition, the operational time is another information that can influence both the environmental and economic aspects because the biodiesel from algae plant cannot operate over the entire year.

Dynamic LCA can be of interest for policy and decision making because different time horizons may be chosen (Levasseur et al., 2010).

Surprisingly, 15 studies have not specified the life time for analysis and the remaining has reported the various time horizon as seen in Table 2-1. As the potential impacts of

emissions and extractions can be sensitive to timing, Pinsonnault et al. (Pinsonnault et al., 2014) focused on the aggregation of temporal distribution of the background system inventory to foreground processes, 50% of studies not only benefited from temporal differentiation of background systems but also missed the time horizon. It seems that the foreground time horizon, dynamic LCA approaches, and temporal aggregation of life cycle inventory (LCI) data are three parameters that should be evaluated in upcoming investigations.

6.4 Land use in LCA

The potential to grow the microalgae on non-tillable land at high surface productivity makes it a promising feature, implying it does not require significant land use (Collet et al., 2014). Clarens et al. (Clarens et al., 2011) compared the algae-derived transportation energy in term of the land use efficiency and demonstrated its superiority over other benchmark crops. The algae cultivation systems can be ranked in terms of their land usage requirement as (Resurreccion et al., 2012): (OP–Fresh water) > (PBR–Fresh water) > (OP–Brackish/salt water) > (PBR–Brackish/salt water).

6.5 Infrastructure in LCA

Chowdhury et al. (Chowdhury et al., 2012) claimed that the construction and maintenance have minor effects on the overall environmental impacts during the operation of algae to biodiesel. On the contrary, Gao et al. estimated that about 35% of the total energy input is due to the infrastructure construction materials (Gao et al., 2013). Campbell et al. (Campbell et al., 2011) set their system boundaries not to include infrastructure and maintenance, due to the lack of exact updated details of subsystems and rarity of the literature regarding their embodied costs and environmental impacts. However, it is obvious that byproduct utilization cannot offset the burden embedded with infrastructure. In the majority (about 75%) (Table 2-1) of the research works on algae to biodiesel production process, the effect of infrastructure on LCA is overlooked, and a realistic impact of algae biodiesel production process on the environment is not assessed.

6.6 Co-product allocation in LCA

Cherubini and his colleagues studied the influences of five different allocation methods (substitution, mass, energy, exergy, and economic allocations) to monitor the environmental impacts by plant products (Cherubini et al., 2011).

Co-product treatment or allocation (as it is known in the LCA terminology) has been reported as one of the possible roots for controversial results in the literature of algae to biodiesel process (van der Voet et al., 2010). The aim of allocation is to quantitatively determine the contribution of biodiesel and its co-products to environmental impacts in the LCA (Stephenson et al., 2010). Evaluating the impact of each allocation method is a critical task which can facilitate decision-making because the choice of allocation will significantly influence the process environmental impacts through energy consumption, GHG emissions, and environmental footprints (Zaimes and Khanna, 2014). When discrepancies exist between the mass, market value, or/and energy content of the co-products, the impact of each allocation approach will be unrealistic (Zaimes et al., 2013; Zaimes and Khanna, 2014). To avoid the allocation, the system boundary expansion (SBE) (known as the displacement method) can be applied (Weidema, 2000; Zaimes and Khanna, 2014). The ISO 14040-series suggest that the allocation should be avoided whenever possible (ISO, 2006; Weidema, 2014). In the displacement method, the conventional product is replaced by a co-product which is produced in the biodiesel production chain. For example, the extracted microalgae biomass, heat or electricity (from AD or HT), recovered nutrients, glycerol, and animal feed (biofertilizer) will be replaced by the external heat, electricity, input nutrient, and petroleum-derived glycerol, respectively (Batan et al., 2010). The credits will reduce the total energy demand and burdens associated with the biodiesel production process. It should be noted that not all the allocations are relevant to the biodiesel production process, and extra care/analysis should be taken as some of the allocation methods may produce misleading results (Zaimes and Khanna, 2014). For instance, the mass allocation may unrealistically lower the biodiesel associated burdens by assigning high amounts of credit to the non-lipid share of biomass (Collet et al., 2014). Similarly, considering the energy allocation of the

biofertilizers, while in reality, they are not valuable for their energy contents (Zaimes and Khanna, 2014).

The displacement method may result in negative values for environmental impacts categories (Zaimes and Khanna, 2014). Zaimes and Khanna (Zaimes and Khanna, 2014) conducted a comprehensive study on the impact of allocation and co-product choice on the biodiesel environmental impacts, and compared the results to a base scenario. For the base scenario, the energy return on investment (EROI) varied from 0.15 to 0.40, and the overall GHG emissions were in the range of 142 to 352 (g CO_2 eq./MJ fuel). In the improved process, the EROI increased to values in the range of 0.39 - 1.18 and the GHG emissions decreased to 35 to 141 (g CO_2 (eq)/MJ fuel). Figure 2-10 illustrates the contribution of different allocation methods in the analysis of LCA in the process of algae to biodiesel. As it can be concluded from Figure 2-10, about 24 % of the studies did not use the allocation methods; 36 % of studies used the energy allocation and 29 % employed the substitution method. Detailed information on their particular treatments of allocation is provided in Table 2-1.

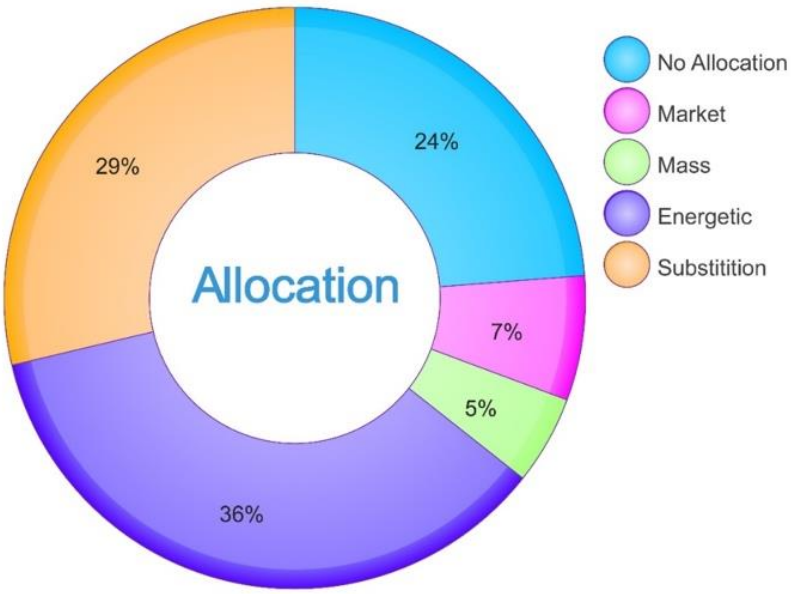


Figure 2-10: Contribution of different allocation methods in the literature studies of algae to biofuel

6.7 Life cycle impact assessment (LCIA)

Translating the environmental interventions into environment impact of a process is a critical step in the LCA that is conducted by classifying numerous interventions into impact categories (van der Voet et al., 2010). The real data from available database and research papers, and the estimated data from simulation studies are included in the software packages for studying the impact (Ciroth, 2007). Different measures and guidelines were employed in the literature to quantify the environmental impacts by a process; one measure may be used as equivalent of another one. For example, the energy input (MJ) can be converted to equivalent CO_2 emissions (g CO_2). Each method (of quantification) measures the impact by a different gas mixture. For instance, EU Renewable Energy Directive (RED) covers three long-lived greenhouse gases: CO_2, CH_4, and N_2O; ReCiPe includes 93 gases; TRACI 2.1 and ReCiPe 2013 both cover more gases than CML 2013 (Bradley et al., 2015). The discrepancy that exists between the characterizing models (and their depth of coverage) will significantly influence the LCA results; a weak measure may suggest a process that is not optimal in terms of its environmental impacts (Owsianiak et al., 2014). ReCiPe midpoint, EDIP (2003), IPCC guidelines, CML method, Eco-Indicator 2002+, TRACI, and CA LCFS are the methods employed to assess the environmental burdens. European Commission (Pant et al., 2010) gathered the corresponding data and compared the most frequently used Life Cycle Impact Assessment methodologies. For a comprehensive review, you may refer to Pant et al. (Pant et al., 2010). GREET, GaBi, SimaPro, and OpenLCA are among the popular software packages which can be used to estimate the impacts of different process variables in the academic and industrial applications. To reasonably quantify the LCA impacts, secondary data (provided by databases such as raw material commodities and end-of-life scenarios) are required which are not based on measurements of the respective process. Their allocations are however, costly and time-consuming if at all accessible (Sayan, 2011). A list of different software packages along with the list of databases for the algae biodiesel processes was given by Sayan (Sayan, 2011). In Figure 2-11, the case specific software implemented in the reviewed papers and employed different life cycle impact assessment (LCIA) methodologies are presented. About 65 % of the studies publicized their used methods.

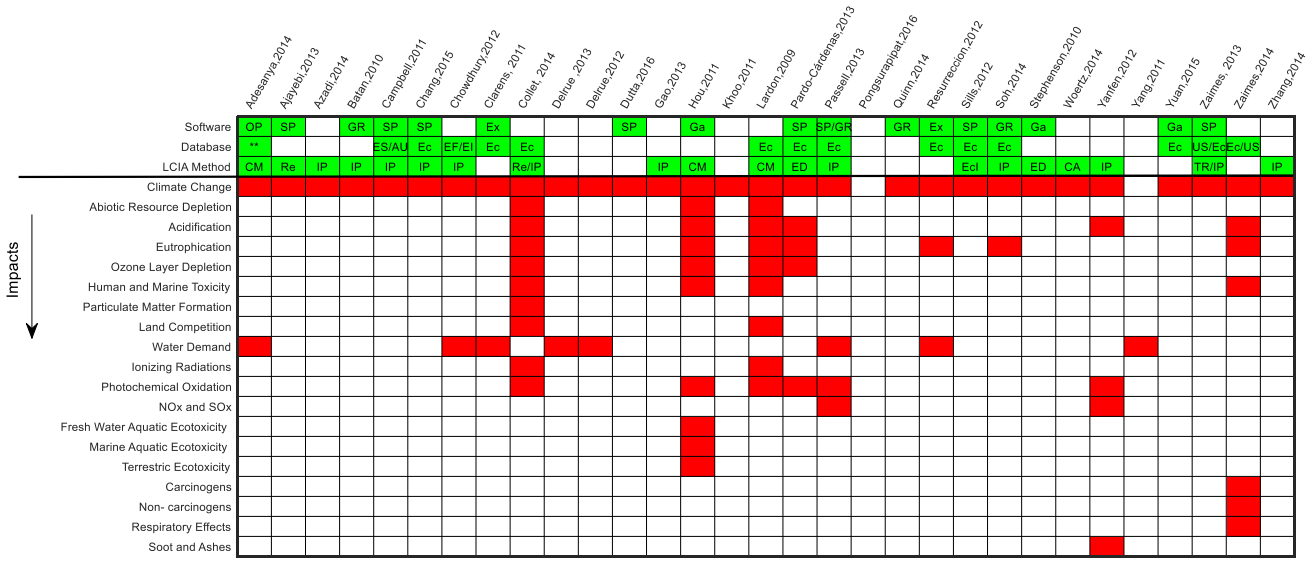


Figure 2-11: Software, database, LCIA methodology, along with the inclusion of different impact categories in the literature studies of algae to biodiesel process. The plot is divided into two main sections; First, the alliance of software, database, LCIA method in which the addressed information highlighted by green color, Second, partly colored matrix allocated for comparison of studied impacts in which the red blocks belong to the addressed impacts. The white blocks refer to non-reported impacts and information.

(Abbreviation: *Software*: **Ex**=Excel, **Ga**=Gabi, **SP**=SimaPro, **GR**=Greet, **OP**=OpenLCA; *Database*: **Ec**=Ecoinvent, **ES**=Energy Supply Association of Australia, **AU**=AusLCI, **EF**=EFMA, **EI**=EIOLCA, **US**=US-LCI, ******=Ecoinvent/ELC/NREL; *LCIA method*: **RE**=ReCiPe midpoint, **IP**=IPCC, **ED**=EDIP (2013), **CA**=CA LCFS, **TR**=TRACI, **CM**=CML, **EcI**=Eco-Indicator 2002+)

7 Energy performance metrics

The energy requirement of algae to biodiesel process is one of the most important factors, dominating the viability of process. It also has a great influence on the environmental impacts of the process such as the GHG emissions (Collet et al., 2014). Three energy metrics are introduced to quantify the energy efficiency of the algal biodiesels, including:

1) energy requirement, 2) energy return on investment (EROI), and 3) net energy ratio (NER).

Energy requirement: This criterion is defined as the amount of energy (MJ) which is required to produce 1 unit of the functional unit (FU) that is being assessed.

Energy return on investment (EROI): This metric is defined as the ratio of biofuel (such as biodiesel) energy output to energy input to produce 1 unit of the functional unit. EROI greater than unity is favorable because more fuel energy is generated per unit energy that is consumed (Zaimes and Khanna, 2014). Two relationships/formulas are proposed for the EROI in the literature. In the first one, the energy demand (input energy) is governed by the primary source of input energy that can be either renewable or non-renewable. The second formulation uses the total cumulative non-renewable input energy for the energy demand. The second approach is more frequently used in the literature of biodiesel.

Net energy ratio (NER): This parameter is defined as the ratio of primary energy input to total energy produced. The energy included in the product (biodiesel) and by-products are considered for the total energy produced. Primary energy includes the electricity, natural gas, and that used to produce the nutrients (Delrue et al., 2012). Collet et al., 2014 suggested that the contribution of upstream process in the NER should be taken into consideration. For this purpose, they have distinguished total energy demand (during entire process) and local energy demand (for one-unit process) and found that the NER decreases from 1.07 (unfavorable) using total energy demand to 0.62 (favorable) using local energy demand for the biodiesel production process. Clarens et al. (Clarens et al., 2011) suggested that NER can be either more or less than unity, depending on the specific combination processes adapted. A summary of energy requirement (MJ/FU), EROI (MJ/FU), and NER along with their equivalent amount of GHG emissions (g CO_2 (eq)/FU) for the algae to biodiesel process is provided in Figure 2-12.

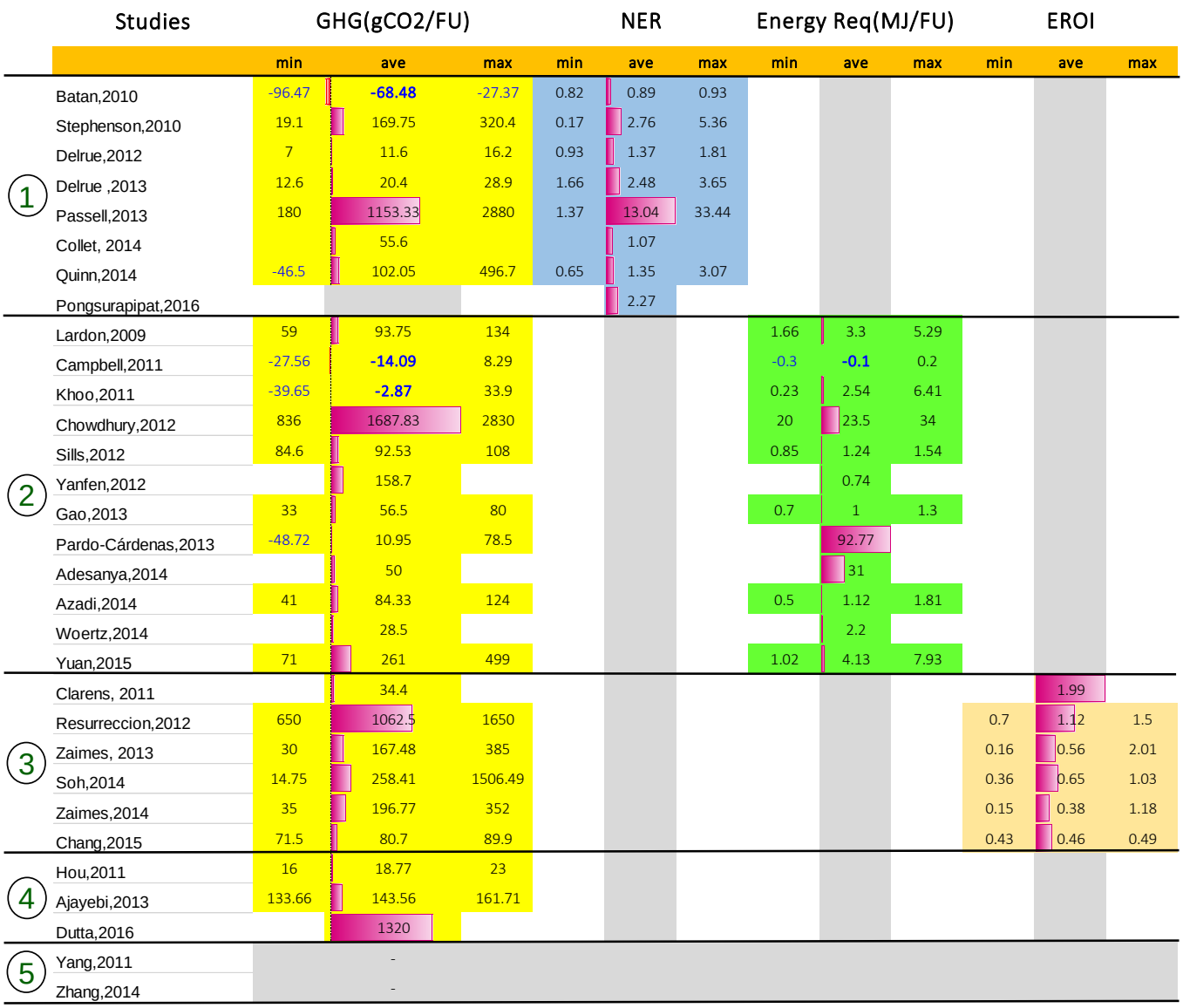


Figure 2-12: Summary of energy metrics in the algae to biodiesel process in literature: (a) energy requirement and its equivalent CO_2 emission, (b) EROI and its equivalent CO_2 emission, and (c) NER and its equivalent CO_2 emission.

Due to lack of consensus on the functional units, the metrics are represented per functional unit. Since, some researchers reported one value, compared to others with a list or range of data, we grouped the data and represented their variation as the minimum, maximum, and average values where the purple bars depict the relative amount of

quantities. The detailed data associated with each study can be accessed via supplementary file. For example, Quinn et al. (2014) conducted analysis of several pathways with even seemingly contradictory results, implying the selection of optimal and appropriate route and technologies drastically affects the LCA outcomes. The grey columns show that there is no reported data for the mentioned metric. The data are classified into five clusters; namely, GHG-NER, GHG-Energy Requirement, GHG-EROI, only GHG, and no information available. To compare the LCA advances of algae biodiesel over the time, the references for each cluster are sorted chronically. To compare the sustainability and environmental impact of biodiesel, specific metrics should be established because every study followed its own way and protocol, and consequently, various results and even contradictory were achieved (see Figure 2-12). Accordingly, Figure 2-12 demonstrates that even for evaluating the energy efficiency of algal biodiesel, there is no unique way of representing outputs. Thus, three criteria are employed to quantify energy performance of algal biodiesel production. These discrepancies on evaluating metrics cast the doubts on procedures for life cycle analysis. Based on Figure 2-12, we can ensure that algal biodiesel introduces serious sustainability concerns or not. As long as these inconsistencies exist, policy makers, entrepreneurs, stakeholder, and investors have less propensity to rely on the available data and invest in this field.

8 Environmental impact measures

The LCA studies have reported a variety of different impact categories, and sometimes the results from literature studies on the environmental impacts are not conclusive. For example, contrary to others, Zaimes and Khanna (Zaimes and Khanna, 2014) claimed that the production of microalgal biodiesel has higher environmental impacts for the majority of impact categories relative to the petroleum diesel.

GHG Balance: It is not surprising that the greenhouse emission is used as an important measure in most of the research in the algae biodiesel literature, owing to their growing impact on global warming and climate change because of human activity (Cherubini and Strømman, 2011). The emission can be quantified in terms of their global warming potential (GWP) in equivalent g of CO_2 that is emitted to the environment. Several emission metrics and time horizons have been introduced based on the application and

policy stream. However, there is no best individual option working for all targets (IPCC, 2014). As shown in Figure 2-12, GHG emissions are estimated by the majority of the published scientific research studies in the algae biodiesel process. In Campbell et al. (Campbell et al., 2011), some GHG values are negative because the generated electricity via biogas combustion compensates the GHG emissions from the electricity source. Without process integration, there will be a higher demand for the fossil fuel energy. As the lipid content of algae increases, the GWP decreases accordingly. The GWP will also be low by recycling the nutrient and by integrating the heat in the process (Chowdhury et al., 2012). For the process scenarios without allocation, the GHG emissions are found to be less sensitive to the lipid content, but in the process scenarios with the allocations, the lipid content is found to inversely affect the GWP. At a higher lipid content, the amount of residue slurry to be processed per mass of biodiesel produced will be less (Chowdhury et al., 2012).

Eutrophication and Acidification (Acidifying gas emission and Acidification of land and water)**:** Eutrophication (EP) is defined as the enrichment of the surface waters by the nutrient. The dissolution of inorganic substances such as sulfates, nitrates, and phosphates will alter the acidity of water. The acid formation potential is commonly expressed by kg SO_2-eq or moles H^+-eq. Major acidifying emissions are caused by NOx, NH_3, and SO_2 (Goedkoop et al., 2009). The nitrogen losses in the algae cultivation ponds are in the forms of evaporation of ammonia and N_2O emissions. About 4 % of the total N input will vaporize as ammonia. The N_2O emissions from the open ponds are estimated to be 0.002 % of the N inputs, which is remarkably lower than the default IPCC emission factor of 1 % of total N inputs (Yuan et al., 2015). Zaimes and Khanna (Zaimes and Khanna, 2014) account for the nitrogen fertilizer and electricity productions as the main sources for the freshwater eutrophication. Their results revealed that the eutrophication is more severe in the algae biodiesel production process, compared to petroleum diesel (Zaimes and Khanna, 2014). The inclusion of GHG, eutrophication, and acidification (in literature) as LCA impacts in the algae to biodiesel process is summarized in Figure 2-11. As it is clear from Figure 2-11, eutrophication and acidification are not considered as much as the GHG in the literature, and they have been investigated only in a few researches. Other environmental metrics have been discussed rarely which is also a

drawback for LCA studies. Besides eutrophication and acidification, human and marine toxicity, land competition, water demand, and freshwater/marine aquatic ecotoxicity are of great interest because of the need for proper topography and immediacy to sustainable water resource as well as logical concerns about potential effect on terrestrial biodiversity, marine, and aquatic resources.

9 Electricity sources used in the algae to biodiesel process

A remarkable portion of the energy and environmental demands is dedicated to the production of electricity to be used in the process (Batan et al., 2010; Collet et al., 2014). Gao et al. (Gao et al., 2013) showed that about 85 % of the total fossil-energy input and 83 % of the GWP of the cultivation step are used for electric power generation itself. A similar study by Stephenson et al. (Stephenson et al., 2010) reported that about 74 % of the fossil energy consumed and 65 % of the GWP impact in the algae cultivation are utilized for electricity generation. Hence, the origin of electricity that is used in the biodiesel process can significantly affect its environmental impacts. Collet et al. (Collet et al., 2014) found that about half of the energy and environmental impacts in the biodiesel production process are due to processing of the co-products such as the algae bio-cake and glycerol. However, the residual biomass can be used to produce bioelectricity which is capable of supplying 52 % of the on-site electricity requirements (Ajayebi et al., 2013). Renewable sources of electricity can significantly influence the energy and environmental impacts of the algae biodiesel process. Wind turbines or photovoltaic panels with reasonable energy ratio as the renewable resources will increase the energy efficiency. Collet et. al. (Collet et al., 2014) supplied 45 % of their required electricity from local renewable source (25 % from regional wind turbines and 20 % by Photovoltaic Panels) and supplied the remaining from coal-based electricity production. By increasing the contribution of renewable energy sources for electricity production, the environmental impacts of algae biodiesel will decrease considerably. In contrary, Zaimes and Khanna did not found a significant change on the environmental impacts by utilization of electricity mix (Zaimes and Khanna, 2013).

In the LCA, Collet et. al (Collet et al., 2014) outlined that the effect of the electricity origin on GWP criterion has the same effect as enhancing the productivity from 10 to 30

(gr/m^2.d). They highlighted that modifying the composition of the input electricity mix will significantly influence the LCA outcomes. Batan et al. (Batan et al., 2010) implemented a sensitivity analysis to compare the average US electricity mix, Northeast electricity mix, and California electricity mix. The compositions were reported as follows:

- The average US electricity mix: 50.4 % coal, 20 % nuclear power, 18.3 % natural gas, and 11.3 % biomass, residual oil, and others.
- Northeast (NE) mix: 33.9 % nuclear, 29.9 % coal, 21.7 % natural gas, and 14.5 % biomass, residual oil, and others.
- The California mix: 36.6 % natural gas, 28.3 % a variety of renewable sources, 20.5 % nuclear, 13.3 % coal, and 1.3 % biomass.

In other studies, the average German electricity grid (55 % share of fossil fuels) was used by Passell et al. (Passell et al., 2013), and the average US energy mix (70 % coal and 30 % natural gas) was used by Chowdhury et al. (Chowdhury et al., 2012).

10 Transportation in LCA

For a precise investigation of LCA, both upstream and downstream processes should be considered. The contribution of transportation in biodiesel production is often overlooked (Van Boxtel et al., 2015). It was observed that, in most impact categories, especially in ozone depletion (ODP), GWP, and abiotic depletion (ADP), the transportation distance negatively damages the environmental impact of process (Hou et al., 2011). A longer transportation distance means higher diesel consumption in trucks, which results in higher CO_2 emissions due to the fuel consumption and more depletion of fossil fuel resources. An assumption has been made in most studies where the entire process from the culture to production and combustion are co-located and close. Thus, no transportation is required for carrying the products (Passell et al., 2013). In Table 2-1, the information about the transportation item included in the studies is reported.

11 Conclusions

A comprehensive review of the algae to biodiesel process (and formation of co-products) was prepared in this manuscript. The literature reveals that there are contradictory results

related to the efficiency of biodiesel when compared to its competing petroleum diesel. Thus, some of the environmental benefits may sound ambiguous. A number of researchers provided a standard framework for the environmental impacts of the algal biodiesel production. Discrepancies in the LCA assumptions, LCI included items, and system boundaries are the major parameters that are subjective and contribute to the variability in the LCA outcomes. A more comprehensive framework is required to truly evaluate the environmental benefits from the algal biodiesel production as a replacement of conventional fossil-fuels. In addition, factors such as infrastructure construction, systematic maintenance, transportation, and waste management should also be considered in future studies; these variables can appreciably affect the life cycle analysis outcome which are commonly overlooked in the literature. Other variables such as the temporal units, choice of allocation, land use, and biogenic carbon source should be redefined. Furthermore, the economic aspect of the algae biodiesel process, including its up-stream and down-stream processing should also be included. One of the major challenges in the algae biodiesel is that the research studies are lack of large scale plant data, and uncertainties related to the technical and economic aspects of the process scale up for commercial applications—despite lab-scale successes. Another challenge is the competition with alternative fossil fuel (petroleum diesel) for which the process has the advantage of maturity. For the conventional fuel, the process has experienced ongoing optimizations, resulting in an increase in the efficiency. The algae biodiesel and other alternative biofuels are still at the development stages. With the depletion of hydrocarbon reservoirs, the sensitivity of society about the environmental concerns and hopefully with the breakthrough of new technologies, the algal biodiesel may be more attractive than its fossil fuel alternatives. Improvements such as upgrading the unit performances, integrating the process with post processing technologies, growing algae under nitrogen-deprived conditions, recovering the nutrients and energy, utilizing flue gas and wastewater, applying genetic engineering techniques for more productive algae, and using renewable sources of electricity improve the future perspectives of algae biodiesel.

References

Abbaszaadeh, A., Ghobadian, B., Omidkhah, M.R., Najafi, G., 2012. Current biodiesel production technologies: a comparative review. Energy Conversion and Management 63, 138-148.

Abdullah, H., Mourant, D., Li, C.-Z., Wu, H., 2010. Bioslurry as a fuel. 3. Fuel and rheological properties of bioslurry prepared from the bio-oil and biochar of mallee biomass fast pyrolysis. Energy & Fuels 24(10), 5669-5676.

Abdullah, H., Wu, H., 2011. Bioslurry as a fuel. 4. Preparation of bioslurry fuels from biochar and the bio-oil-rich fractions after bio-oil/biodiesel extraction. Energy & Fuels 25(4), 1759-1771.

Abdullah, M.A., Usman, S.M., Shah, A.A., El-Sayed, H., 2014. Algal Biotechnology For Bioenergy, Environmental Remediation And High-Value Biochemicals. Biotechnology and Bioinformatics: Advances and Applications for Bioenergy, Bioremediation and Biopharmaceutical Research, 301.

Adesanya, V.O., Cadena, E., Scott, S.A., Smith, A.G., 2014. Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system. Bioresource technology 163, 343-355.

Aguirre, A.-M., Bassi, A., Saxena, P., 2013. Engineering challenges in biodiesel production from microalgae. Critical reviews in biotechnology 33(3), 293-308.

Ajayebi, A., Gnansounou, E., Raman, J.K., 2013. Comparative life cycle assessment of biodiesel from algae and jatropha: A case study of India. Bioresource technology 150, 429-437.

Alba, L.G., Torri, C., Fabbri, D., Kersten, S.R., Brilman, D.W.W., 2013. Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae. Chemical engineering journal 228, 214-223.

Alcántara, C., García-Encina, P.A., Muñoz, R., 2013. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. Chemical engineering journal 221, 238-246.

Amin, S., 2009. Review on biofuel oil and gas production processes from microalgae. Energy conversion and management 50(7), 1834-1840.

Andrich, G., Zinnai, A., Nesti, U., Venturi, F., 2006. Supercritical fluid extraction of oil from microalga Spirulina (Arthrospira) platensis. Acta Alimentaria 35(2), 195-203.

Araújo, K., Mahajan, D., Kerr, R., Silva, M.d., 2017. Global Biofuels at the Crossroads: An Overview of Technical, Policy, and Investment Complexities in the Sustainability of Biofuel Development. Agriculture 7(4), 32.

Atabani, A.E., Silitonga, A.S., Badruddin, I.A., Mahlia, T., Masjuki, H., Mekhilef, S., 2012. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renewable and sustainable energy reviews 16(4), 2070-2093.

Azadi, P., Brownbridge, G., Mosbach, S., Smallbone, A., Bhave, A., Inderwildi, O., Kraft, M., 2014. The carbon footprint and non-renewable energy demand of algae-derived biodiesel. Applied Energy 113, 1632-1644.

Aziz, M., Oda, T., Kashiwagi, T., 2014. Integration of energy-efficient drying in microalgae utilization based on enhanced process integration. Energy 70, 307-316.

Balasubramanian, S., Allen, J.D., Kanitkar, A., Boldor, D., 2011. Oil extraction from Scenedesmus obliquus using a continuous microwave system–design, optimization, and quality characterization. Bioresource Technology 102(3), 3396-3403.

Balli, O., Aras, H., Hepbasli, A., 2008. Exergoeconomic analysis of a combined heat and power (CHP) system. International Journal of Energy Research 32(4), 273-289.

Barreiro, D.L., Prins, W., Ronsse, F., Brilman, W., 2013. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. Biomass and Bioenergy 53, 113-127.

Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C., 2015. Harvesting techniques applied to microalgae: a review. Renewable and Sustainable Energy Reviews 41, 1489-1500.

Basu, P., 2013. Biomass gasification, pyrolysis and torrefaction: practical design and theory. Academic press.

Batan, L., Quinn, J., Willson, B., Bradley, T., 2010. Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae. Environmental science & technology 44(20), 7975-7980.

Beckman, E.J., 2004. Supercritical and near-critical CO_2 in green chemical synthesis and processing. The Journal of Supercritical Fluids 28(2-3), 121-191.

Biller, P., Ross, A.B., Skill, S., Lea-Langton, A., Balasundaram, B., Hall, C., Riley, R., Llewellyn, C., 2012. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Research 1(1), 70-76.

Bohutskyi, P., Betenbaugh, M.J., Bouwer, E.J., 2014. The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains. Bioresource technology 155, 366-372.

Boocock, D.G., Konar, S.K., Mao, V., Lee, C., Buligan, S., 1998. Fast formation of high-purity methyl esters from vegetable oils. Journal of the American Oil Chemists' Society 75(12), 1167-1172.

Borbely, A.-M., Kreider, J.F., 2001. Distributed generation: the power paradigm for the new millennium. CRC press.

Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of biotechnology 70(1), 313-321.

Bradley, T., Maga, D., Antón, S., 2015. Unified approach to Life Cycle Assessment between three unique algae biofuel facilities. Applied Energy 154, 1052-1061.

Brennan, L., Owende, P., 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and sustainable energy reviews 14(2), 557-577.

Brentner, L.B., Eckelman, M.J., Zimmerman, J.B., 2011. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. Environmental science & technology 45(16), 7060-7067.

Bribián, I.Z., Capilla, A.V., Usón, A.A., 2011. Life cycle assessment of building materials: Comparative analysis of energy and environmental impacts and evaluation of the eco-efficiency improvement potential. Building and Environment 46(5), 1133-1140.

British Petroleum, 2017. Statistical review of world energy 2017.

Broch, A., Jena, U., Hoekman, S.K., Langford, J., 2013. Analysis of solid and aqueous phase products from hydrothermal carbonization of whole and lipid-extracted algae. Energies 7(1), 62-79.

Brown, A.P., Slabas, A.R., Rafferty, J.B., 2009. Fatty acid biosynthesis in plants—metabolic pathways, structure and organization, Lipids in photosynthesis. Springer, pp. 11-34.

Bussemaker, M.J., Zhang, D., 2013. Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications. Industrial & Engineering Chemistry Research 52(10), 3563-3580.

Cai, T., Park, S.Y., Racharaks, R., Li, Y., 2013. Cultivation of Nannochloropsis salina using anaerobic digestion effluent as a nutrient source for biofuel production. Applied energy 108, 486-492.

Callahan, D.L., Martin, G.J., Hill, D.R., Olmstead, I.L., Dias, D.A., 2015. Analytical approaches for the detailed characterization of microalgal lipid extracts for the production of biodiesel. Marine Algae Extracts: Processes, Products, and Applications, 331-346.

Campbell, P.K., Beer, T., Batten, D., 2011. Life cycle assessment of biodiesel production from microalgae in ponds. Bioresource technology 102(1), 50-56.

Canakci, M., Sanli, H., 2008. Biodiesel production from various feedstocks and their effects on the fuel properties. Journal of industrial microbiology & biotechnology 35(5), 431-441.

Chang, K.J.L., Rye, L., Dunstan, G.A., Grant, T., Koutoulis, A., Nichols, P.D., Blackburn, S.I., 2015. Life cycle assessment: heterotrophic cultivation of thraustochytrids for biodiesel production. Journal of Applied Phycology 27(2), 639-647.

Chen, M., Liu, T., Chen, X., Chen, L., Zhang, W., Wang, J., Gao, L., Chen, Y., Peng, X., 2012. Subcritical co-solvents extraction of lipid from wet microalgae pastes of Nannochloropsis sp. European Journal of Lipid Science and Technology 114(2), 205-212.

Chen, W.-H., Huang, M.-Y., Chang, J.-S., Chen, C.-Y., Lee, W.-J., 2015. An energy analysis of torrefaction for upgrading microalga residue as a solid fuel. Bioresource technology 185, 285-293.

Cheng, J., Huang, R., Li, T., Zhou, J., Cen, K., 2014. Biodiesel from wet microalgae: extraction with hexane after the microwave-assisted transesterification of lipids. Bioresource technology 170, 69-75.

Cherubini, F., Bird, N.D., Cowie, A., Jungmeier, G., Schlamadinger, B., Woess-Gallasch, S., 2009. Energy-and greenhouse gas-based LCA of biofuel and bioenergy systems: Key issues, ranges and recommendations. Resources, conservation and recycling 53(8), 434-447.

Cherubini, F., Strømman, A.H., 2011. Life cycle assessment of bioenergy systems: state of the art and future challenges. Bioresource technology 102(2), 437-451.

Cherubini, F., Strømman, A.H., Ulgiati, S., 2011. Influence of allocation methods on the environmental performance of biorefinery products—A case study. Resources, Conservation and Recycling 55(11), 1070-1077.

Cheung, P.C., 1999. Temperature and pressure effects on supercritical carbon dioxide extraction of n-3 fatty acids from red seaweed. Food Chemistry 65(3), 399-403.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnology advances 25(3), 294-306.

Chisti, Y., 2013. Constraints to commercialization of algal fuels. Journal of biotechnology 167(3), 201-214.

Cho, S., Lee, D., Luong, T.T., Park, S., Oh, Y.-K., Lee, T., 2011. Effects of carbon and nitrogen sources on fatty acid contents and composition in the green microalga, Chlorella sp. 227. J Microbiol Biotechnol 21(10), 1073-1080.

Chowdhury, R., Viamajala, S., Gerlach, R., 2012. Reduction of environmental and energy footprint of microalgal biodiesel production through material and energy integration. Bioresource technology 108, 102-111.

Christensen, T.H., Gentil, E., Boldrin, A., Larsen, A.W., Weidema, B.P., Hauschild, M., 2009. C balance, carbon dioxide emissions and global warming potentials in LCA-modelling of waste management systems. Waste Management & Research 27(8), 707-715.

Ciroth, A., 2007. ICT for environment in life cycle applications openLCA—A new open source software for life cycle assessment. The International Journal of Life Cycle Assessment 12(4), 209-210.

Clarens, A.F., Nassau, H., Resurreccion, E.P., White, M.A., Colosi, L.M., 2011. Environmental impacts of algae-derived biodiesel and bioelectricity for transportation. Environmental science & technology 45(17), 7554-7560.

Collet, P., Hélias, A., Lardon, L., Steyer, J.-P., Bernard, O., 2015. Recommendations for Life Cycle Assessment of algal fuels. Applied Energy 154, 1089-1102.

Collet, P., Lardon, L., Hélias, A., Bricout, S., Lombaert-Valot, I., Perrier, B., Lépine, O., Steyer, J.-P., Bernard, O., 2014. Biodiesel from microalgae–Life cycle assessment and recommendations for potential improvements. Renewable Energy 71, 525-533.

Cooney, M., Young, G., Nagle, N., 2009. Extraction of bio-oils from microalgae. Separation & Purification Reviews 38(4), 291-325.

Costa, J.A.V., De Morais, M.G., 2011. The role of biochemical engineering in the production of biofuels from microalgae. Bioresource technology 102(1), 2-9.

Crampon, C., Mouahid, A., Toudji, S.-A.A., Lépine, O., Badens, E., 2013. Influence of pretreatment on supercritical CO 2 extraction from Nannochloropsis oculata. The Journal of Supercritical Fluids 79, 337-344.

Davis, S.C., Anderson-Teixeira, K.J., DeLucia, E.H., 2009. Life-cycle analysis and the ecology of biofuels. Trends in plant science 14(3), 140-146.

de Boer, K., Moheimani, N.R., Borowitzka, M.A., Bahri, P.A., 2012. Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption. Journal of Applied Phycology 24(6), 1681-1698.

De Schamphelaire, L., Verstraete, W., 2009. Revival of the biological sunlight-to-biogas energy conversion system. Biotechnology and Bioengineering 103(2), 296-304.

DeCicco, J.M., Liu, D.Y., Heo, J., Krishnan, R., Kurthen, A., Wang, L., 2016. Carbon balance effects of US biofuel production and use. Climatic Change 138(3-4), 667-680.

Delrue, F., Li-Beisson, Y., Setier, P.-A., Sahut, C., Roubaud, A., Froment, A.-K., Peltier, G., 2013. Comparison of various microalgae liquid biofuel production pathways based on energetic, economic and environmental criteria. Bioresource technology 136, 205-212.

Delrue, F., Setier, P.-A., Sahut, C., Cournac, L., Roubaud, A., Peltier, G., Froment, A.-K., 2012. An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresource technology 111, 191-200.

Demibras, A., 2008. Biodiesel a realistic fuel alternative for diesel engines. springer.

Demibras, A., 2009. Biofuels - Securing the Planet's Future Energy Needs. Springer, Heidelberg, Germany.

Demirbas, A., 2010. Use of algae as biofuel sources. Energy conversion and management 51(12), 2738-2749.

Demirbas, A., Demirbas, M.F., 2011. Importance of algae oil as a source of biodiesel. Energy conversion and management 52(1), 163-170.

Demirbas, M.F., 2011. Biofuels from algae for sustainable development. Applied Energy 88(10), 3473-3480.

Dhyani, V., Bhaskar, T., 2017. A comprehensive review on the pyrolysis of lignocellulosic biomass. Renewable Energy.

Díaz-Reinoso, B., Moure, A., Domínguez, H., Parajó, J.C., 2006. Supercritical CO_2 extraction and purification of compounds with antioxidant activity. Journal of Agricultural and Food Chemistry 54(7), 2441-2469.

Diebold, J., Bridgwater, A., 1997. Overview of fast pyrolysis of biomass for the production of liquid fuels, Developments in thermochemical biomass conversion. Springer, pp. 5-23.

Diebold, J., Milne, T., Czernik, S., Oasmaa, A., Bridgwater, A., Cuevas, A., Gust, S., Huffman, D., Piskorz, J., 1997. Proposed specifications for various grades of pyrolysis oils, Developments in thermochemical biomass conversion. Springer, pp. 433-447.

Dincer, I., 2000. Renewable energy and sustainable development: a crucial review. Renewable and Sustainable Energy Reviews 4(2), 157-175.

Dominguez-Faus, R., Powers, S.E., Burken, J.G., Alvarez, P.J., 2009. The water footprint of biofuels: A drink or drive issue? ACS Publications.

Dong, T., Knoshaug, E.P., Pienkos, P.T., Laurens, L.M., 2016. Lipid recovery from wet oleaginous microbial biomass for biofuel production: a critical review. Applied Energy 177, 879-895.

Doucha, J., Lívanský, K., 2008. Influence of processing parameters on disintegration of Chlorella cells in various types of homogenizers. Applied microbiology and biotechnology 81(3), 431.

Dutta, S., Neto, F., Coelho, M.C., 2016. Microalgae biofuels: A comparative study on techno-economic analysis & life-cycle assessment. Algal Research 20, 44-52.

Ebadi, A.G., Hisoriev, H., 2017. Gasification of algal biomass (Cladophora glomerata L.) with CO_2 /H_2O/O_2 in a circulating fluidized bed. Environmental technology, 1-7.

Elliott, D.C., 2007. Historical developments in hydroprocessing bio-oils. Energy & Fuels 21(3), 1792-1815.

Elliott, D.C., Biller, P., Ross, A.B., Schmidt, A.J., Jones, S.B., 2015. Hydrothermal liquefaction of biomass: developments from batch to continuous process. Bioresource technology 178, 147-156.

Evans, A., Strezov, V., Evans, T.J., 2009. Assessment of sustainability indicators for renewable energy technologies. Renewable and sustainable energy reviews 13(5), 1082-1088.

Fahy, E., Cotter, D., Sud, M., Subramaniam, S., 2011. Lipid classification, structures and tools. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1811(11), 637-647.

Fields, M.W., Hise, A., Lohman, E.J., Bell, T., Gardner, R.D., Corredor, L., Moll, K., Peyton, B.M., Characklis, G.W., Gerlach, R., 2014. Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Applied microbiology and biotechnology 98(11), 4805-4816.

Franco, C.J., Zapata, S., Dyner, I., 2015. Simulation for assessing the liberalization of biofuels. Renewable and Sustainable Energy Reviews 41, 298-307.

Frank, E., Han, J., Palou-Rivera, I., Elgowainy, A., Wang, M., 2011. Life-cycle analysis of algal lipid fuels with the greet model. Center for Transportation Research, Energy Systems Division, Argonne National Laboratory, Oak Ridge, 11-15.

Frank, E.D., Elgowainy, A., Han, J., Wang, Z., 2013. Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. Mitigation and Adaptation Strategies for Global Change 18(1), 137-158.

Gao, X., Yu, Y., Wu, H., 2013. Life cycle energy and carbon footprints of microalgal biodiesel production in Western Australia: a comparison of byproducts utilization strategies. ACS Sustainable Chemistry & Engineering 1(11), 1371-1380.

Gerardo, M.L., Van Den Hende, S., Vervaeren, H., Coward, T., Skill, S.C., 2015. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. Algal Research 11, 248-262.

Gerbens-Leenes, W., Hoekstra, A.Y., van der Meer, T.H., 2009. The water footprint of bioenergy. Proceedings of the National Academy of Sciences 106(25), 10219-10223.

Gerde, J.A., Wang, T., Yao, L., Jung, S., Johnson, L.A., Lamsal, B., 2013. Optimizing protein isolation from defatted and non-defatted Nannochloropsis microalgae biomass. Algal Research 2(2), 145-153.

Ghasemi Naghdi, F., González González, L.M., Chan, W., Schenk, P.M., 2016. Progress on lipid extraction from wet algal biomass for biodiesel production. Microbial biotechnology 9(6), 718-726.

Goedkoop, M., Heijungs, R., Huijbregts, M., De Schryver, A., Struijs, J., Van Zelm, R., 2009. ReCiPe 2008. A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level 1.

Gog, A., Roman, M., Toşa, M., Paizs, C., Irimie, F.D., 2012. Biodiesel production using enzymatic transesterification–current state and perspectives. Renewable Energy 39(1), 10-16.

Gollakota, A., Kishore, N., Gu, S., 2017. A review on hydrothermal liquefaction of biomass. Renewable and Sustainable Energy Reviews.

Gouveia, L., Oliveira, A.C., 2009. Microalgae as a raw material for biofuels production. Journal of industrial microbiology & biotechnology 36(2), 269-274.

Griffiths, M.J., Harrison, S.T., 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology 21(5), 493-507.

Grima, E.M., Belarbi, E.-H., Fernández, F.A., Medina, A.R., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology advances 20(7), 491-515.

Groom, M.J., Gray, E.M., Townsend, P.A., 2008. Biofuels and biodiversity: principles for creating better policies for biofuel production. Conservation biology 22(3), 602-609.

Gunaseelan, V.N., 1997. Anaerobic digestion of biomass for methane production: a review. Biomass and bioenergy 13(1-2), 83-114.

Gupta, S.K., Ansari, F., Bauddh, K., Singh, B., Nema, A., Pant, K., 2017. Harvesting of Microalgae for Biofuels: Comprehensive Performance Evaluation of Natural, Inorganic, and Synthetic Flocculants, Green Technologies and Environmental Sustainability. Springer, pp. 131-156.

Guschina, I.A., Harwood, J.L., 2006. Lipids and lipid metabolism in eukaryotic algae. Progress in lipid research 45(2), 160-186.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. Oil extraction from microalgae for biodiesel production. Bioresource technology 102(1), 178-185.

Harto, C., Meyers, R., Williams, E., 2010. Life cycle water use of low-carbon transport fuels. Energy Policy 38(9), 4933-4944.

Heilmann, S.M., Davis, H.T., Jader, L.R., Lefebvre, P.A., Sadowsky, M.J., Schendel, F.J., Von Keitz, M.G., Valentas, K.J., 2010. Hydrothermal carbonization of microalgae. Biomass and Bioenergy 34(6), 875-882.

Heilmann, S.M., Jader, L.R., Harned, L.A., Sadowsky, M.J., Schendel, F.J., Lefebvre, P.A., Von Keitz, M.G., Valentas, K.J., 2011. Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Applied Energy 88(10), 3286-3290.

Herrero, M., Cifuentes, A., Ibañez, E., 2006. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food chemistry 98(1), 136-148.

Hischier, R., Weidema, B., Althaus, H.-J., Bauer, C., Doka, G., Dones, R., Frischknecht, R., Hellweg, S., Humbert, S., Jungbluth, N., 2010. Implementation of life cycle impact assessment methods. Final report ecoinvent v2 2.

Hou, J., Zhang, P., Yuan, X., Zheng, Y., 2011. Life cycle assessment of biodiesel from soybean, jatropha and microalgae in China conditions. Renewable and Sustainable Energy Reviews 15(9), 5081-5091.

Htet, M.Z., Ling, L.Y., Yun, S.H., Olaganathan, R., 2013. Biofuel from microalgae: a review on the current status and future trends. International Journal of Advanced Biotechnology and Research 4, 329-341.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The plant journal 54(4), 621-639.

IEA Statistics, 2017. CO_2 emissions from fuel combustion-highlights. IEA, Paris https://www.iea.org/publications/freepublications/publication/CO2EmissionsfromFuelCombustionHighlights2017.pdf.

IEA/IRENA 2017. Perspectives for the Energy Transition – investment needs for a low-carbon energy system.

http://www.irena.org/media/Files/IRENA/Agency/Publication/2017/Mar/Perspectives_for_the_Energy_Transition_2017.pdf.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the

Intergovernmental Panel on Climate Change, [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. Geneva, Switzerland, , p. 151.

IPCC, 2015. Climate change 2014: mitigation of climate change. Cambridge University Press.

Islam, M.A., Brown, R.J., O'Hara, I., Kent, M., Heimann, K., 2014. Effect of temperature and moisture on high pressure lipid/oil extraction from microalgae. Energy Conversion and Management 88, 307-316.

ISO, 2006. Environmental Management: Life Cycle Assessment: Principles and Framework. ISO.

Jeon, K., Suresh, A., Kim, Y.-C., 2013. Highly efficient molecular delivery into Chlamydomonas reinhardtii by electroporation. Korean Journal of Chemical Engineering 30(8), 1626-1630.

Joannes, C., Sipaut, C.S., Dayou, J., Yasir, S.M., Mansa, R.F., 2015. The potential of using pulsed electric field (pef) technology as the cell disruption method to extract lipid from microalgae for biodiesel production. International Journal of Renewable Energy Research (IJRER) 5(2), 598-621.

Kendall, A., 2012. Time-adjusted global warming potentials for LCA and carbon footprints. The International Journal of Life Cycle Assessment 17(8), 1042-1049.

Kendall, A., Price, L., 2012. Incorporating time-corrected life cycle greenhouse gas emissions in vehicle regulations. Environmental science & technology 46(5), 2557-2563.

Keymer, P., Ruffell, I., Pratt, S., Lant, P., 2013. High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. Bioresource technology 131, 128-133.

Khoo, H., Sharratt, P., Das, P., Balasubramanian, R., Naraharisetti, P., Shaik, S., 2011. Life cycle energy and CO 2 analysis of microalgae-to-biodiesel: preliminary results and comparisons. Bioresource technology 102(10), 5800-5807.

Kim, J., Yoo, G., Lee, H., Lim, J., Kim, K., Kim, C.W., Park, M.S., Yang, J.-W., 2013. Methods of downstream processing for the production of biodiesel from microalgae. Biotechnology advances 31(6), 862-876.

Kim, Y.-H., Choi, Y.-K., Park, J., Lee, S., Yang, Y.-H., Kim, H.J., Park, T.-J., Kim, Y.H., Lee, S.H., 2012. Ionic liquid-mediated extraction of lipids from algal biomass. Bioresource technology 109, 312-315.

Kiwjaroun, C., Tubtimdee, C., Piumsomboon, P., 2009. LCA studies comparing biodiesel synthesized by conventional and supercritical methanol methods. Journal of Cleaner Production 17(2), 143-153.

Kligerman, D.C., Bouwer, E.J., 2015. Prospects for biodiesel production from algae-based wastewater treatment in Brazil: A review. Renewable and Sustainable Energy Reviews 52, 1834-1846.

Knuckey, R.M., Brown, M.R., Robert, R., Frampton, D.M., 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering 35(3), 300-313.

König, R.B., Sales, R., Roselet, F., Abreu, P.C., 2014. Harvesting of the marine microalga Conticribra weissflogii (Bacillariophyceae) by cationic polymeric flocculants. biomass and bioenergy 68, 1-6.

Kovacevic, V., Wesseler, J., 2010. Cost-effectiveness analysis of algae energy production in the EU. Energy Policy 38(10), 5749-5757.

Kralova, I., Sjöblom, J., 2010. Biofuels–renewable energy sources: a review. Journal of Dispersion Science and Technology 31(3), 409-425.

Kruse, A., 2009. Hydrothermal biomass gasification. The Journal of Supercritical Fluids 47(3), 391-399.

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., Van Langenhove, H., 2010. Enhanced CO 2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in biotechnology 28(7), 371-380.

Kumar, A., Jones, D.D., Hanna, M.A., 2009. Thermochemical biomass gasification: a review of the current status of the technology. Energies 2(3), 556-581.

Kusdiana, D., Saka, S., 2004. Effects of water on biodiesel fuel production by supercritical methanol treatment. Bioresource technology 91(3), 289-295.

Lacaze, J.-P.C., Stobo, L.A., Turrell, E.A., Quilliam, M.A., 2007. Solid-phase extraction and liquid chromatography–mass spectrometry for the determination of free fatty acids in shellfish. Journal of Chromatography A 1145(1), 51-57.

Lakaniemi, A.-M., Tuovinen, O.H., Puhakka, J.A., 2013. Anaerobic conversion of microalgal biomass to sustainable energy carriers–a review. Bioresource technology 135, 222-231.

Lam, M.K., Lee, K.T., Mohamed, A.R., 2009. Life cycle assessment for the production of biodiesel: a case study in Malaysia for palm oil versus jatropha oil. Biofuels, Bioproducts and Biorefining 3(6), 601-612.

Lardon, L., Helias, A., Sialve, B., Steyer, J.-P., Bernard, O., 2009. Life-cycle assessment of biodiesel production from microalgae. ACS Publications.

Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A.C., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Jackson, R.B., Boden, T.A., Tans, P.P., Andrews, O.D., Arora, V.K., Bakker, D.C.E., Barbero, L., Becker, M., Betts, R.A., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Cosca, C.E., Cross, J., Currie, K., Gasser, T., Harris, I., Hauck, J., Haverd, V., Houghton, R.A., Hunt, C.W., Hurtt, G., Ilyina, T., Jain, A.K., Kato, E., Kautz, M., Keeling, R.F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metzl, N., Millero, F., Monteiro, P.M.S., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S.I., Nojiri, Y., Padín, X.A., Peregon, A., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B.D., Tian, H., Tilbrook, B., van der Laan-Luijkx, I.T., van der Werf, G.R., van Heuven, S., Viovy, N., Vuichard, N., Walker,

A.P., Watson, A.J., Wiltshire, A.J., Zaehle, S., Zhu, D., 2017. Global Carbon Budget 2017. Earth Syst. Sci. Data Discuss. 2017, 1-79.

Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., Oh, H.-M., 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresource technology 101(1), S75-S77.

Lee, Y.-K., 2001. Microalgal mass culture systems and methods: their limitation and potential. Journal of applied phycology 13(4), 307-315.

Léonard, C., Alsteens, D., Dumitru, A.C., Mingeot-Leclercq, M.-P., Tyteca, D., 2017. Lipid Domains and Membrane (Re) Shaping: From Biophysics to Biology, The Biophysics of Cell Membranes. Springer, pp. 121-175.

Levasseur, A., Brandão, M., Lesage, P., Margni, M., Pennington, D., Clift, R., Samson, R., 2012. Valuing temporary carbon storage. Nature Climate Change 2(1), 6.

Levasseur, A., Lesage, P., Margni, M., Deschenes, L., Samson, R., 2010. Considering time in LCA: dynamic LCA and its application to global warming impact assessments. Environmental science & technology 44(8), 3169-3174.

Levasseur, A., Lesage, P., Margni, M., Samson, R., 2013. Biogenic carbon and temporary storage addressed with dynamic life cycle assessment. Journal of Industrial Ecology 17(1), 117-128.

Li, Y., Naghdi, F.G., Garg, S., Adarme-Vega, T.C., Thurecht, K.J., Ghafor, W.A., Tannock, S., Schenk, P.M., 2014. A comparative study: the impact of different lipid extraction methods on current microalgal lipid research. Microbial cell factories 13(1), 14.

Liu, X., Clarens, A.F., Colosi, L.M., 2012. Algae biodiesel has potential despite inconclusive results to date. Bioresource technology 104, 803-806.

Liu, X., Saydah, B., Eranki, P., Colosi, L.M., Mitchell, B.G., Rhodes, J., Clarens, A.F., 2013. Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. Bioresource technology 148, 163-171.

Lotze-Campen, H., Lampe, M., Kyle, P., Fujimori, S., Havlik, P., Meijl, H., Hasegawa, T., Popp, A., Schmitz, C., Tabeau, A., 2014. Impacts of increased bioenergy demand on global food markets: an AgMIP economic model intercomparison. Agricultural Economics 45(1), 103-116.

Louw, T.M., Griffiths, M.J., Jones, S.M., Harrison, S.T., 2016. Techno-economics of algal biodiesel, Algae Biotechnology. Springer, pp. 111-141.

Lucas, S., Alonso, E., Sanz, J., Cocero, M., 2001. Safety Study in a Supercritical Extraction Plant. Chemie Ingenieur Technik 73(6), 725-725.

Luo, L., Van der Voet, E., Huppes, G., De Haes, H.A.U., 2009. Allocation issues in LCA methodology: a case study of corn stover-based fuel ethanol. The International Journal of Life Cycle Assessment 14(6), 529-539.

Lv, J.-M., Cheng, L.-H., Xu, X.-H., Zhang, L., Chen, H.-L., 2010. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresource Technology 101(17), 6797-6804.

Makareviciene, V., Skorupskaite, V., Andruleviciute, V., 2013. Biodiesel fuel from microalgae-promising alternative fuel for the future: a review. Reviews in Environmental Science and Bio/Technology 12(2), 119-130.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renewable and sustainable energy reviews 14(1), 217-232.

Math, M., Kumar, S.P., Chetty, S.V., 2010. Technologies for biodiesel production from used cooking oil—A review. Energy for sustainable Development 14(4), 339-345.

Mercer, P., Armenta, R.E., 2011. Developments in oil extraction from microalgae. European Journal of Lipid Science and Technology 113(5), 539-547.

Milledge, J.J., Heaven, S., 2013. A review of the harvesting of micro-algae for biofuel production. Reviews in Environmental Science and Bio/Technology 12(2), 165-178.

Minowa, T., Sawayama, S., 1999. A novel microalgal system for energy production with nitrogen cycling. Fuel 78(10), 1213-1215.

Mo, W., Soh, L., Werber, J.R., Elimelech, M., Zimmerman, J.B., 2015. Application of membrane dewatering for algal biofuel. Algal Research 11, 1-12.

Møller, J., Boldrin, A., Christensen, T.H., 2009. Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. Waste management & research 27(8), 813-824.

Msanne, J., Xu, D., Konda, A.R., Casas-Mollano, J.A., Awada, T., Cahoon, E.B., Cerutti, H., 2012. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae Chlamydomonas reinhardtii and Coccomyxa sp. C-169. Phytochemistry 75, 50-59.

Muñoz, R., Navia, R., Ciudad, G., Tessini, C., Jeison, D., Mella, R., Rabert, C., Azócar, L., 2015. Preliminary biorefinery process proposal for protein and biofuels recovery from microalgae. Fuel 150, 425-433.

National Research Council, 2012. Sustainable Development of Algal Biofuels in the United States. The National Academies Press, Washington, DC.

O'Hare, M., Plevin, R.J., Martin, J.I., Jones, A.D., Kendall, A., Hopson, E., 2009. Proper accounting for time increases crop-based biofuels' greenhouse gas deficit versus petroleum. Environmental Research Letters 4(2), 024001.

Olivier, J., Schure, K., Peters, J., 2017. TRENDS IN GLOBAL CO_2 AND TOTAL GREENHOUSE GAS EMISSIONS.

Omer, A.M., 2008. Energy, environment and sustainable development. Renewable and sustainable energy reviews 12(9), 2265-2300.

Owsianiak, M., Laurent, A., Bjørn, A., Hauschild, M.Z., 2014. IMPACT 2002+, ReCiPe 2008 and ILCD's recommended practice for characterization modelling in life cycle impact assessment: a case study-based comparison. The International Journal of Life Cycle Assessment 19(5), 1007-1021.

Pahl, G., 2008. Biodiesel: growing a new energy economy. Chelsea Green Publishing.

Pahl, S.L., Lee, A.K., Kalaitzidis, T., Ashman, P.J., Sathe, S., Lewis, D.M., 2013. Harvesting, thickening and dewatering microalgae biomass, Algae for biofuels and energy. Springer, pp. 165-185.

Pant, D., Singh, A., Van Bogaert, G., Gallego, Y.A., Diels, L., Vanbroekhoven, K., 2011. An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: relevance and key aspects. Renewable and Sustainable Energy Reviews 15(2), 1305-1313.

Pant, R., Bersani, R., Pennington, D.W., Brandao, M., 2010. ILCD Handbook-Analysis of existing environmental impact assessment methodologies for use in life cycle assessment-background document.

Pardo-Cárdenas, Y., Herrera-Orozco, I., González-Delgado, Á.-D., Kafarov, V., 2013. Environmental assessment of microalgae biodiesel production in Colombia: Comparison of three oil extraction systems. CT&F-Ciencia, Tecnología y Futuro 5(2), 85-100.

Park, J., Craggs, R., Shilton, A., 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresource technology 102(1), 35-42.

Passell, H., Dhaliwal, H., Reno, M., Wu, B., Amotz, A.B., Ivry, E., Gay, M., Czartoski, T., Laurin, L., Ayer, N., 2013. Algae biodiesel life cycle assessment using current commercial data. Journal of environmental management 129, 103-111.

Pearsall, R.V., Connelly, R.L., Fountain, M.E., Hearn, C.S., Werst, M.D., Hebner, R.E., Kelley, E.F., 2011. Electrically dewatering microalgae. IEEE Transactions on Dielectrics and Electrical Insulation 18(5).

Peccia, J., Haznedaroglu, B., Gutierrez, J., Zimmerman, J.B., 2013. Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends in biotechnology 31(3), 134-138.

Perez, V.H., Junior, E.G.S., Cubides, D.C., David, G.F., Justo, O.R., Castro, M.P., Sthel, M.S., de Castro, H.F., 2014. Trends in biodiesel production: Present status and future directions, Biofuels in Brazil. Springer, pp. 281-302.

Pernet, F., Tremblay, R., 2003. Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. Lipids 38(11), 1191-1195.

Pienkos, P.T., Darzins, A., 2009. The promise and challenges of microalgal-derived biofuels. Biofuels, Bioproducts and Biorefining 3(4), 431-440.

Pinsonnault, A., Lesage, P., Levasseur, A., Samson, R., 2014. Temporal differentiation of background systems in LCA: relevance of adding temporal information in LCI databases. The International Journal of Life Cycle Assessment 19(11), 1843-1853.

Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresource technology 102(1), 17-25.

Pokoo-Aikins, G., Nadim, A., El-Halwagi, M.M., Mahalec, V., 2010. Design and analysis of biodiesel production from algae grown through carbon sequestration. Clean Technologies and Environmental Policy 12(3), 239-254.

Pongsurapipat, Y., Areeprasert, C., Takahashi, F., Tokimatsu, K., Yoshikawa, K., 2016. Life cycle analysis of low-temperature hydrothermal treatment pathway to produce biodiesel from microalgae. Biofuels, 1-9.

Pragya, N., Pandey, K.K., Sahoo, P., 2013. A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renewable and Sustainable Energy Reviews 24, 159-171.

Putt, R., Singh, M., Chinnasamy, S., Das, K., 2011. An efficient system for carbonation of high-rate algae pond water to enhance CO 2 mass transfer. Bioresource technology 102(3), 3240-3245.

Qu, Y., Wei, X., Zhong, C., 2003. Experimental study on the direct liquefaction of Cunninghamia lanceolata in water. Energy 28(7), 597-606.

Quinn, J.C., Smith, T.G., Downes, C.M., Quinn, C., 2014. Microalgae to biofuels lifecycle assessment—multiple pathway evaluation. Algal Research 4, 116-122.

Quispe, C.A., Coronado, C.J., Carvalho Jr, J.A., 2013. Glycerol: production, consumption, prices, characterization and new trends in combustion. Renewable and Sustainable Energy Reviews 27, 475-493.

Rabl, A., Benoist, A., Dron, D., Peuportier, B., Spadaro, J.V., Zoughaib, A., 2007. How to account for CO_2 emissions from biomass in an LCA. The International Journal of Life Cycle Assessment 12(5), 281-281.

Randhawa, K.S., Relph, L.E., Armstrong, M.C., Rahman, P.K., 2017. Biofuel production: tapping into microalgae despite challenges. Biofuels 8(2), 261-271.

Ranjith Kumar, R., Hanumantha Rao, P., Arumugam, M., 2015. Lipid extraction methods from microalgae: a comprehensive review. Frontiers in Energy Research 2, 61.

Ras, M., Lardon, L., Bruno, S., Bernet, N., Steyer, J.-P., 2011. Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. Bioresource Technology 102(1), 200-206.

REN21, 2017. Renewables 2017—Global Status Report. 2017. Available online: http://www.ren21.net/wp-content/uploads/2017/06/170607_GSR_2017_Full_Report.pdf (accessed on 12 June 2017).

Resurreccion, E.P., Colosi, L.M., White, M.A., Clarens, A.F., 2012. Comparison of algae cultivation methods for bioenergy production using a combined life cycle assessment and life cycle costing approach. Bioresource technology 126, 298-306.

Richmond, A., 2008. Handbook of microalgal culture: biotechnology and applied phycology. John Wiley & Sons.

Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., Pohl, C., 1996. Accelerated solvent extraction: a technique for sample preparation. Analytical Chemistry 68(6), 1033-1039.

Ridgway, N., McLeod, R., 2015. Biochemistry of lipids, lipoproteins and membranes. Elsevier.

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and bioengineering 102(1), 100-112.

Rösch, C., Skarka, J., Wegerer, N., 2012. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresource technology 107, 191-199.

Rosenberg, J.N., Oyler, G.A., Wilkinson, L., Betenbaugh, M.J., 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Current opinion in Biotechnology 19(5), 430-436.

Salim, S., Bosma, R., Vermuë, M.H., Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. Journal of applied phycology 23(5), 849-855.

Samorì, C., Barreiro, D.L., Vet, R., Pezzolesi, L., Brilman, D.W., Galletti, P., Tagliavini, E., 2013. Effective lipid extraction from algae cultures using switchable solvents. Green chemistry 15(2), 353-356.

Sander, K., Murthy, G.S., 2010. Life cycle analysis of algae biodiesel. The International Journal of Life Cycle Assessment 15(7), 704-714.

Santana, A., Jesus, S., Larrayoz, M., 2012. Supercritical carbon dioxide extraction of algal lipids for the biodiesel production. Procedia Engineering 42, 1755-1761.

Sarin, A., 2012. Biodiesel: production and properties. Royal Society of Chemistry, Cambridge, UK.

Sayan, B., 2011. The Contribution of Open Frameworks to Life Cycle Assessment.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy research 1(1), 20-43.

Schlesinger, A., Eisenstadt, D., Bar-Gil, A., Carmely, H., Einbinder, S., Gressel, J., 2012. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Biotechnology advances 30(5), 1023-1030.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G., 2010. Biodiesel from algae: challenges and prospects. Current opinion in biotechnology 21(3), 277-286.

Serrano-Ruiz, J.C., Ramos-Fernández, E.V., Sepúlveda-Escribano, A., 2012. From biodiesel and bioethanol to liquid hydrocarbon fuels: new hydrotreating and advanced microbial technologies. Energy & Environmental Science 5(2), 5638-5652.

Sheets, J.P., Ge, X., Park, S.Y., Li, Y., 2014. Effect of outdoor conditions on Nannochloropsis salina cultivation in artificial seawater using nutrients from anaerobic digestion effluent. Bioresource technology 152, 154-161.

Shen, Y., Pei, Z., Yuan, W., Mao, E., 2009. Effect of nitrogen and extraction method on algae lipid yield. International Journal of Agricultural and Biological Engineering 2(1), 51-57.

Show, K.-Y., Lee, D.-J., Mujumdar, A.S., 2015. Advances and challenges on algae harvesting and drying. Drying Technology 33(4), 386-394.

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology advances 27(4), 409-416.

Sikarwar, V.S., Zhao, M., Clough, P., Yao, J., Zhong, X., Memon, M.Z., Shah, N., Anthony, E.J., Fennell, P.S., 2016. An overview of advances in biomass gasification. Energy & Environmental Science 9(10), 2939-2977.

Sikarwar, V.S., Zhao, M., Fennell, P.S., Shah, N., Anthony, E.J., 2017. Progress in biofuel production from gasification. Progress in Energy and Combustion Science 61, 189-248.

Sills, D.L., Paramita, V., Franke, M.J., Johnson, M.C., Akabas, T.M., Greene, C.H., Tester, J.W., 2012. Quantitative uncertainty analysis of life cycle assessment for algal biofuel production. Environmental science & technology 47(2), 687-694.

Singh, A., Nigam, P.S., Murphy, J.D., 2011. Renewable fuels from algae: an answer to debatable land based fuels. Bioresource technology 102(1), 10-16.

Soh, L., Montazeri, M., Haznedaroglu, B.Z., Kelly, C., Peccia, J., Eckelman, M.J., Zimmerman, J.B., 2014. Evaluating microalgal integrated biorefinery schemes: empirical controlled growth studies and life cycle assessment. Bioresource technology 151, 19-27.

Soh, L., Zimmerman, J., 2011. Biodiesel production: the potential of algal lipids extracted with supercritical carbon dioxide. Green Chemistry 13(6), 1422-1429.

Sorguven, E., Özilgen, M., 2010. Thermodynamic assessment of algal biodiesel utilization. Renewable Energy 35(9), 1956-1966.

Speranza, L.G., Ingram, A., Leeke, G.A., 2015. Assessment of algae biodiesel viability based on the area requirement in the European Union, United States and Brazil. Renewable Energy 78, 406-417.

Stephenson, A.L., Kazamia, E., Dennis, J.S., Howe, C.J., Scott, S.A., Smith, A.G., 2010. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors. Energy & Fuels 24(7), 4062-4077.

Stichnothe, H., Azapagic, A., 2009. Bioethanol from waste: Life cycle estimation of the greenhouse gas saving potential. Resources, Conservation and Recycling 53(11), 624-630.

Su, Y., Song, K., Zhang, P., Su, Y., Cheng, J., Chen, X., 2017. Progress of microalgae biofuel's commercialization. Renewable and Sustainable Energy Reviews 74, 402-411.

Suehara, K.-i., Kawamoto, Y., Fujii, E., Kohda, J., Nakano, Y., Yano, T., 2005. Biological treatment of wastewater discharged from biodiesel fuel production plant with alkali-catalyzed transesterification. Journal of Bioscience and Bioengineering 100(4), 437-442.

Taher, H., Al-Zuhair, S., Al-Marzouqi, A.H., Haik, Y., Farid, M., 2014a. Effective extraction of microalgae lipids from wet biomass for biodiesel production. biomass and bioenergy 66, 159-167.

Taher, H., Al-Zuhair, S., Al-Marzouqi, A.H., Haik, Y., Farid, M., Tariq, S., 2014b. Supercritical carbon dioxide extraction of microalgae lipid: process optimization and laboratory scale-up. The Journal of Supercritical Fluids 86, 57-66.

Tan, K.T., Lee, K.T., Mohamed, A.R., 2009. Production of FAME by palm oil transesterification via supercritical methanol technology. Biomass and bioenergy 33(8), 1096-1099.

Tenney, M.W., Echelberger, W.F., Schuessler, R.G., Pavoni, J.L., 1969. Algal flocculation with synthetic organic polyelectrolytes. Applied microbiology 18(6), 965-971.

Troldborg, M., Heslop, S., Hough, R.L., 2014. Assessing the sustainability of renewable energy technologies using multi-criteria analysis: Suitability of approach for national-scale assessments and associated uncertainties. Renewable and Sustainable Energy Reviews 39, 1173-1184.

Tzanetis, K.F., Posada, J.A., Ramirez, A., 2017. Analysis of biomass hydrothermal liquefaction and biocrude-oil upgrading for renewable jet fuel production: The impact of reaction conditions on production costs and GHG emissions performance. Renewable Energy 113, 1388-1398.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. Journal of renewable and sustainable energy 2(1), 012701.

Valdez, P.J., Nelson, M.C., Wang, H.Y., Lin, X.N., Savage, P.E., 2012. Hydrothermal liquefaction of Nannochloropsis sp.: Systematic study of process variables and analysis of the product fractions. biomass and bioenergy 46, 317-331.

Valenzuela, J., Carlson, R., Gerlach, R., Cooksey, K., Peyton, B.M., Bothner, B., Fields, M.W., 2013. Nutrient resupplementation arrests bio-oil accumulation in Phaeodactylum tricornutum. Applied microbiology and biotechnology 97(15), 7049-7059.

Van Boxtel, A., Perez-Lopez, P., Breitmayer, E., Slegers, P., 2015. The potential of optimized process design to advance LCA performance of algae production systems. Applied Energy 154, 1122-1127.

Van der Stelt, M., Gerhauser, H., Kiel, J., Ptasinski, K., 2011. Biomass upgrading by torrefaction for the production of biofuels: A review. Biomass and bioenergy 35(9), 3748-3762.

van der Voet, E., Lifset, R.J., Luo, L., 2010. Life-cycle assessment of biofuels, convergence and divergence. Biofuels 1(3), 435-449.

Van Gerpen, J., Shanks, B., Pruszko, R., Clements, D., Knothe, G., 2004. Biodiesel analytical methods. National Renewable Energy Laboratory, Colorado, 37-47.

Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends in biotechnology 31(4), 233-239.

Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO_2 bio-mitigation using microalgae. Applied microbiology and biotechnology 79(5), 707-718.

Wang, M., 2005. Updated energy and greenhouse gas emission results of fuel ethanol, The 15th International Symposium on Alcohol Fuels, San Diego. pp. 26-28.

Ward, A., Lewis, D., Green, F., 2014. Anaerobic digestion of algae biomass: a review. Algal Research 5, 204-214.

Weidema, B., 2000. Avoiding co-product allocation in life-cycle assessment. Journal of industrial ecology 4(3), 11-33.

Weidema, B., 2014. Has ISO 14040/44 failed its role as a standard for life cycle assessment? Journal of Industrial Ecology 18(3), 324-326.

Wijffels, R.H., Barbosa, M.J., 2010. An outlook on microalgal biofuels. Science 329(5993), 796-799.

Wiley, P.E., Campbell, J.E., McKuin, B., 2011. Production of biodiesel and biogas from algae: a review of process train options. Water Environment Research 83(4), 326-338.

Williams, P.J.l.B., Laurens, L.M., 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy & Environmental Science 3(5), 554-590.

Woertz, I.C., Benemann, J.R., Du, N., Unnasch, S., Mendola, D., Mitchell, B.G., Lundquist, T.J., 2014. Life cycle GHG emissions from microalgal biodiesel–a CA-GREET model. Environmental science & technology 48(11), 6060-6068.

Wu, H., Yu, Y., Yip, K., 2010. Bioslurry as a fuel. 1. Viability of a bioslurry-based bioenergy supply chain for mallee biomass in Western Australia. Energy & Fuels 24(10), 5652-5659.

Wu, L.F., Chen, P.C., Huang, A.P., Lee, C.M., 2012. The feasibility of biodiesel production by microalgae using industrial wastewater. Bioresource Technology 113, 14-18.

Xu, L., Brilman, D.W.W., Withag, J.A., Brem, G., Kersten, S., 2011. Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis. Bioresource technology 102(8), 5113-5122.

Yanfen, L., Zehao, H., Xiaoqian, M., 2012. Energy analysis and environmental impacts of microalgal biodiesel in China. Energy Policy 45, 142-151.

Yang, C., Li, R., Cui, C., Liu, S., Qiu, Q., Ding, Y., Wu, Y., Zhang, B., 2016. Catalytic hydroprocessing of microalgae-derived biofuels: a review. Green Chemistry 18(13), 3684-3699.

Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresource technology 102(1), 159-165.

Yoo, G., Park, W.-K., Kim, C.W., Choi, Y.-E., Yang, J.-W., 2012. Direct lipid extraction from wet Chlamydomonas reinhardtii biomass using osmotic shock. Bioresource technology 123, 717-722.

Young, G., Nippgen, F., Titterbrandt, S., Cooney, M.J., 2010. Lipid extraction from biomass using co-solvent mixtures of ionic liquids and polar covalent molecules. Separation and Purification Technology 72(1), 118-121.

Yu, Y., Wu, H., 2010. Bioslurry as a fuel. 2. Life-cycle energy and carbon footprints of bioslurry fuels from mallee biomass in Western Australia. Energy & Fuels 24(10), 5660-5668.

Yuan, J., Kendall, A., Zhang, Y., 2015. Mass balance and life cycle assessment of biodiesel from microalgae incorporated with nutrient recycling options and technology uncertainties. Gcb Bioenergy 7(6), 1245-1259.

Yusuf, N., Kamarudin, S.K., Yaakub, Z., 2011. Overview on the current trends in biodiesel production. Energy conversion and management 52(7), 2741-2751.

Zaimes, G., Borkowski, M., Khanna, V., 2013. Life-cycle environmental impacts of biofuels and Co-products, Biofuel Technologies. Springer, pp. 471-499.

Zaimes, G.G., Khanna, V., 2013. Microalgal biomass production pathways: evaluation of life cycle environmental impacts. Biotechnology for biofuels 6(1), 88.

Zaimes, G.G., Khanna, V., 2014. The role of allocation and coproducts in environmental evaluation of microalgal biofuels: How important? Sustainable Energy Technologies and Assessments 7, 247-256.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresource technology 102(2), 1149-1158.

Zeng, D., Li, R., Yan, T., Fang, T., 2014. Perspectives and advances of microalgal biodiesel production with supercritical fluid technology. RSC Advances 4(75), 39771-39781.

Zeng, X., Danquah, M.K., Chen, X.D., Lu, Y., 2011. Microalgae bioengineering: from CO 2 fixation to biofuel production. Renewable and Sustainable Energy Reviews 15(6), 3252-3260.

Zhang, Y., Kendall, A., Yuan, J., 2014. A comparison of on-site nutrient and energy recycling technologies in algal oil production. Resources, Conservation and Recycling 88, 13-20.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water research 47(13), 4294-4302.

(Dutta et al., 2016; Pardo-Cárdenas et al., 2013)

CHAPTER 3

Dynamic Thermodynamic Flux Balance Analysis for Modeling Genome Scale Metabolism

1 ABSTRACT

Genome scale models have been a deserving representative of biological species and their functional states. The constraints hold on solutions space are attributed to one aspect of physiological and environmental capacity. The integration of thermodynamic data help to deciphering internal complexity of cell metabolism. Also, knowledge of how far in reaction from equilibrium can help us in regulation of metabolism.

In this study, we incorporated the thermodynamic data into iJO1366 genome scale metabolic network, and in the next step, we performed dynamic thermodynamic flux balance analysis (DT-FBA) to dynamically simulate cell phenotype under changing environment. The cases studied are diauxic growth of glucose and xylose under aerobic

and aerobic-anaerobic condition. In parallel, dynamic flux balance analysis (DFBA) implemented on model and results were compared. DT-FBA captures cell behavior better than DFBA. In addition, flux variability analysis (FVA) and thermodynamic variability analysis (TVA) were performed to check the permissible range of fluxes. Surprisingly, the results showed that for 46 reactions FVA predicts to be unidirectional while TVA reveals them as bidirectional and reversible.

Keywords: Dynamic Thermodynamic Flux Balance Analysis, Dynamic Flux Balance Analysis, Thermodynamic Variability Analysis, Flux Variability Analysis, Diauxic Growth

2 Introduction

Understanding of cell metabolism and how it reacts to numerous stresses and environmental condition will help us to take advantages of cells as bio-refineries, monitor cell behavior to treat diseases, and demystify complexity of life.

In order to apply the techniques and tools for studying metabolism, we need a model organism that is easy and cheap to grow. The most common model organism is E. coli. It is able to grow aerobically and aerobically which make it a perfect model for research (Chen et al., 2011; Kim et al., 2009; Lin and Tanaka, 2006).

Deep insight into cell requires a comprehensive model to deduce phenotype from the genotype and extracellular condition (Covert et al., 2003; Price et al., 2004a; Price et al., 2004b). To satisfy this demand, genome-scale metabolic models emerged as they provide detailed picture of metabolic systems (Edwards et al., 2002; Palsson, 2000; Reed and Palsson, 2003) in which phenotypes depicted as flux distributions through metabolic networks(Edwards et al., 2002).

A genome-scale network models can integrates physiological functional information and high-throughput large-scale omics such as genomics, transcriptomics, bioinformatics, and metabolomics and (Brunk et al., 2018). The first *in silico* metabolic reconstruction of *E. coli* MG1655 were reconstructed in 2000 to evaluate its metabolic capabilities.(Edwards and Palsson, 2000).

All possible expressed behavior must abide by imposed constraints such as environmental, physicochemical, topobiological, self-imposed regulatory, and evolutionary constraints (Covert et al., 2003).

Flux Balance Analysis (FBA) is the most prevalent constraints-based modeling tool to study metabolite flux flow through metabolic networks (O'brien et al., 2013; Orth et al., 2010; Palsson and Palsson, 2015). FBA paradigm only fulfils mass conservation without considering energy conservation or second law of thermodynamics (Fleming et al., 2012). In another term, biological processes oblige an energy to drive them forward and away from thermodynamic equilibrium state (Soh and Hatzimanikatis, 2010). Revealing that thermodynamics governs many interactions in metabolic systems, it will fundamentally help us to predict the phenotype in more concise way.

Occasionally happens that a reaction mathematically generates flux but is not necessarily thermodynamically feasible (Fleming et al., 2012). Successively, thermodynamically constrained stoichiometric-based models were proposed to additionally force reactions to conform the laws of thermodynamics (Angeles-Martinez and Theodoropoulos, 2016).

Imposing thermodynamic constraints reduces the solution space because it will delete fluxes in opposite direction of Gibbs free energy change (Angeles-Martinez and Theodoropoulos, 2016).

Thermodynamics has been utilized in numerous studies (Noor, 2018), with two major aspects focusing on imposition of loop law and reaction reversibility/directionality.

In **first** category, they used energy balance to remove thermodynamically infeasible loops, and subsequently reduced feasible solution space. According to Kirchhoff's loop law and second law of thermodynamics expressing the positive entropy production for reaction, the multiplication of flux of reaction and chemical potential differences associated with it must be less than zero (Beard et al., 2004; Beard et al., 2002; Kümmel et al., 2006; Yang et al., 2005). Due to nonlinearity and non-convexity of constraints, their implementation on large scale models will be computationally intensive and problematic (Fleming et al., 2012; Schellenberger et al., 2011; Yang et al., 2005). Loop-law constraints were incorporated into *loopless* FBA (also known as ll-COBRA) in form of general mixed

integer programming approach were defined to eliminate solutions incompatible with the loop law. Semi-thermodynamic FBA (stFBA) enforces stronger thermodynamic constrains on the flux solution compared to ll-COBRA (Noor, 2018).

In the **last** category, by estimating Gibbs energies of reactions and thermodynamic constraints, they determined reaction reversibility and directionality, and subsequently eliminate thermodynamically infeasible reaction (Henry et al., 2007; Hoppe et al., 2007; Senger and Papoutsakis, 2008). The representative of this category is thermodynamic flux balance analysis (TFBA).

3 Why Dynamic Modeling?

Intracellular metabolism and cell growth as complex systems behave highly dynamics because of dynamic nature of fed-batch and batch cultures (Antoniewicz, 2013) . In fact, cell needs to dynamically adapt itself to changing extracellular (environment). A key assumption made for intracellular metabolism is pseudo-steady state, while the time for extracellular is longer to equilibrate with cell environment (Jouhten et al., 2012). Flux balance analysis only allows study of intracellular flux distributions but a methodology is needed to simulate cell metabolism under dynamic condition by combining extracellular with intracellular metabolism. Unstructured models are not able to portray a detailed representation of cell whereas dynamic genome-scale models inherently reflect cellular dynamics and eventually lead to optimal control profile of processes (Hjersted and Henson, 2006). Macroscopic kinetic models threat cell as a black box and have been used for simple phenomena such as bacterial growth or substrate/product inhibition (Anesiadis et al., 2013). Dynamic flux balance analysis (DFBA) (Mahadevan et al., 2002) as a microscopic framework was introduced to overcome the shortcoming associated with macroscopic unstructured models by providing a detailed metabolic models as well as the no need of enzyme kinetic (Hjersted and Henson, 2006; Jeong et al., 2016). Also, due to its dependability on genome scale models, it can be used in larger operational range (Jeong et al., 2016).

4 Flux Balance Analysis

It is assumed that there is no accumulation or depletion of metabolite inside the cell, therefore, the rate of concentration is equal to zero, or the system is at pseudo-steady state (Schilling et al., 1999; Varma and Palsson, 1994; Wiback et al., 2004). In mathematical form, mass balance equations of metabolites in network are represented as

$$\frac{d[\mathrm{c}]}{dt} = S.V \xrightarrow{@\ \text{Steady state}} S.V = 0 \qquad (1)$$

where c is the metabolite concentration, V is an r×1 vector of fluxes through the r reactions, and S is m×r matrix of the stoichiometric coefficients for the r reactions and m metabolites reactions in the network. By the way, each individual variable (here, fluxes) has a minimum and maximum flux rates limit as constraint:

$$v_{min} \le v \le v_{max}$$

By imposing the constraints, the feasible solution space will be reduced dramatically. Through linear optimization, a flux distribution that optimize an objective function (e.g., maximize growth rate or ATP production) - a single optimal point lies on the edge of feasible solution space- will be obtained by FBA but obviously mass balance constraints are neither sufficient to represent all kind of constraints nor uniquely predict the flux, thus, the system are always underdetermined (Orth et al., 2010; Reed and Palsson, 2004; Varma and Palsson, 1994).

$$\begin{aligned} &Max\ v_i \quad \text{(e.g., growth rate or ATP)} \\ &s.t \\ &S.V = 0, \\ &v_{min} \le v \le v_{max} \end{aligned} \qquad (2)$$

5 Thermodynamic Flux Balance Analysis (TFBA)

By setting thermodynamic constraints on genome scale metabolic models, it would be more possible to identify the fluxes abide by the physiological condition (Kiparissides and Hatzimanikatis, 2017). The thermodynamics-augmented versions of FBA were introduced (Kiparissides and Hatzimanikatis, 2017) as thermodynamics-based flux analysis (TFA)(Ataman and Hatzimanikatis, 2015) or so called thermodynamics-based metabolic flux analysis (TMFA) (Hamilton et al., 2013; Henry et al., 2007), and thermodynamics-based flux balance analysis (TFBA) (Soh and Hatzimanikatis, 2014; Soh et al., 2012).

Despite the lack of $\Delta_f G°$ experimental data for most compound in metabolic networks (Henry et al., 2006), Gibbs free energy of all reactions will be provided using published data or computational methods such as group contribution method (Jankowski et al., 2008; Mavrovouniotis, 1991; Noor et al., 2012) or component contribution (Noor et al., 2013). These computational methods first estimate standard Gibbs energies of formation ($\Delta_f G°$) of metabolites, and eventually yield standard Gibbs free energy of reaction ($\Delta_r G°$) (Henry et al., 2007; Henry et al., 2006):

$$\Delta_r G'^{0}_{est} = \sum_{i=1}^{m} n_i \Delta_f G'^{0}_{est_i} \qquad (3)$$

To be precise, these approximations have been defined for standard conditions (298.15 K, 1 atm, pH 7.0, zero ionic strength, all compounds at 1 M), rather than physiological conditions (Boghigian et al., 2010). Consequently, these conditions are applied to both the extracellular and intracellular environment even for reactions contribute in metabolite transport of metabolites across the cellular membrane.

6 Gibbs free energy change of reaction (ΔrG ´) for transport reactions

Following assumption of 1mM activity, both parameters of electrochemical potential, $\Delta\psi$, and pH gradient, ΔpH ($pH_{\text{intracellular}} - pH_{\text{extracellular}}$), across the cell membrane are considered zero.

The energy required to transport a metabolite across membrane is sum of the driving force of the transmembrane differential pH to transport H^+ into the compartment, $\Delta_{\Delta pH}G$, and the energy associated with the transport of an ion across the membrane, $\Delta_{\Delta\psi}G$ (Henry et al., 2007),

$$\Delta_r G'_{\text{transport}} = \Delta_{\Delta\psi}G + \Delta_{\Delta pH}G \quad (4)$$

It is clear that at standard condition (pH=7 and ionic-strength =0) means $\Delta pH = 0$ and $\Delta\psi = 0$, and consequently $\Delta_r G'_{\text{transport}} = 0$. Nonetheless, under physiological conditions ΔpH, $\Delta\psi$ and $\Delta_r G'_{\text{transport}}$ are not equal to zero (Henry et al., 2007; Henry et al., 2006; Raetz, 1996).

$$\Delta_{\Delta\psi}G(\text{kcal/mol}) = nF\Delta\psi \quad (5)$$

$$\Delta\psi(\text{mV}) = 33.33\Delta pH - 143.33 \quad (6)$$

$$\Delta_{\Delta pH}G(\text{kcal/mol}) = -2.3\text{hRT}\Delta pH \quad (7)$$

Where n is the transported net charge across membrane, *F* is the Faraday constant in kcal/mV mol*,* and *h* is the number of transported protons across the membrane.

Gibbs free energy due to an intracellular ***biochemical reaction*** is formulated as well:

$$\Delta_r G'^{m}_{\text{intracellular}} = \sum_{i=1}^{\text{Products}} n_i \left[\Delta_f G_i'^{0} + RT\ln(C_i)\right] - \sum_{i=1}^{\text{Reactants}} n_i \left[\Delta_f G_i'^{0} + RT\ln(C_i)\right] \quad (8)$$

The overall $\Delta_r G'^{m}$ of a reaction across the compartment membrane is combination of

$$\Delta_r G'^{m} = \Delta_r G'^{m}_{\text{transport}} + \Delta_r G'^{m}_{\text{intracellular}} \quad (9)$$

7 Thermodynamic FBA Formulation

The general assumption is no intracellular metabolite accumulation (or steady state) which is called mass balance constraints:

$$S \cdot V = 0 \quad (10)$$

Integration of the second law of thermodynamics into constrain reaction directionality relates $\Delta_r G$ to sign of flux-carrying reactions (i.e., if $\Delta_r G > 0$, then $v_{net} < 0$ and vice versa). First, we reformed the mathematical form of reversible reaction by separating it into two reactions: forward and backward reaction.

$$lb_i \le v_i \le ub_i$$

After imposing the reaction directionality constraints, in a reversible reaction, if one direction is active, the opposite direction should be inactive to prevent simultaneous use of constraints. In addition, if $\Delta_r G$ of flux-carrying reaction is positive in forward direction, its associated flux must be zero whereas the flux associated with backward reaction is active.

$$LB_i \left(1 - \mathbf{z}_i\right) \le v_i \le UB_i . \mathbf{z}_i \qquad (11)$$

$$-M . \mathbf{z}_i - \varepsilon \le \Delta_r G'_i \le M . \left(1 - \mathbf{z}_i\right) + \varepsilon \qquad (12)$$

Where z_i is independent binary variable associated with reaction i, M is a constant selected to be big enough to continuously guarantee if v_i and z_i are zero, LB_j and UB_j are the lower and upper bounds, respectively. The term M is to constrain reactions with a non-zero flux (Schellenberger et al., 2011). Practically, to avoid degeneracy in LP/MILP solution, $\Delta_r G'_i$ should be confined in firm positive or firm negative, to this aim, very small value of epsilon is employed.

The Gibbs free energy of reaction, $\Delta_r G'$:

$$\Delta_r G' = \Delta_r G'^{0}_{est} + RT \ln\left(\prod_{i=1}^{m} a_i^{n_i}\right) \qquad \text{with } a_i = c_i \gamma_i \qquad (13)$$

In another words, solution is supposed to be ideal, as well as volumeless metabolites, thus, the activity is $a_i = C_i / C_{standard}$, where $C_{standard}$ is the standard concentration of the 1 M metabolites (Angeles-Martinez and Theodoropoulos, 2016).

$$\Delta_r G' = \Delta_r G'^{0}_{est} + RT \ln\left(\prod_{i=1}^{m} C_i^{n_i}\right) \qquad (14)$$

Where R is the universal gas constant, T is the temperature assumed to be 298 K, m is the number of compounds involved in the reaction, a_i is the activity of compound i, n_i is the stoichiometric coefficient of compound i in the reaction (n_i is negative for reactants and positive for products), γ_i is dimensionless activity coefficients, and c_i is intermediate concentration. Because the activities of most compounds in intracellular are not available, we agreed to use mean activity of 1mM in the cell (Albe et al., 1990; Henry et al., 2006). The energy required for transmembrane transport is also accounted into $\Delta_r G'^{\circ}$ term as described in transport reaction section.

8 Dynamic Thermodynamic FBA (DTFBA) and Dynamic FBA (DFBA)

In DFBA formulation, the batch time is discretized into intervals and tries to instantaneously solve the optimization for each time step to find the optimal flux distribution at that particular time, and eventually integrates over entire time horizon. This methodology is referred to as Static Optimization Approach (SOA) proposed by (Mahadevan et al., 2002).

DFBA is a framework with three interconnected subunits which are solved iteratively (Figure 3-1) (Kelly et al., 2018; Saitua et al., 2017): (i) the substrate uptake kinetics unit, (ii) intracellular metabolism unit and (iii) the dynamic unit:

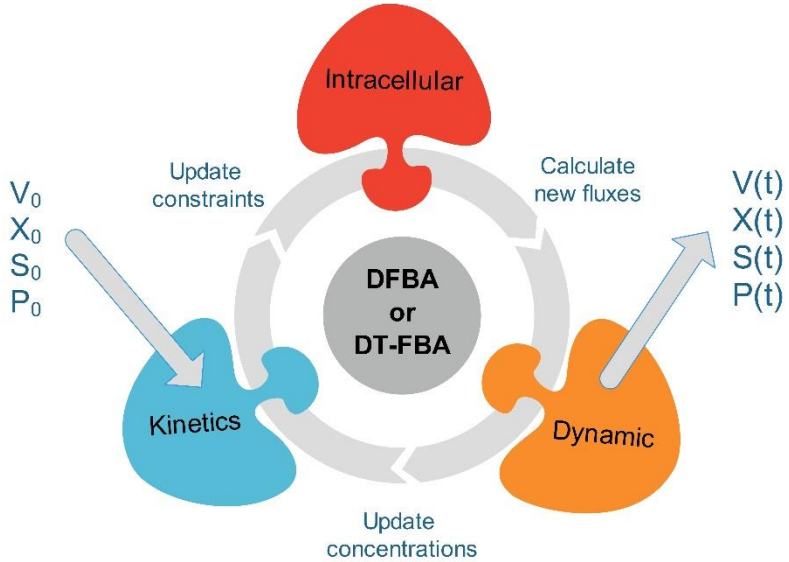


Figure 3-1: Schematic of DFBA framework

First, initial conditions are fed to kinetic units to ensure that the constraints adhere to physiological constraints and inhibition (Saitua et al., 2017). These constraints are capable of being dynamically changed over times as well. Then, the constrained model is ready for static flux balance analysis (FBA) or static thermodynamic flux balance analysis (TFBA) in intracellular flux calculation unit. In this block, the calculated objective (here, biomass growth rate) and other desired fluxes are selected to pass along to dynamic units. Finally, concentration of the state variables is updated through solving ODE equations and integrated into kinetic units for next iteration. This cycle keeps repeating until it reaches to favorite growth rate or end of simulation.

8.1 Kinetic Unit

Technically, intracellular and extracellular environment are linked by cellular biomass growth rate and substrate uptake kinetics (Hjersted and Henson, 2006). The kinetic uptake expressions for glucose (v_g), xylose (v_z) and oxygen (v_o) are determined using Michaelis-Menten kinetics:

$$v_g = v_{g,\max} \frac{G}{K_g + G} \frac{1}{1 + \frac{E}{K_{ie}}} \tag{15}$$

$$v_z = v_{z,\max} \frac{Z}{K_Z + Z} \frac{1}{1 + \frac{G}{K_{ig}}} \frac{1}{1 + \frac{E}{K_{ie}}} \tag{16}$$

$$v_o = v_{o,\max} \frac{O}{K_o + O} \tag{17}$$

where G, Z, E and O are the glucose, xylose, ethanol and dissolved oxygen concentrations in the medium [g/L], respectively, K_g, K_z and K_o are saturation constants, $v_{g,max}$, $v_{z,max}$ and $v_{o,max}$ are maximum uptake rates [mmol/g_{DCW}· h] for glucose, xylose and dissolved oxygen, and K_{ie} and K_{ig} are an inhibition constant. The glucose uptake rate adheres to Michaelis –Menten kinetics with an extra regulatory

expression stem from growth rate inhibition caused by ethanol presence in medium (Hjersted and Henson, 2009; Sainz et al., 2003).

The lower and upper bound of fluxes, and parameters employed in Michaelis-Menten kinetics are presented in Table 3-1.

TABLE 3-1: Substrate uptake parameter values

SYMBOL	VALUE
$V_{G,MAX}$	10.5
K_G	0.0027
$V_{Z,MAX}$	6
K_Z	0.0165
$V_{O,MAX}$	15
K_O	0.024
K_{IE}	20
K_{IG}	0.005

8.2 Dynamic unit

In this unit, ordinary differential equations (ODEs) are being solved for variable states of batch volume, biomass growth, and exchange species:

$$\frac{dV}{dt} = F_{in} - F_{out} \tag{18}$$

$$\frac{dX}{dt} = \mu_i \cdot X_i + \frac{\left(X_{feed,i} \cdot F_{in} - X_i \cdot F_{out}\right)}{V} \tag{19}$$

$$\frac{dS_i}{dt} = \upsilon_i \cdot X_i + \frac{\left(S_{feed,i} \cdot F_{in} - S_i \cdot F_{out}\right)}{V} \tag{20}$$

Where V is volume [L], t is time [h], F_{in} and F_{out} is the feed and output rate in [L/h]. X is the biomass concentration [g/L], μ is the specific growth rate [h^{-1}], S is the substrate concentration [g/L].

8.3 Diauxic growth

Cellulose and hemicellulose are the main component of lignocellulosic biomass (Gonzalez et al., 2017). From the breakdown of these two classes, monomers such as glucose and xylose are derived (Gírio et al., 2010; Kim et al., 2015). In another word, glucose and xylose are the most abundant sugar source from terrestrial plant biomass. Therefore, their conversion into value-added products and biofuels has grabbed the attention of many metabolic engineer (Gonzalez et al., 2017; Liu et al., 2012).

When microbial species are grown in a chemically defined media nourished with two sugars (i.e., mixed-substrate media: glucose and xylose), two type of behaviours has been reported (Hermsen et al., 2015):

1- Sequential utilization of substrates, which results in diauxic growth.
2- Simultaneous consumption of sugars

However, when glucose and xylose are provided as carbon source, E. coli is not able to metabolize both simultaneously until glucose is depleted. Sequential consumption commonly is subjected to carbon catabolite repression (CCR)(Deutscher et al., 2006; Hermsen et al., 2015; Müller-Hill, 1996; Narang and Pilyugin, 2007). However, some studies questioned the importance of CCR for preferential sugar uptake mechanism (Chu and Barnes, 2016; Inada et al., 1996; Okada et al., 1981).By the way, two main mechanism are responsible for CCR (Chu and Barnes, 2016):

1- Metabolic gene regulation by transcription regulators, mostly by cAMP-Crp regulatory system
2- Direct repression of second carbon uptake (here, xylose) by glucose.

This preferential selectivity leads to lower yield of products from mixed hydrolyzates substrates. For, ethanol production, the low productivity is the major obstacle for economic feasibility of cellulosic ethanol production (Kim et al., 2012).

Technically speaking, diauxic growth is reprogramming of a metabolic network (Mahadevan et al., 2002) in order to adapt itself to maximize population growth. Two growth stage are separated by a lengthy time which is called lag-phase (Chu and Barnes, 2016) which is subjected to loss of growth during switch. In contrast to diauxic growth (bi-phase growth) which is related to cell behavior, lag phase is attributed to “unequal distribution of growth rates within the population” and not cell phenotype (Boulineau et al., 2013; Chu and Barnes, 2016; Kotte et al., 2014; van Heerden et al., 2014) and can not be assumed as time required for metabolic gene switch.

It is worthy to note, although due to diauxic growth of wildtype E. coli, it is not an ideal host strain for chemicals from lignocellulosic biomass (Deutscher, 2008; Görke and Stülke, 2008; Kim et al., 2015) , genetically manipulated E. coli are still on interests. Many attempts have been made to optimally engineer microorganisms capable of metabolizing both carbon simultaneously (Kim et al., 2012).

9 Results and discussion

9.1 Simulation Procedure

First, thermodynamic constraints in TFBA require having $\Delta_r G'^{\circ}$ of the reactions which could be estimated experimentally or theoretically. In our paper, we used ModelSEED database (http://modelseed.org) to obtain the desired $\Delta_f G^{\circ}$ of each compound. Because these data are in their standard form, it is necessary to adjust them into *in vivo* conditions to reflect cell conditions. We followed the procedure implemented in Alberty’s textbooks (Alberty, 2005;2006) to transform standard Gibbs energy of formation for each metabolite species to desired ionic strength, temperature and *pH.*

We assumed an ionic strength of 0.25 M for Cytoplasmic and zero for next two compartments, and temperature at 310.15 K (37 °C) because generally for biochemical reactions occur at body temperature. pH for Cytoplasmic, Periplasmic, and Extracellular set to 7.5, 7, and 7, respectively. Then using equations 2-9, we calculated the transformed standard Gibbs free energy of reaction ($\Delta_r G'^{\circ}$).

Now the thermodynamics-integrated model is ready to undergo DT-FBA presented schematically in Figure 3-1. For intracellular model, the model maximizes the biomass growth by considering the constraints mentioned in equations 10, 11, 12, and 14.

The form of TFBA problem is called mixed integer linear programming (MILP) in which the variables are combination of real and integer numbers, and its objective function and the constraints should be linear. MILP calculations were performed using the CPLEX Optimization Version 12.8.0 (IBM ILOG CPLEX Optimization Studio) solver in MATLAB (The MathWorks Inc., Natick, MA) with the COBRA Toolbox.

9.2 Model Description:

The updated version of metabolic network of common laboratory strain Escherichia coli K-12 MG1655 (known as *E. coli* iJO1366) were used in this study (Orth et al., 2011). This model contains 1366 genes, 2251 metabolic reactions, and 1136 unique metabolites. The model consists of three compartments: Cytoplasmic, Periplasmic, and Extracellular. The visualized pathways of this model can be found in (**https://escher.github.io**) using "Escher" web application (King et al., 2015) as followed here:

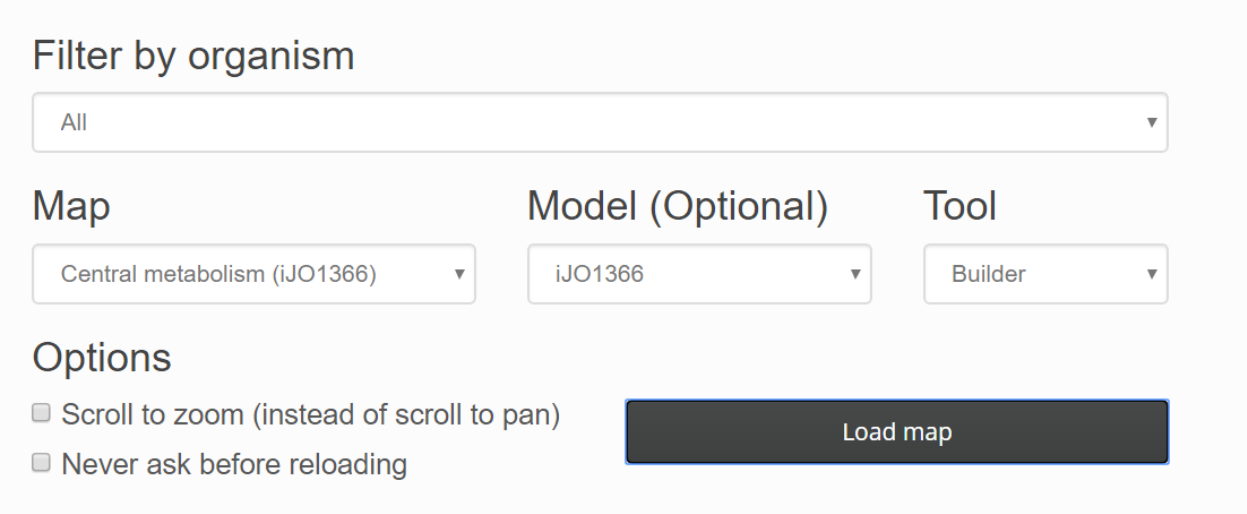


Figure 3-2: The "Escher" web application for visualizing pathway and biochemical reaction of E. coli iJO1366.

9.3 Batch Culture Medium

The initial concentration of glucose and xylose was assumed to be 15.3 and 8 g/L. The initial inoculum (biomass) set to 0.03 g/L. Also, for *in silico* microorganisms, we assume environment adaptation (Joy and Kremling, 2010). Simulation time started from initial time of 0 hours to a time course of 10 hours. For long term aerobic condition, dissolved oxygen concentration was regulated at 0.24 mmol/L over simulation. In the case of reparative system to fermentative system simulation or transition from aerobic to anaerobic simulation, initial dissolved oxygen concentration fixed to 0.24 mmol/L.

Maximum glucose uptake flux was restricted to 10.5 $mmolgDW^{-1}hr^{-1}$ where the maximum uptake of xylose was imposed to 6 $mmolgDW^{-1}hr^{-1}$.

Physiologically reasonable bounds between 10^{-5} M and 0.02 M will constrain the intracellular reactions as observed in cell (Albe et al., 1990) except for hydrogen ion H^+ concentration was fixed to 10^{-7} M (Henry et al., 2007). Furthermore, fluxes through reactions for FBA and TFBA simulation was set to typical values of [-1000 1000] mmol/gm DW/h as upper and lower limit of fluxes.

9.4 Distribution of $\Delta_f G$ °, $\Delta_r G$ °, and $\Delta_r G'$° values for reactions in iJO1366

The distribution of standard Gibbs energies of formation ($\Delta_f G°$), transformed standard Gibbs free energy of reaction ($\Delta_r G'°$), and transformed standard Gibbs free energy of formation ($\Delta_f G'°$) are presented in figures 3-(3-5).

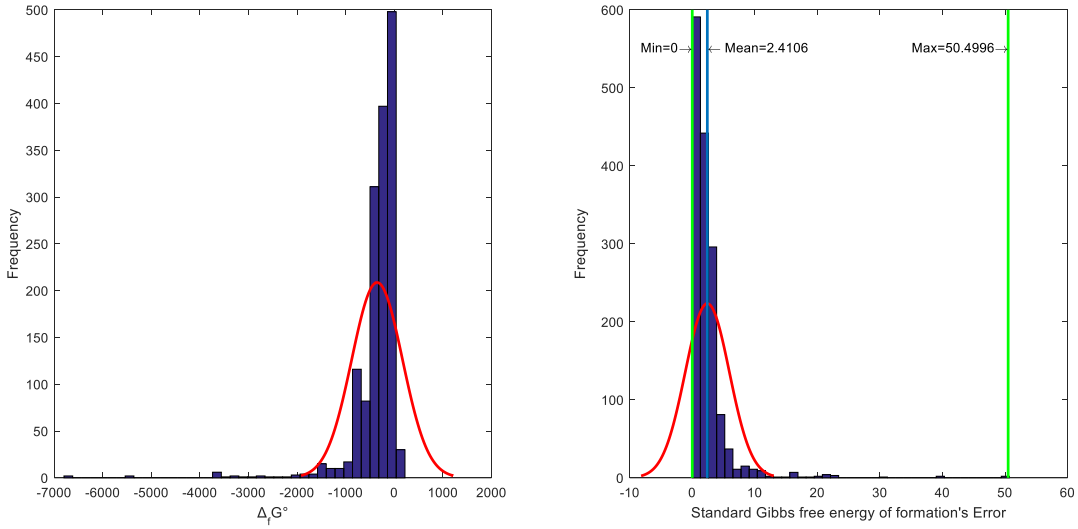


Figure 3-3: Distribution of standard Gibbs energies of formation

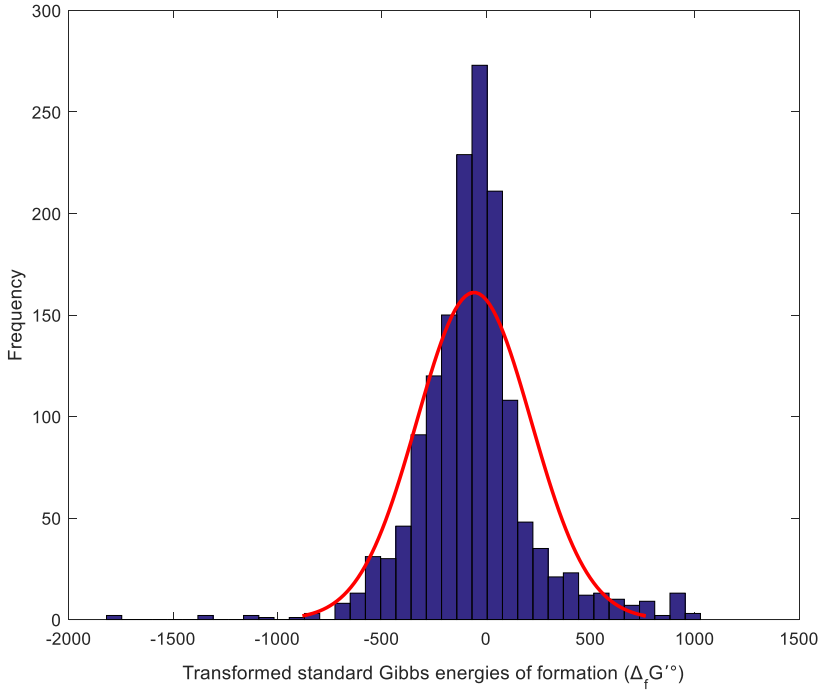


Figure 3-4: The Gaussian distribution of transformed standard Gibbs free energy of formation.

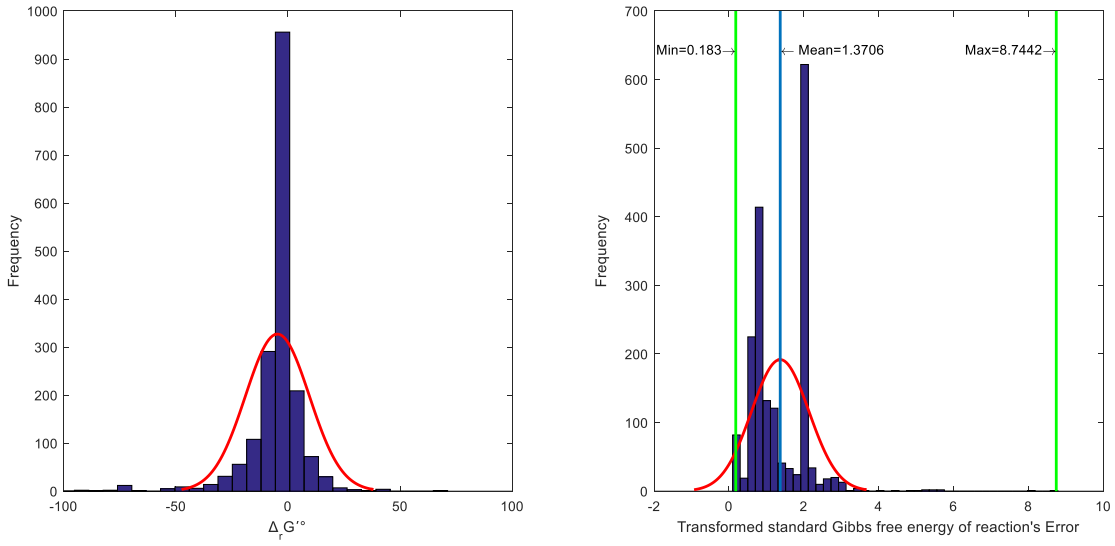


Figure 3-5: The frequency and distribution of transformed standard Gibbs free energy of reaction beside associated errors

The statistics indicate that about 77.1% of all reactions whose thermodynamics properties have been predicted in model have zero or negative transformed standard Gibbs free energy of reaction. Also, among 1807 metabolites in model, the standard Gibbs free energy of formation data have been predicted and observed for 1517 metabolite, it means that 83.9% of compound have defined properties. Among these values, 1438 compounds have negative values.

9.5 Case Study 1: Diauxic growth simulation under aerobic simulation

Using DFBA and DT-FBA, we are able to simulate mixed sugar metabolism and diauxic growth for E. coli under aerobic condition in media containing glucose and xylose.

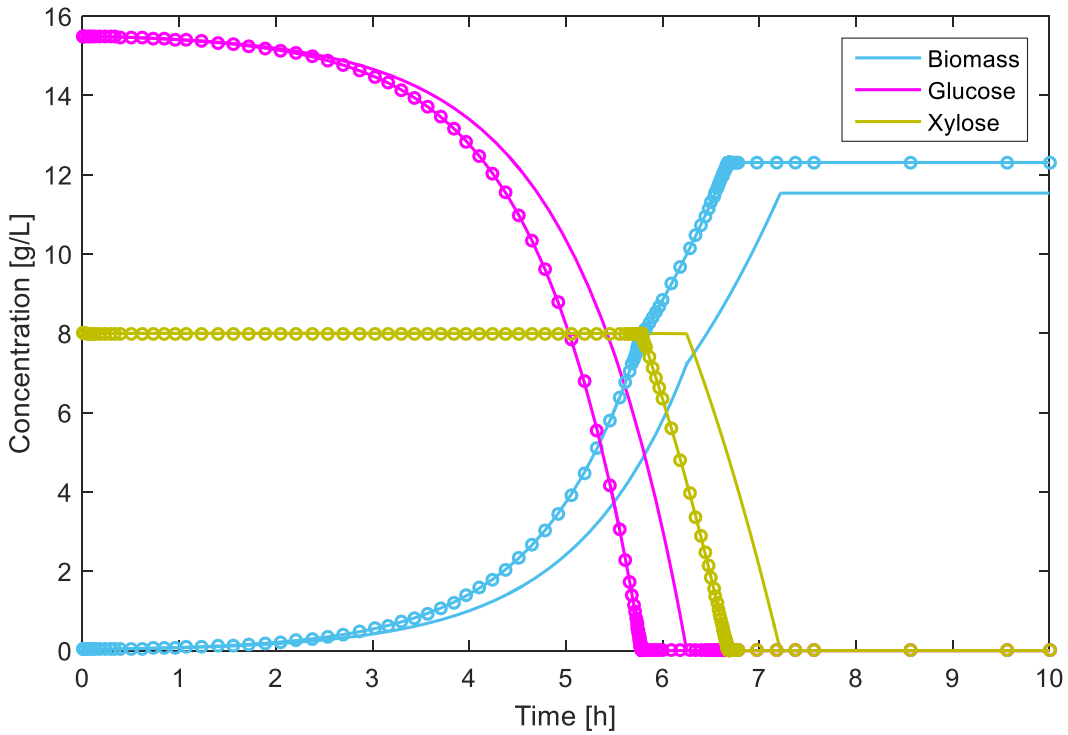


Figure 3-6: Dynamic profile of glucose, xylose, and biomass. The line with a marked circle belongs to DFBA while the other line belongs to DT-FBA

Based on Figure 3-6, it can be seen that since glucose is present in media, the constraints on glucose are active and limit the growth (up to 5.7 and 6.2 hr for DFBA and DT-FBA, respectively). Once glucose is depleted, microbe faces a nutrient-deficient situation, it activates the regulatory signal. Meanwhile, as reported by Chu and Barnes (Chu and Barnes, 2016), cell tries to find the best trade-off between two objectives: adapt itself fast to new environment and maintain growth high. According to this assumption, objective function in lag phase is not the only objective. After glucose is consumed, E. coli begins growing on xylose.

According to Figure 3-7, DFBA predicts glucose consumption and xylose consumption time sooner than DT-FBA. Also, Biomass predicted by DFBA is higher than DT-FBA.

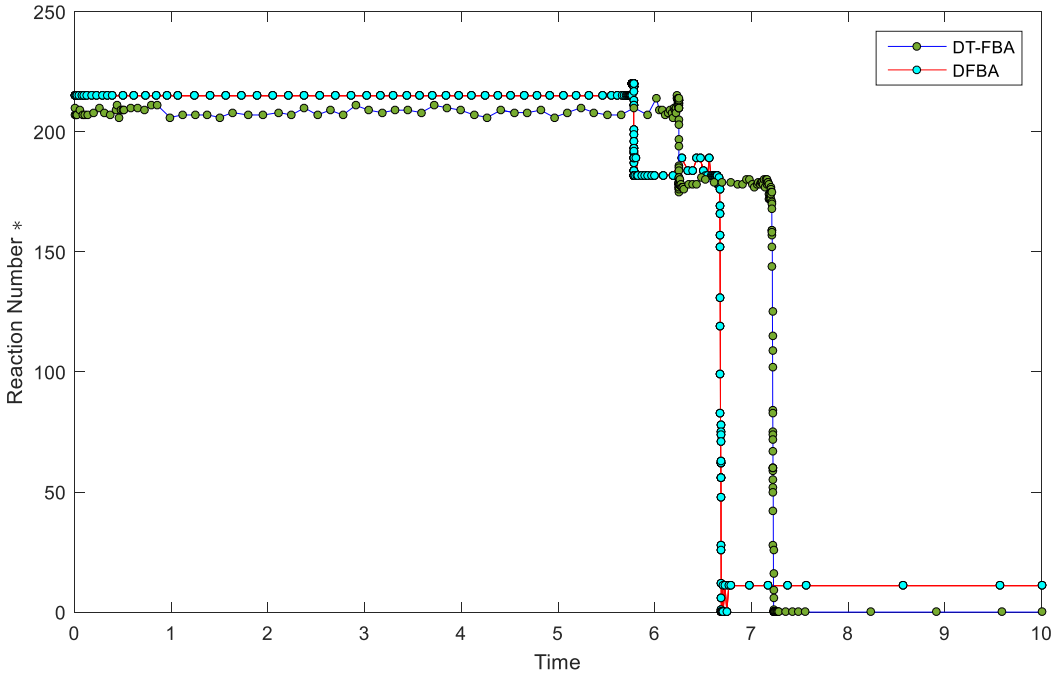


Figure 3-7: Number of valid reaction plotted versus time.

Through mining information from solutions over batch simulations, we eliminated the flux with zero flux, upper and lower limit of 1000 and -1000 as well as fluxes with fluctuation less than 0.1 value (Max-Min<0.1). In this study we call them valid reaction with acceptable and available flux. The figure clearly shows that the number of valid reaction for DT-FBA is lower than DFBA both glucose and xylose were consumed. This stems from the thermodynamic constraints which reduce the solution space.

We implemented a FVA and TVA to determine the permissible range of biochemical reaction (Figure 3-8). In both methodologies, the range for net flux is obtained by maximizing and minimizing a special reaction in optimal growth rate. Based on this information we can later classify the reactions to see which one is reversible or near of far from equilibrium under physiological condition. A cut-off of 1 mM was used as tolerance to exclude uncertain data from analysis and the values lower than this value were changed to zero.

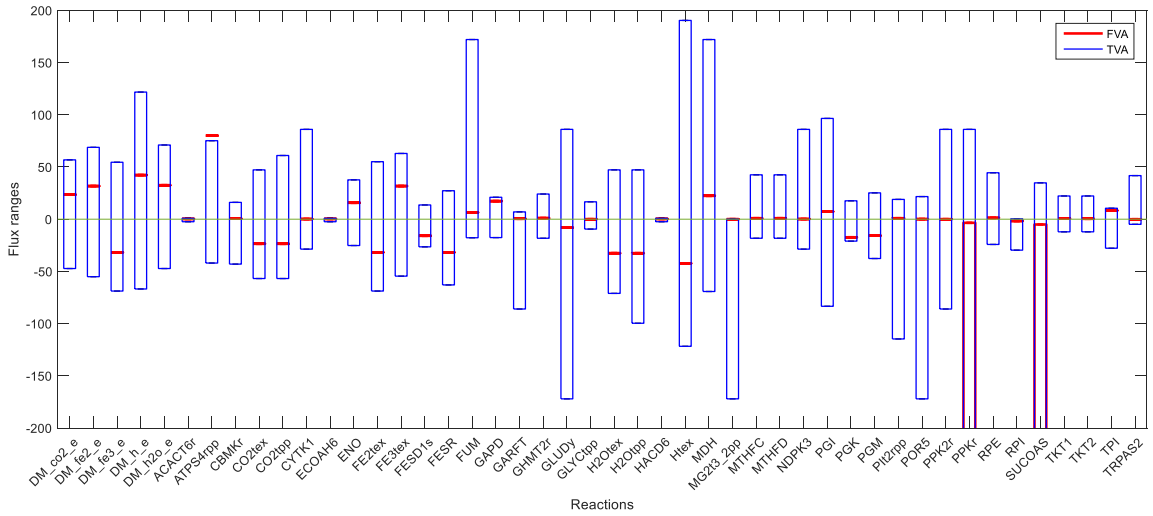


Figure 3-8: Flux variability analysis and thermodynamic variability analysis to classify reactions.

It shows that for all the reaction mentioned on plot, FVA estimates them as unidirectional whereas TVA analysis indicates all depicted reactions are bidirectional and reversible. In addition, except PPKr and SUCOAS, all remaining reaction on plot does not predict variability based on FVA while TVA predicts variability for all reactions.

9.6 Case Study 2: Diauxic growth simulation under aerobic condition and transition to anaerobic

The initial conditions are same as for aerobic simulation except oxygen concentration in batch is not regulated at constant concentration (Figure 3-9).

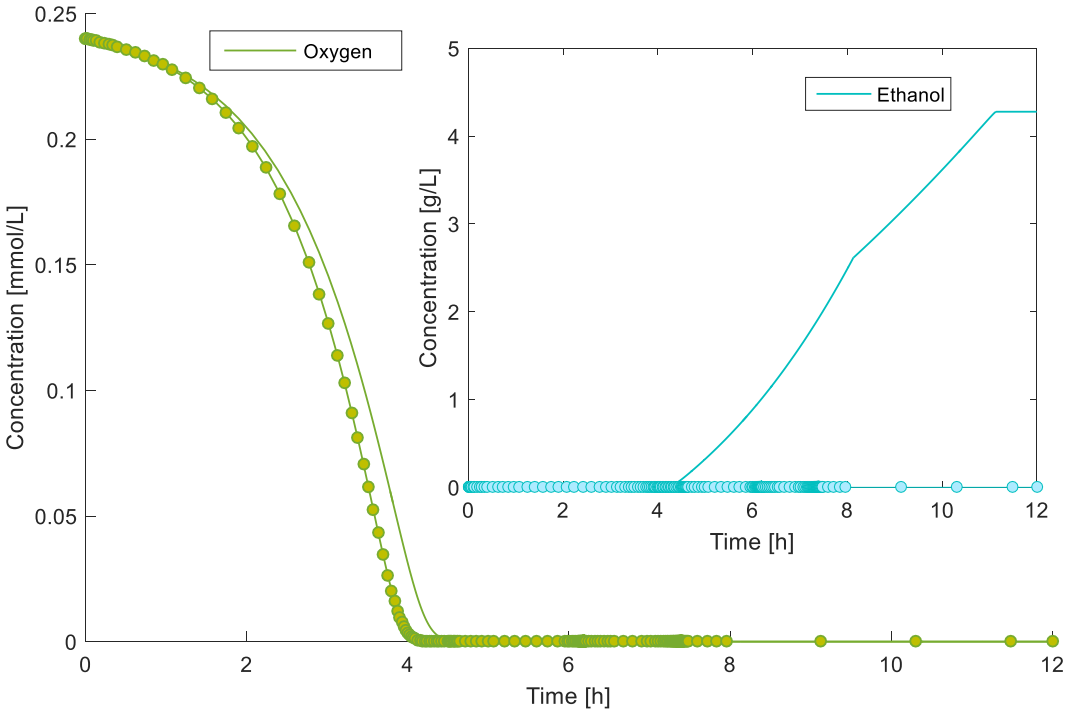


Figure 3-9: Oxygen and ethanol profile under anaerobic to aerobic transition for DFBA and DT-FBA. The line with circle belongs to DFBA

According to this figure, DFBA predicts no ethanol production even in anaerobic condition while DT-FBA reflects the diauxic growth and mixed-sugar preferential utilization into ethanol production as well.

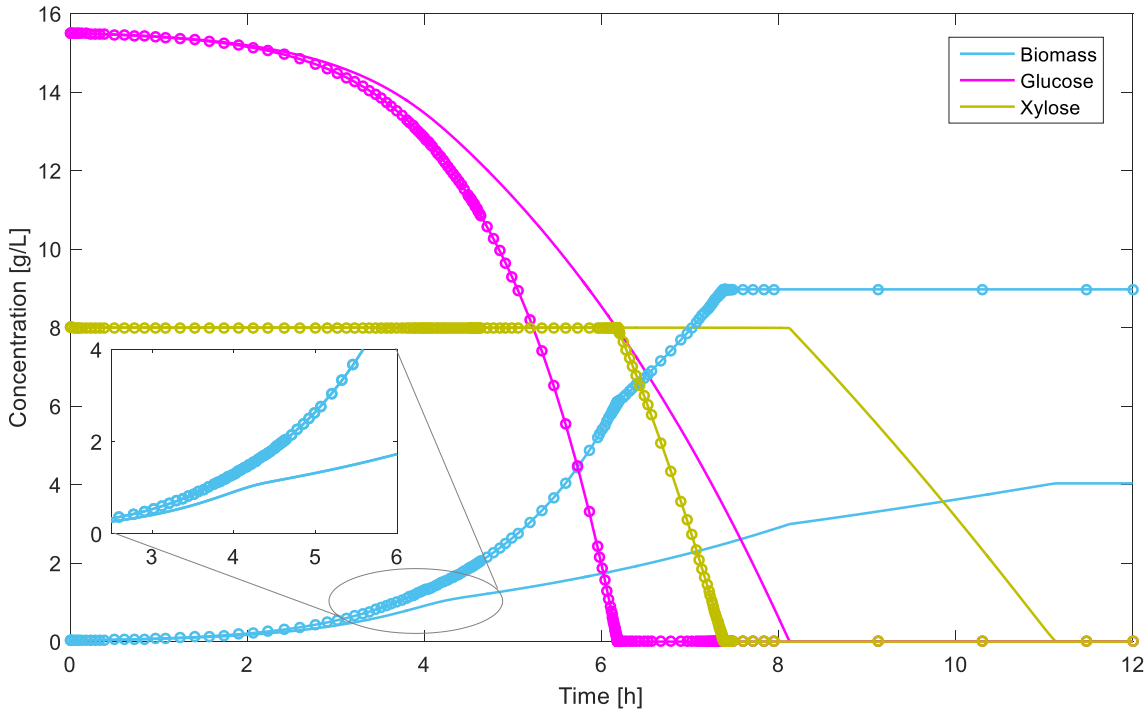


Figure 3-10: Concentration profile for glucose, xylose, and biomass under diauxic growth and transition from respiration to fermentation

Similar to previous simulation, DFBA predicts the consumption times sooner than DT-FBA. An interesting event was the capability of DT-FBA to capture transition from aerobic to anaerobic (Figure 3-10). Simulation shows DFBA fails to detect transition and its biomass grows same as before transition. When oxygen is present, aerobic respiration allows the complete oxidation of a growth substrate (i.e., glucose), leading to maximum energy conservation. Therefore, aerobic respiration is the most preferred mode of cell (Partridge et al., 2006; Trotter et al., 2011). In the absence of oxygen, it behaves two alternative metabolic modes: first, anaerobic respiration, if there is a terminal electron acceptor, such as NO^{-}_{3}, is available. Second: fermentation, if there is no terminal electron acceptor (Yasid et al., 2016). In the case of fermentation, overflow metabolites such as ethanol is secreted into media. DT-FBA also adapt itself to anaerobic situation into fermentation which mostly guide cellular metabolism to metabolite secretion rather than energy conservation.

Another evidence that confirm DT-FBA's predictability is Figure 3-11. It clearly shows that after transition from aerobic to anaerobic, number of valid reactions drastically fall. It implies the quiescence of intracellular reaction contribute in cell growth.

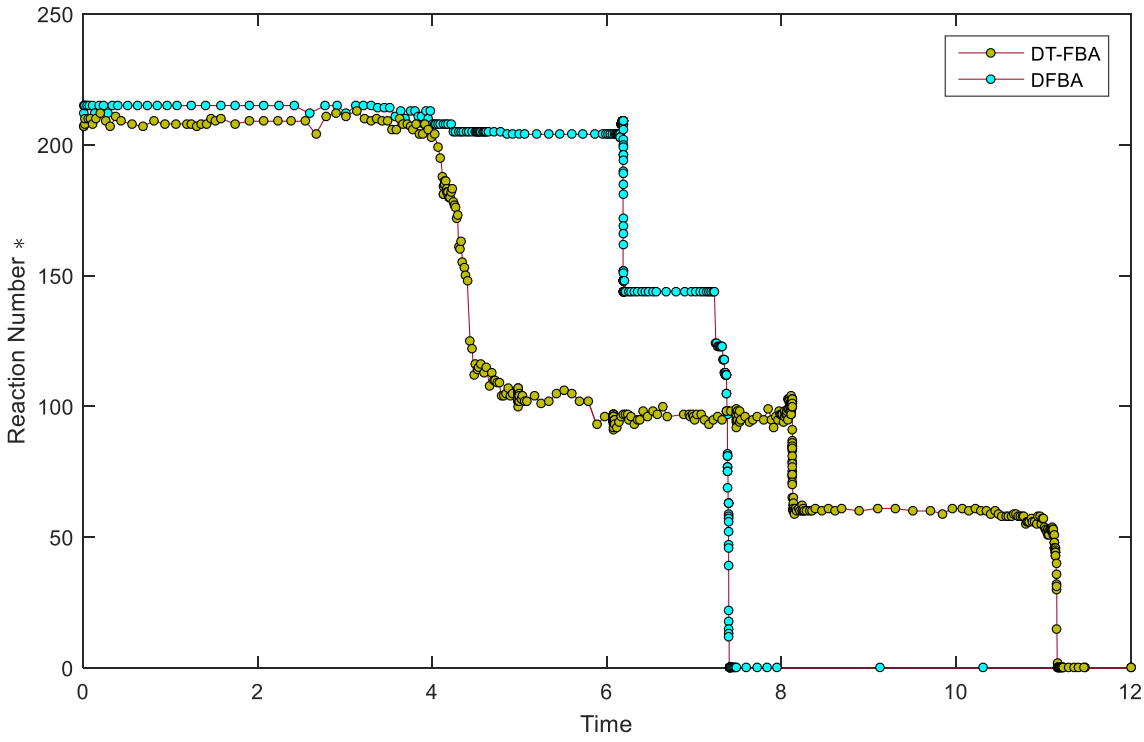


Figure 3-11: Number of valid reaction suddenly drops after transition from aerobic to anaerobic

All in all, the simulations by DT-FBA outperforms DFBA in detecting active reactions, diagnosing nutrient change and consumption, and production of ethanol in anaerobic condition.

TVA which is based on thermodynamic FBA recognizes the variations on fluxes while FVA (based on FBA) assumes these fluxes as unidirectional. All these findings are because of imposing an extra physiological constraint on models which makes it stronger in capturing cell phenotype.

10 Conclusion

To increase the ability of models in capturing phenotype behavior, thermodynamic data were integrated and Thermodynamic FBA (TFBA) was formulated. Consequently, we

used dynamic TFBA to model E. coli behaviour under diauxic growth for two condition. A condition with complete aerobic, and a condition in which oxygen would deplete meantime and system would be anaerobic. Dynamic TFBA were compared to FBA to check which approach predict cell response better. Finally, Flux variability analysis and thermodynamic variability analysis were performed. The results were interesting because TVA diagnosed reactions as reversible whereas FVA had considered them as bidirectional. Using this information, metabolic engineer can find the best pathway or reaction for genetic manipulation or in silico analysis and design.

Reference

Albe, K.R., Butler, M.H., Wright, B.E., 1990. Cellular concentrations of enzymes and their substrates. Journal of theoretical biology 143(2), 163-195.

Anesiadis, N., Kobayashi, H., Cluett, W.R., Mahadevan, R., 2013. Analysis and design of a genetic circuit for dynamic metabolic engineering. ACS synthetic biology 2(8), 442-452.

Angeles-Martinez, L., Theodoropoulos, C., 2016. Estimation of flux distribution in metabolic networks accounting for thermodynamic constraints: The effect of equilibrium vs. blocked reactions. Biochemical Engineering Journal 105, 347-357.

Antoniewicz, M.R., 2013. Dynamic metabolic flux analysis—tools for probing transient states of metabolic networks. Current opinion in biotechnology 24(6), 973-978.

Ataman, M., Hatzimanikatis, V., 2015. Heading in the right direction: thermodynamics-based network analysis and pathway engineering. Current opinion in biotechnology 36, 176-182.

Beard, D.A., Babson, E., Curtis, E., Qian, H., 2004. Thermodynamic constraints for biochemical networks. Journal of theoretical biology 228(3), 327-333.

Beard, D.A., Liang, S.-d., Qian, H., 2002. Energy balance for analysis of complex metabolic networks. Biophysical journal 83(1), 79-86.

Boghigian, B.A., Shi, H., Lee, K., Pfeifer, B.A., 2010. Utilizing elementary mode analysis, pathway thermodynamics, and a genetic algorithm for metabolic flux determination and optimal metabolic network design. BMC systems biology 4(1), 49.

Boulineau, S., Tostevin, F., Kiviet, D.J., ten Wolde, P.R., Nghe, P., Tans, S.J., 2013. Single-cell dynamics reveals sustained growth during diauxic shifts. PLoS One 8(4), e61686.

Brunk, E., Sahoo, S., Zielinski, D.C., Altunkaya, A., Dräger, A., Mih, N., Gatto, F., Nilsson, A., Gonzalez, G.A.P., Aurich, M.K., 2018. Recon3D enables a three-dimensional view of gene variation in human metabolism. Nature biotechnology 36(3), 272.

Chen, X., Alonso, A.P., Allen, D.K., Reed, J.L., Shachar-Hill, Y., 2011. Synergy between 13C-metabolic flux analysis and flux balance analysis for understanding metabolic adaption to anaerobiosis in E. coli. Metabolic engineering 13(1), 38-48.

Chu, D., Barnes, D.J., 2016. The lag-phase during diauxic growth is a trade-off between fast adaptation and high growth rate. Scientific reports 6, 25191.

Covert, M.W., Famili, I., Palsson, B.O., 2003. Identifying constraints that govern cell behavior: a key to converting conceptual to computational models in biology? Biotechnology and Bioengineering 84(7), 763-772.

Deutscher, J., 2008. The mechanisms of carbon catabolite repression in bacteria. Current opinion in microbiology 11(2), 87-93.

Deutscher, J., Francke, C., Postma, P.W., 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiology and Molecular Biology Reviews 70(4), 939-1031.

Edwards, J., Palsson, B., 2000. The Escherichia coli MG1655 in silico metabolic genotype: its definition, characteristics, and capabilities. Proceedings of the National Academy of Sciences 97(10), 5528-5533.

Edwards, J.S., Covert, M., Palsson, B., 2002. Metabolic modelling of microbes: the flux-balance approach. Environmental microbiology 4(3), 133-140.

Fleming, R.M., Maes, C.M., Saunders, M.A., Ye, Y., Palsson, B.Ø., 2012. A variational principle for computing nonequilibrium fluxes and potentials in genome-scale biochemical networks. Journal of theoretical biology 292, 71-77.

Gírio, F.M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S., Bogel-Łukasik, R., 2010. Hemicelluloses for fuel ethanol: a review. Bioresource technology 101(13), 4775-4800.

Gonzalez, J.E., Long, C.P., Antoniewicz, M.R., 2017. Comprehensive analysis of glucose and xylose metabolism in Escherichia coli under aerobic and anaerobic conditions by 13 C metabolic flux analysis. Metabolic engineering 39, 9-18.

Görke, B., Stülke, J., 2008. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nature Reviews Microbiology 6(8), 613.

Hamilton, J.J., Dwivedi, V., Reed, J.L., 2013. Quantitative assessment of thermodynamic constraints on the solution space of genome-scale metabolic models. Biophysical journal 105(2), 512-522.

Henry, C.S., Broadbelt, L.J., Hatzimanikatis, V., 2007. Thermodynamics-based metabolic flux analysis. Biophysical journal 92(5), 1792-1805.

Henry, C.S., Jankowski, M.D., Broadbelt, L.J., Hatzimanikatis, V., 2006. Genome-scale thermodynamic analysis of Escherichia coli metabolism. Biophysical journal 90(4), 1453-1461.

Hermsen, R., Okano, H., You, C., Werner, N., Hwa, T., 2015. A growth-rate composition formula for the growth of E. coli on co-utilized carbon substrates. Molecular systems biology 11(4), 801.

Hjersted, J., Henson, M., 2009. Steady-state and dynamic flux balance analysis of ethanol production by Saccharomyces cerevisiae. IET systems biology 3(3), 167-179.

Hjersted, J.L., Henson, M.A., 2006. Optimization of Fed-Batch Saccharomyces cerevisiae Fermentation Using Dynamic Flux Balance Models. Biotechnology progress 22(5), 1239-1248.

Hoppe, A., Hoffmann, S., Holzhütter, H.-G., 2007. Including metabolite concentrations into flux balance analysis: thermodynamic realizability as a constraint on flux distributions in metabolic networks. BMC systems biology 1(1), 23.

Inada, T., Kimata, K., Aiba, H., 1996. Mechanism responsible for glucose–lactose diauxie in Escherichia coli: challenge to the cAMP model. Genes to Cells 1(3), 293-301.

Jankowski, M.D., Henry, C.S., Broadbelt, L.J., Hatzimanikatis, V., 2008. Group contribution method for thermodynamic analysis of complex metabolic networks. Biophysical journal 95(3), 1487-1499.

Jeong, D.H., Yoo, S.J., Kim, J.H., Lee, J.M., 2016. Computationally efficient dynamic simulation of cellular kinetics via explicit solution of flux balance analysis: xDFBA

modelling and its biochemical process applications. Chemical Engineering Research and Design 113, 85-95.

Jouhten, P., Wiebe, M., Penttilä, M., 2012. Dynamic flux balance analysis of the metabolism of Saccharomyces cerevisiae during the shift from fully respirative or respirofermentative metabolic states to anaerobiosis. The FEBS journal 279(18), 3338-3354.

Joy, J., Kremling, A., 2010. Study of the growth of Escherichia coli on mixed substrates using dynamic flux balance analysis, 11th IFAC symposium on computer applications in biotechnology. pp. 401-406.

Kelly, W., Veigne, S., Li, X., Subramanian, S.S., Huang, Z., Schaefer, E., 2018. Optimizing performance of semi-continuous cell culture in an ambr15™ microbioreactor using dynamic flux balance modeling. Biotechnology progress 34(2), 420-431.

Kim, S., Seol, E., Oh, Y.-K., Wang, G., Park, S., 2009. Hydrogen production and metabolic flux analysis of metabolically engineered Escherichia coli strains. international journal of hydrogen energy 34(17), 7417-7427.

Kim, S.M., Choi, B.Y., Ryu, Y.S., Jung, S.H., Park, J.M., Kim, G.-H., Lee, S.K., 2015. Simultaneous utilization of glucose and xylose via novel mechanisms in engineered Escherichia coli. Metabolic engineering 30, 141-148.

Kim, S.R., Ha, S.-J., Wei, N., Oh, E.J., Jin, Y.-S., 2012. Simultaneous co-fermentation of mixed sugars: a promising strategy for producing cellulosic ethanol. Trends in biotechnology 30(5), 274-282.

King, Z.A., Dräger, A., Ebrahim, A., Sonnenschein, N., Lewis, N.E., Palsson, B.O., 2015. Escher: a web application for building, sharing, and embedding data-rich visualizations of biological pathways. PLoS computational biology 11(8), e1004321.

Kiparissides, A., Hatzimanikatis, V., 2017. Thermodynamics-based Metabolite Sensitivity Analysis in metabolic networks. Metabolic engineering 39, 117-127.

Kotte, O., Volkmer, B., Radzikowski, J.L., Heinemann, M., 2014. Phenotypic bistability in Escherichia coli's central carbon metabolism. Molecular systems biology 10(7), 736.

Kümmel, A., Panke, S., Heinemann, M., 2006. Systematic assignment of thermodynamic constraints in metabolic network models. BMC bioinformatics 7(1), 512.

Lin, Y., Tanaka, S., 2006. Ethanol fermentation from biomass resources: current state and prospects. Applied microbiology and biotechnology 69(6), 627-642.

Liu, L., Zhang, L., Tang, W., Gu, Y., Hua, Q., Yang, S., Jiang, W., Yang, C., 2012. Phosphoketolase pathway for xylose catabolism in Clostridium acetobutylicum revealed by 13C-metabolic flux analysis. Journal of bacteriology, JB. 00713-00712.

Mahadevan, R., Edwards, J.S., Doyle III, F.J., 2002. Dynamic flux balance analysis of diauxic growth in Escherichia coli. Biophysical journal 83(3), 1331-1340.

Mavrovouniotis, M.L., 1991. Estimation of standard Gibbs energy changes of biotransformations. Journal of Biological Chemistry 266(22), 14440-14445.

Müller-Hill, B., 1996. The lac operon: a short history of a genetic paradigm Walter de Gruyter.

Narang, A., Pilyugin, S.S., 2007. Bacterial gene regulation in diauxic and non-diauxic growth. Journal of theoretical biology 244(2), 326-348.

Noor, E., 2018. Removing both Internal and Unrealistic Energy-Generating Cycles in Flux Balance Analysis. arXiv preprint arXiv:1803.04999.

Noor, E., Bar-Even, A., Flamholz, A., Lubling, Y., Davidi, D., Milo, R., 2012. An integrated open framework for thermodynamics of reactions that combines accuracy and coverage. Bioinformatics 28(15), 2037-2044.

Noor, E., Haraldsdóttir, H.S., Milo, R., Fleming, R.M., 2013. Consistent estimation of Gibbs energy using component contributions. PLoS computational biology 9(7), e1003098.

O'brien, E.J., Lerman, J.A., Chang, R.L., Hyduke, D.R., Palsson, B.Ø., 2013. Genome-scale models of metabolism and gene expression extend and refine growth phenotype prediction. Molecular systems biology 9(1), 693.

Okada, T., Ueyama, K., Niiya, S., Kanazawa, H., Futai, M., Tsuchiya, T., 1981. Role of inducer exclusion in preferential utilization of glucose over melibiose in diauxic growth of Escherichia coli. Journal of bacteriology 146(3), 1030-1037.

Orth, J.D., Conrad, T.M., Na, J., Lerman, J.A., Nam, H., Feist, A.M., Palsson, B.Ø., 2011. A comprehensive genome-scale reconstruction of Escherichia coli metabolism—2011. Molecular systems biology 7(1), 535.

Orth, J.D., Thiele, I., Palsson, B.Ø., 2010. What is flux balance analysis? Nature biotechnology 28(3), 245.

Palsson, B., 2000. The challenges of in silico biology. Nature biotechnology 18(11), 1147.

Palsson, B., Palsson, B.Ø., 2015. Systems biology. Cambridge university press.

Partridge, J.D., Scott, C., Tang, Y., Poole, R.K., Green, J., 2006. Escherichia coli transcriptome dynamics during the transition from anaerobic to aerobic conditions. Journal of Biological Chemistry 281(38), 27806-27815.

Price, N.D., Reed, J.L., Palsson, B.Ø., 2004a. Genome-scale models of microbial cells: evaluating the consequences of constraints. Nature Reviews Microbiology 2(11), 886.

Price, N.D., Schellenberger, J., Palsson, B.O., 2004b. Uniform sampling of steady-state flux spaces: means to design experiments and to interpret enzymopathies. Biophysical journal 87(4), 2172-2186.

Raetz, C., 1996. Escherichia coli and Salmonella cellular and molecular biology. Escherichia coli and Salmonella: Cellular and Molecular Biology 1, 1035-1063.

Reed, J.L., Palsson, B.Ø., 2003. Thirteen years of building constraint-based in silico models of Escherichia coli. Journal of bacteriology 185(9), 2692-2699.

Reed, J.L., Palsson, B.Ø., 2004. Genome-scale in silico models of E. coli have multiple equivalent phenotypic states: assessment of correlated reaction subsets that comprise network states. Genome research 14(9), 1797-1805.

Sainz, J., Pizarro, F., Pérez-Correa, J.R., Agosin, E., 2003. Modeling of yeast metabolism and process dynamics in batch fermentation. Biotechnology and Bioengineering 81(7), 818-828.

Saitua, F., Torres, P., Pérez-Correa, J.R., Agosin, E., 2017. Dynamic genome-scale metabolic modeling of the yeast Pichia pastoris. BMC systems biology 11(1), 27.

Schellenberger, J., Lewis, N.E., Palsson, B.Ø., 2011. Elimination of thermodynamically infeasible loops in steady-state metabolic models. Biophysical journal 100(3), 544-553.

Schilling, C.H., Schuster, S., Palsson, B.O., Heinrich, R., 1999. Metabolic pathway analysis: basic concepts and scientific applications in the post-genomic era. Biotechnology progress 15(3), 296-303.

Senger, R.S., Papoutsakis, E.T., 2008. Genome-scale model for Clostridium acetobutylicum: Part I. Metabolic network resolution and analysis. Biotechnology and bioengineering 101(5), 1036-1052.

Soh, K.C., Hatzimanikatis, V., 2010. Network thermodynamics in the post-genomic era. Current opinion in microbiology 13(3), 350-357.

Soh, K.C., Hatzimanikatis, V., 2014. Constraining the flux space using thermodynamics and integration of metabolomics data, Metabolic Flux Analysis. Springer, pp. 49-63.

Soh, K.C., Miskovic, L., Hatzimanikatis, V., 2012. From network models to network responses: integration of thermodynamic and kinetic properties of yeast genome-scale metabolic networks. FEMS yeast research 12(2), 129-143.

Trotter, E.W., Rolfe, M.D., Hounslow, A.M., Craven, C.J., Williamson, M.P., Sanguinetti, G., Poole, R.K., Green, J., 2011. Reprogramming of Escherichia coli K-12 metabolism during the initial phase of transition from an anaerobic to a micro-aerobic environment. PloS one 6(9), e25501.

van Heerden, J.H., Wortel, M.T., Bruggeman, F.J., Heijnen, J.J., Bollen, Y.J., Planqué, R., Hulshof, J., O'Toole, T.G., Wahl, S.A., Teusink, B., 2014. Lost in transition: start-up of glycolysis yields subpopulations of nongrowing cells. Science 343(6174), 1245114.

Varma, A., Palsson, B.O., 1994. Metabolic flux balancing: basic concepts, scientific and practical use. Nature Biotechnology 12(10), 994.

Wiback, S.J., Famili, I., Greenberg, H.J., Palsson, B.Ø., 2004. Monte Carlo sampling can be used to determine the size and shape of the steady-state flux space. Journal of theoretical biology 228(4), 437-447.

Yang, F., Qian, H., Beard, D.A., 2005. Ab initio prediction of thermodynamically feasible reaction directions from biochemical network stoichiometry. Metabolic engineering 7(4), 251-259.

Yasid, N.A., Rolfe, M.D., Green, J., Williamson, M.P., 2016. Homeostasis of metabolites in Escherichia coli on transition from anaerobic to aerobic conditions and the transient secretion of pyruvate. Royal Society open science 3(8), 160187.

CHAPTER 4

Conclusion and Future Works

In recent years, the use of high-throughput sequencing and gene expression profiling techniques has allowed researchers to map the structure of different biological networks (regulatory, signaling, metabolic) for several cell types and to understand many of their properties.

Several efforts resulted in the reconstructed and refined genome-scale metabolic network models. These metabolic networks include all known biochemical reactions occurring in a specific cell and represent a structured database of the totality of known metabolic processes that take place in the cell, including the metabolites involved, the enzymes catalyzing each of the reactions and the genes that code for the necessary machinery for these processes.

Among the different methodologies to reconstructed genome-scale metabolic networks, the constraint-based reconstruction and analysis (COBRA) approach has proven quite useful for studying cell metabolism at the genome scale using constraints to narrow down the range of feasible flux distributions to recapitulate real pathway usage. Genome-scale metabolic network models enable the quantitative analysis of intracellular metabolic fluxes “in silico” and the prediction of phenotype from genotype.

In metabolic networks many simulations carry out in “static” state whereas our interest is to predict the behavior in a “dynamical” approach and to understand how environment and intracellular interact.

In addition, the metabolic phenotype of cell systems often involves high levels of nutrient uptake and excessive byproduct secretion. Negative correlations between some by-products and cell growth were found, suggesting a way to increase cell viability by reducing the concentrations of some media components. For example, when cultured mammalian cells grow with excess glucose, lactate dehydrogenase activity increases, leading to a high turnover of intracellular pyruvate and subsequent secretion of lactate into the extracellular medium. As lactate accumulates, both cell growth and cell productivity decrease and certain enzymes in the glycolytic pathway are downregulated. So, an important objective in bioprocess control is to reduce lactate secretion in mammalian cell culture as prevention of excessive accumulation of harmful metabolic byproducts.

Achieving the prevention of excessive accumulation, determination of the optimal concentration of input nutrients and producing maximum biomass as well as the direct influence pf metabolic fluxes on cell physiology, convince us to modulate metabolic pathways via media optimization.

It has been demonstrated that co-culture can assist in improving the yield and productivity compared to the mono-culture of organisms because of the presence of synergy between these two species. However, the metabolic interactions in the algal co-cultures are not well understood. In order to understand these metabolic interactions in the co-cultures, genome-scale metabolic analysis of cells can be recruited to shed a light on this way as this models can successfully predict the chemostat growth and byproduct secretion with substrate.

Developing methods for the analysis of metabolism in co-cultures, and investigating the inter-species metabolic interactions for enhancing the metabolic rate and biofuel synthesis is an interesting objective. Metabolic models can be used to address pertinent questions on how to optimally co-culture microalgae which is the desired aim in this consolidated bioprocessing approach.

In addition, we have submitted a proposal for incorporating Microarray and RNA-seq data into metabolic networks. The build network is fed into a Multi-level and multi objective

problem which dynamically simulate the cell growth. For this project, metaheuristic and evolutionary algorithms are utilized for optimization.

Due to various industry and research experiences, using quantitative and dynamic models of the metabolic phenotypes can help us to optimize the cell culture and more accurately monitor the cell behavior and determine optimal metabolic/regulatory performance of cell under different conditions. The development of a validated computational metabolic/regulatory model will provide a better understanding of metabolic alterations and develop therapies for example to inhibit tumor by identifying the optimal metabolic enzymes to target or directing stem cell fate towards desired specialized cell types.