

CRANFIELD UNIVERSITY

Domoyi Castro Mathew

IMPROVING MICROALGAE BIOFUEL PRODUCTION: AN  
ENGINEERING MANAGEMENT APPROACH

School of Engineering  
Energy & Power Engineering Division

PhD  
Academic Year: 2013- 2014

Supervisors: Dr Giuseppina Di Lorenzo & Prof Pericles Pilidis  
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This thesis is submitted in fulfilment of the requirements for the  
degree of PhD

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## ABSTRACT

The use of microalgae culture to convert CO<sub>2</sub> from power plant flue gases into biomass that are readily converted into biofuels offers a new frame of opportunities to enhance, compliment or replace fossil-fuel-use. Apart from being renewable, microalgae also have the capacity to utilise materials from a variety of wastewater and the ability to yield both liquid and gaseous biofuels. However, the processes of cultivation, incorporation of a production system for power plant waste flue gas use, algae harvesting, and oil extraction from the biomass have many challenges. Using SimaPro software, Life cycle Assessment (LCA) of the challenges limiting the microalgae (*Chlorella vulgaris*) biofuel production process was performed to study algae-based pathway for producing biofuels. Attention was paid to material use, energy consumed and the environmental burdens associated with the production processes. The goal was to determine the weak spots within the production system and identify changes in particular data-set that can lead to and lower material use, energy consumption and lower environmental impacts than the baseline microalgae biofuel production system. The analysis considered a hypothetical transesterification and Anaerobic Digestion (AD) transformation of algae-to-biofuel process. Life cycle Inventory (LCI) characterisation results of the baseline biodiesel (BD) transesterification scenario indicates that heating to get the biomass to 90% DWB accounts for 64% of the total input energy, while electrical energy and fertilizer obligations represents 19% and 16% respectively. Also, Life Cycle Impact Assessment (LCIA) results of the baseline BD production scenario show high proportional contribution of electricity and heat energy obligations for most impact categories considered relative to other resources. This is attributed to the concentration/drying requirement of algae biomass in order to ease downstream processes of lipid extraction and subsequent transesterification of extracted lipids into BD. Thus, four prospective alternative production scenarios were successfully characterised to evaluate the extent of their impact scenarios on the production system with regards to lowering material use, lower energy consumption and lower environmental burdens than the standard algae biofuel production system. A 55.3% reduction

in mineral use obligation was evaluated as the most significant impact reduction due to the integration of 100% recycling of production harvest water for the AD production system. Recycling also saw water demand reduced from 3726 kg (freshwater).kgBD<sup>-1</sup> to 591kg (freshwater).kgBD<sup>-1</sup> after accounting for evaporative losses/biomass drying for the BD transesterification production process. Also, the use of wastewater/sea water as alternative growth media for the BD production system, indicated potential savings of: 4.2 MJ (11.8%) in electricity/heat obligation, 10.7% reductions for climate change impact, and 87% offset in mineral use requirement relative to the baseline production system. Likewise, LCIA characterisation comparison results comparing the baseline production scenarios with that of a set-up with co-product economic allocation consideration show very interesting outcomes. Indicating -12 MJ surplus (-33%) reductions for fossil fuels resource use impact category, 52.7% impact reductions for mineral use impact and 56.6% reductions for land use impact categories relative to the baseline BD production process model. These results show the importance of allocation consideration to LCA as a decision support tool. Overall, process improvements that are needed to optimise economic viability also improve the life cycle environmental impacts or sustainability of the production systems. Results obtained have been observed to agree reasonably with Monte Carlo sensitivity analysis, with the production scenario proposing the exploitation of wastewater/sea water to culture algae biomass offering the best result outcome. This study may have implications for additional resources such as production facility and its construction process, feedstock processing logistics and transport infrastructure which are excluded. Future LCA study will require extensive consideration of these additional resources such as: facility size and its construction, better engineering data for water transfer, combined heat and power plant efficiency estimates and the fate of long-term emissions such as organic nitrogen in the AD digestate. Conclusions were drawn and suggestions proffered for further study.

Keywords: CO<sub>2</sub>; biomass; fossil fuel; Life Cycle Assessment (LCA); *Chlorella vulgaris*; Anaerobic Digestion (AD); Transesterification; Monte Carlo sensitivity analysis.

## **ACKNOWLEDGEMENTS**

My foremost acknowledgement goes to my supervisors Dr Giuseppina Di Lorenzo and Professor Pericle Pilidis for their supervision, support and useful commentaries over the past three years and particularly in the course of writing this thesis. Dr Giuseppina and Dr Ofem Usani were particularly immensely helpful with editorial comments and advice while the writing of this Thesis was in progress. Many thanks also to the Petroleum Technology Development Fund (PTDF) Nigeria, for providing the financial sponsorship throughout the course of my PhD research.

Extra-Special thanks also to my family and good friends, as I have been very fortunate to be associated with you and your support all these years. My very special thanks to Chief Dan Etete, chairman of Dan Etete Foundations for his financial support which came just-in-time. You are well appreciated and many thanks. Thanks also to Dr Kolios Athanasios and Ms Kowalewska Alicja for their moral and technical support with SimaPro LCA Software. Finally, I would like to acknowledge all the scholars whose Journal/Conference papers I have read over the years. Without having such value-added intellectual intercourse with their work, I could never have gained the knowledge and experience required to write this thesis.





# TABLE OF CONTENTS

ABSTRACT .....	i
ACKNOWLEDGEMENTS.....	iii
LIST OF FIGURES.....	ix
LIST OF TABLES .....	xiii
LIST OF EQUATIONS.....	xv
LIST OF ABBREVIATIONS .....	xvii
CHAPTER ONE .....	1
GENERAL INTRODUCTION.....	1
1.1 Introduction .....	1
1.2 Aim and Objectives .....	4
1.3 Thesis Structure.....	5
CHAPTER TWO.....	7
LITERATURE REVIEW .....	7
2.1 Overview.....	7
2.2 Types and classes of Microalgae.....	8
2.2.1 Green algae (Chlorophyceae).....	10
2.2.2 Diatoms (Bacillariophyceae).....	10
2.3 Algae biochemical composition.....	10
2.4 Microalgae Growth (Nutritional) and Cultivation Methods.....	11
2.5 Microalgae Growth (Nutritional) modes.....	12
2.5.1 Photoautotrophic .....	12
2.5.2 Heterotrophic.....	13
2.5.3 Mixotrophic.....	13
2.6 Microalgae Cultivation Methods.....	14
2.6.1 Open pond cultivation system .....	14
2.6.2 Photobioreactor (PBR) cultivation system .....	16
2.6.3 Hybrid or coupled cultivation systems .....	18
2.7 Factors affecting Microalgae Cultivation for biofuel production.....	20
2.7.1 Impact of strain.....	20
2.7.2 Environmental factors.....	21
2.7.3 Light regime .....	21
2.7.4 Temperature.....	22
2.7.5 Salinity.....	22
2.8 Nutrient requirements .....	23
2.8.1 Microalgae CO <sub>2</sub> requirement .....	23
2.8.2 Fertilizer requirement option.....	26
2.8.3 Wastewater application option .....	27
2.9 Biotechnological approaches to control cell composition .....	29
2.9.1 Biochemical (metabolic) engineering approach.....	30
2.9.2 Genetic engineering approach .....	31

2.10 Microalgae harvesting and biomass concentration processes.....	33
2.11 Harvesting methods.....	33
2.11.1 Flocculation.....	34
2.11.2 Flotation.....	35
2.11.3 Filtration.....	36
2.11.4 Centrifugal sedimentation.....	36
2.12 Biomass drying processes.....	37
2.12.1 Biomass extraction and purification processes.....	38
2.13 Microalgae biofuel energy production technologies.....	39
2.13.1 Anaerobic Digestion (AD) conversion processes.....	40
2.13.2 Transesterification.....	44
2.14 Thermochemical conversion processes.....	48
2.14.1 Combustion.....	49
2.14.2 Pyrolysis.....	50
2.14.3 Gasification.....	52
2.14.4 Thermochemical liquefaction.....	54
2.15 Comparative LCA studies of previous microalgae based biofuel production systems.....	56
CHAPTER THREE.....	63
METHODOLOGY OF DATA COLLECTION.....	63
3.1 LCA methodology overview.....	63
3.2 Goal and scope definition.....	63
3.2.1 Functional Unit,.....	64
3.2.2 System boundary.....	64
3.2.3 Allocation issues.....	66
3.3 Life Cycle Inventory (LCI).....	67
3.4 Life Cycle Impact Assessment (LCIA).....	67
3.5 Interpretation.....	68
3.6 Model description and case study.....	69
3.7 Microalgae baseline production pathway overview.....	73
3.8 Life Cycle Inventory (LCI) data.....	75
3.8.1 Algae cultivation data.....	75
3.8.2 Harvesting and dewatering process.....	77
3.8.3 Microalgae biomass conversion technology (Dry Process).....	78
3.8.4 Microalgae oil extraction process.....	79
3.8.5 Input flow description for the conversion technologies.....	80
3.9 LCIA using Eco-indicator 99 method in SimaPro.....	80
3.10 Sensitivity/Data Quality (uncertainty) Analysis.....	83
CHAPTER FOUR.....	85
RESULTS AND DISCUSSIONS.....	85
4.1 Framework.....	85
4.2 Microalgae biofuel pathway baseline case.....	86

4.3 LCI results of biodiesel transesterification process .....	87
4.4 LCIA results of baseline biodiesel production model.....	91
4.4.1 LCIA of microalgae biodiesel production with 100% recycling of production harvest water .....	97
4.4.2 LCIA results of prospects of using wastewater/sea water .....	105
4.4.3 LCIA of biodiesel production scenario with co-products economic allocation consideration.....	110
4.4.4 LCIA of biodiesel production assuming a 70% induced increase in algae lipid yield.....	118
4.4.5 LCIA comparison of baseline biodiesel model with all proposed alternative biodiesel production scenarios .....	127
4.5 Energy Recovery via anaerobic digestion (AD) of algae biomass feedstock .....	132
4.5.1 LCIA results of baseline model for the Anaerobic Digestion (AD) of algae biomass to produce 1kg biogas.....	133
4.5.2 LCIA analysis of the impact of 100% recycling of production harvest water on the algae-to-biogas AD transformation system.....	137
4.5.3 LCIA analysis of the prospects of using wastewater/sea water as culture medium for the algae-to-biogas AD production process.....	144
4.5.4 LCIA analysis of the prospective impact of induced increase in algae oil yield on the algae-to-biogas AD transformation process .....	149
4.5.5 Comparing the baseline model of producing 1kg biogas from AD of algae biomass with that of all proposed prospective alternative production scenarios.....	155
CHAPTER FIVE .....	160
CONCLUSIONS AND RECOMMENDATION FOR FURTHER STUDIES.....	160
5.1 Introduction .....	160
5.2 Conclusions .....	161
5.3 Limitations and recommendations for further research .....	169
REFERENCES.....	171



## LIST OF FIGURES

Figure 1 Fuel shares of global energy consumption in 2007 (adapted: IEA, 2007, 2009; BP. 2009) .....	1
Figure 2 Generic Microalgae biofuel process chain (adapted from Mata et al., 2010).....	8
Figure 3 Arial view of a raceway pond (Y. Chisty 2007) .....	15
Figure 4 the Photobioreactor (adopted from Molina et al., 2001) .....	18
Figure 5 Sources of CO <sub>2</sub> for microalgae growth .....	24
Figure 6 Energy Conversion Processes from Biomass (Source: Tsukahara and Sawayama, 2005) .....	40
Figure 7 Transesterification with alkali catalyst .....	46
Figure 8 Transesterification by acid catalyst.....	46
Figure 9 Biomass thermochemical conversion processes (Balat et al., 2009)..	48
Figure 10 Microalgae biofuel production System Boundary .....	65
Figure 11 Microalgal-based Biofuel Production Process Flow Diagram .....	70
Figure 12 Microalgae-to-biofuel process options, (a) the dry process route, and (b) the wet process route (adapted from Yang et al., 2011). The value of the DW denotes the input dry solid weight as a percentage of the designed post-process.....	72
Figure 13 Biomass Harvesting/Dewatering process option .....	77
Figure 14 Process Contribution Analysis for the production of 1kg biodiesel (BD) via Transesterification (Domoyi et al., 2013) .....	87
Figure 15 Pie chart showing Energy Input (EIP) profile for producing 1 kg biodiesel via transesterification .....	90
Figure 16 Analysing 1 kg microalgae biodiesel production (baseline scenario) at impact characterisation level .....	93
Figure 17 Analysing 1 kg microalgae biodiesel production (baseline scenario) at single score impact level .....	94
Figure 18 Graphical representation of uncertainty analysis of 1 kg biodiesel production (base-case scenario) .....	96
Figure 19 Analysing 1kg microalgae biodiesel production with 100% production water recycling at impact characterisation level .....	99
Figure 20 Single score analysis of microalgae biodiesel production with 100% recycling of production harvest water .....	100

Figure 21 Comparing 1 kg microalgae biodiesel production (baseline scenario) v. 1 kg biodiesel production with 100% recycling of production harvest water .....	102
Figure 22 Single score LCIA: comparing 1 kg biodiesel production (baseline scenario) with 1 kg biodiesel production with 100% production water recycling.....	103
Figure 23 Uncertainty analysis of 1 kg microalgae biodiesel production (baseline scenario) v 1 kg microalgae biodiesel production with 100% recycling of production harvest water .....	104
Figure 24 LCIA analysis of 1 kg microalgae biodiesel production using wastewater/sea water .....	105
Figure 25 single score impact analysis of 1 kg microalgae biodiesel production process using wastewater/sea water .....	108
Figure 26 Comparing 1 kg microalgae biodiesel production (base-line scenario) v 1 kg microalgae biodiesel production using wastewater/sea water .....	109
Figure 27 Analysing 1 kg microalgae biodiesel production baseline scenario (with co-products economic allocation consideration) .....	112
Figure 28 Analysing 1 kg microalgae biodiesel production base-case scenario with co-products economic allocation consideration at single score impact level.....	113
Figure 29 Comparing 1kg algae biodiesel production (base-case scenario) with 1kg algae biodiesel production (with co-product economic allocation consideration).....	115
Figure 30 Single score LCIA comparing 1kg microalgae biodiesel production (baseline scenario) with 1 kg biodiesel production integrating co-products economic allocation consideration. ....	116
Figure 31 Uncertainty Analysis of 1kg algae biodiesel production with co-products economic allocation consideration (A) minus 1kg algae biodiesel production (baseline scenario) (B). ....	117
Figure 32 LCIA Analysis of 1 kg microalgae biodiesel production (assuming 70% biomass induced lipid yield) .....	120
Figure 33 Single score LCIA analysing 1 kg algae biodiesel production with 70% induced biomass lipid yield.....	122
Figure 34 Comparing 1kg microalgae biodiesel production assuming a 70% induced lipid yield with that of 1kg algae biodiesel production (baseline scenario) .....	124
Figure 35 Single score LCIA analysis comparing 1kg microalgae biodiesel production (baseline scenario) with 1kg algae biodiesel production assuming a 70% induced increase in lipid yield .....	125

Figure 36 Uncertainty analysis of 1kg microalgae biodiesel production with 70% induced increase in lipid yield vs 1kg algae biodiesel production (baseline scenario) .....	126
Figure 37 LCIA Comparing biodiesel production processes: (1) baseline scenario; (2) 100% recycling of production harvest water; (3) use of wastewater/sea water; (4) co-products economic allocation consideration; and (5) 70% induced increase in lipid yield .....	129
Figure 38 Single score LCIA comparing processes: (1) base-case scenario, (2) 100% recycling of production harvest water, (3) use of wastewater/sea water, (4) co-products economic allocation consideration, and (5) 70% induced lipid yield.....	131
Figure 39 LCIA analysis of Anaerobic Digestion (AD) of algae biomass to produce 1 kg biogas (baseline model) .....	136
Figure 40 LCIA analysis of AD of algae biomass to produce 1kg biogas (with 100% recycling of production harvest water).....	139
Figure 41 Comparing anaerobic digestion (AD) of algae biomass to produce 1kg biogas incorporating 100% recycling of production harvest water with AD of algae biomass to produce 1kg biogas (baseline model) .....	141
Figure 42 Single score LCIA comparing 1kg biogas production from AD of algae biomass (baseline model) with that of set-up with 100% recycling of production harvest water .....	142
Figure 43 Uncertainty analysis of algae-to-biogas AD transformation process (with 100% recycling of production harvest water) vs algae-to-biogas transformation (base-case scenario).....	143
Figure 44 LCIA results for AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium: wastewater here represents secondary effluents characterised by low biological oxygen demand (BOD) and chemical oxygen demand (COD) but high in inorganic N and P. ....	146
Figure 45 LCIA comparison of AD of algae biomass to produce 1kg biogas (baseline model) vs AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium .....	147
Figure 46 Single score LCIA analysis comparing AD of algal biomass to produce 1kg biogas (baseline model) with AD of algal biomass to produce 1kg biogas using wastewater/sea water as growth media .....	148
Figure 47 LCIA analysis of 1kg biogas production via AD of algal biomass with 70% induced lipid yield.....	150
Figure 48 Comparing 1 kg biogas produced from AD of algal biomass (baseline model) with that of producing 1kg biogas via AD of algal biomass (assuming 70% induced increase in lipid yield).....	153

Figure 49 Single score LCIA analysis comparing AD of algal biomass to produce 1kg biogas (baseline model) with AD of algal biomass to produce 1kg biogas assuming a 70% induced increase in lipid synthesis ..... 154

Figure 50 LCIA Comparing processes: (1) AD of algae biomass to produce 1kg biogas assuming 70% induce increase in lipid yield, (2) AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium, (3) AD of algae biomass to produce 1kg biogas with 100% recycling of production harvest water, and (4) baseline AD production model ..... 156

Figure 51 Single score LCIA comparing processes: (L to R) (1) AD of algae biomass to produce 1kg biogas assuming 70% induce increase in lipid yield, (2) AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium, (3) AD of algae biomass to produce 1kg biogas with 100% recycling of production harvest water, and (4) baseline AD production model..... 158



## LIST OF TABLES

Table 1 Comparison of microalgae production methods (adapted from Harun et al., 2010) .....	19
Table 2 Typical concentrations of emissions of flue gases from power generating plants using different types of fuel (Ramachandra et al., 2002) .....	26
Table 3 Merits and demerits of different transesterification processes (adapted from Huang et al., 2010) .....	45
Table 4 Operating conditions and expected products yields for pyrolysis (Brennan and Owende 2010) .....	50
Table 5 Input flows for microalgae cultivation per kg algae .....	76
Table 6 Input flows for harvest and dewatering process per kg algae .....	78
Table 7 Input flow description for oil extraction per kg algae .....	79
Table 8 Input flows for biomass conversion technologies per 1 kg delivered energy (biofuels) .....	80
Table 9 Typical value systems of the three different perspectives ((Goedkoop and Spruiensma, 2001) .....	82
Table 10 Transesterification process Input parameters to produce 1kg Biodiesel (BD), Note: EIP is energy input; while, EOP is energy output. ....	88
Table 11 Transesterification process output parameters .....	89
Table 12 SimaPro Input data sheet for the production of 1 kg biodiesel (baseline scenario), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation .....	92
Table 13 SimaPro Input data sheet for the production of 1 kg biodiesel with 100% recycling of production harvest water .....	98
Table 14 SimaPro Input data sheet for the production of 1 kg biodiesel using wastewater/sea water as culture media .....	107
Table 15 SimaPro Input data sheet for the production of 1 kg biodiesel with co-product allocation .....	111
Table 16 SimaPro Input data sheet for the production of 1 kg biodiesel assuming a 70% induced increase in algae lipid yield .....	119
Table 17 SimaPro Input data sheet for the production of 1 kg biogas (baseline scenario) via Anaerobic Digestion (AD), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation .....	134

Table 18 Single score values of process contributions for the production of 1 kg microalgae biofuel. Excluding infrastructure processes/excluding long-term emissions, Note: N/A means not applicable.....	137
Table 19 SimaPro Input data sheet for the production of 1 kg biogas via Anaerobic Digestion (AD) of algae (with 100% recycling of production harvest water), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation .....	138
Table 20 Single score values of process contributions for the production of 1 kg biogas via AD of microalgae. Excluding infrastructure processes/excluding long-term emissions .....	140
Table 21 SimaPro Input data sheet for the production of 1 kg biogas via Anaerobic Digestion (AD) from algal biomass (using wastewater/sea water as culture media), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation .....	145
Table 22 SimaPro Input data sheet for the production of 1 kg biogas (with 70% induced lipid yield) via Anaerobic Digestion (AD), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation .....	151

## LIST OF EQUATIONS

Equation 1 .....	41
Equation 2 .....	42
Equation 3 .....	53
Equation 4 .....	53
Equation 5 .....	53
Equation 6 .....	53
Equation 7 .....	53



## **LIST OF ABBREVIATIONS**

AD – Anaerobic Digestion

ASTM – American Society for Testing Materials

BD – Biodiesel

BOD – Biological Oxygen Demand

CCS – Carbon Capture and Storage

CCM – Carbon Concentration Mechanism

Ci – Inorganic Carbon

CO<sub>2</sub> – Carbon dioxide

COD – Chemical Oxygen Demand

CH<sub>4</sub> – Methane

CH – Swiss

CHP – Combined Heat and Power

DNA – Deoxyribonucleic acid

DW – Dry Weight

DWB – Dry Weight Basis

FAME – Fatty Acid Methyl esters

Fe – Iron

FFA – free Fatty Acids

GHG – Green House Gas

GWP – Global Warming Potential

HHV – Higher Heating Value

HRAP – High Rate Algae Pond

HRT – Hydraulic Retention Time

LCA – Life Cycle Assessment

LCI – Life Cycle Inventory

LCIA – Life Cycle Impact Assessment

LEA – Lipid Extracted Algae

LC<sub>NER</sub> – Life Cycle Net Energy Ratio

MEA – Mono Ethanolamine

N – Nitrogen

NaCl – Sodium Chloride

N<sub>2</sub>O – Nitrous oxide

NEB – Net Energy Balance

NER – Net energy Ratio

NOX – Nitrogen oxides

OLR – Organic Loading Rate

P – Phosphorous

PBR – Photobioreactor

PFD – Photo flux Density

PUFA – Polyunsaturated Fatty Acids

RER - Europe

SD – Standard Deviation

SOX – Sulphur Oxides

TAG - Triglycerides

TFF – Tangential Flow Filtration

U – Unit Process

VFA – Volatile Fatty Acids

VS – Volatile Solids

WWT – Waste Water Treatment



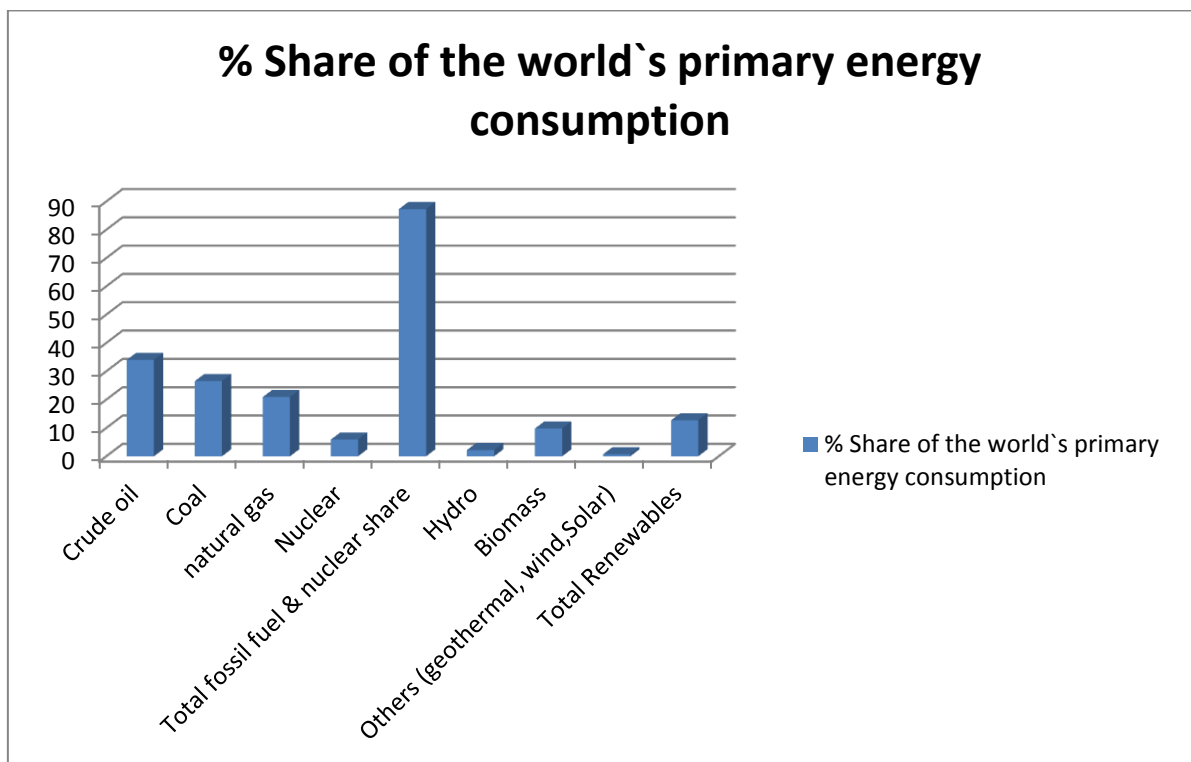


# CHAPTER ONE

## GENERAL INTRODUCTION

### 1.1 Introduction

Over 80% of the world's annual global primary energy consumption to date is accounted for by fossil fuel (Coal, Crude oil, and natural gas) (**Figure 1**), particularly in the areas of transportation, manufacturing and domestic heating (Gouveia and Oliveira, 2009; BP, 2009; IEA (International Energy Agency), 2013).



**Figure 1 Fuel shares of global energy consumption (IEA, 2013)**

However, rapid depletion of fossil fuels reserves coupled with concerns over anthropogenic carbon dioxide (CO<sub>2</sub>) equivalent emissions of greenhouse gases (GHG's), such as CO<sub>2</sub>, methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), arising from

fossil fuel combustion has driven the world towards renewable sources of energy (Brennan and Owende, 2010; Praetorius and Schumacher, 2009; Intergovernmental Panel on Climate Change - IPCC, 2007). Similarly, predictions by some industry sources and researchers (BP, 2009; Khan et al., 2009) that the world's fossil oil reserves would be exhausted in less than 50 years from now and frequent threats to the security of fossil fuel supply (Rogner, 2000) due to global political developments limit fossil fuel dependency. Conversely, others think that fossil fuels dominance will remain and in abundant supply well for a considerable period of time (Brennan and Owende, 2010). This is with regards to the huge technological advancement, potential reserves, and the discoveries of latest unconventional reserves of natural gas (IEA (International Energy Agency), 2003b).

These scenarios has led to increased research interest in a portfolio of climate change effects mitigation options such as: new renewable sources for energy and transportation fuels, the deployment of more efficient fossil-fuel combustion technologies, increasing the efficiency of energy conversion and utilisation, switching to low carbon content fuels and Carbon Capture and Storage (CCS) of CO<sub>2</sub> from fossil-fuel power plants (Herzog and Golomb, 2004; Suebsiri and Wilson, 2011; Korre et al., 2010). In addition, vigorous long-term research aimed at developing advanced and potentially viable model concept of utilising waste CO<sub>2</sub> from power plants as an economic advantage through processes that can profitably utilise captured CO<sub>2</sub> is necessary (Tom, 2011; Kumar et al., 2010).

Microalgae biofuel production is an emerging field that has attracted a lot of attention as a result of its potential for sequestering power plants waste CO<sub>2</sub> (with supplementary nutrients) into biomass at a higher photosynthetic rate (1.83 kg of CO<sub>2</sub> is required to produce 1kg of dry algal biomass) (Brennan and Owende, 2010; Kumar et al., 2010; Yusuf, 2007), far higher than that of dedicated energy food and oil crops. Microalgae can be cultivated all year round giving higher yields than the best oilseed crop (rapeseed). For example, microalgae biodiesel yields 12,000 L ha<sup>-1</sup> (open pond production) compared to

1,190 L ha<sup>-1</sup> for rapeseed (Schenk et al., 2008). Also, its capacity to utilise nutrients particularly Nitrogen (N) and Phosphorous (P) from a variety of wastewater, sets it apart from other biomass resources (Brennan and Owende, 2010). In addition, the biochemical configuration algal biomass could be readily modified via altering culture environment with significantly enhanced lipid synthesis (Yusuf, 2007; Courchesne et al., 2009).

Furthermore, microalgae are a major source of high value co-products (Spolaore et al., 2006) such as proteins (*beta-carotene*) extracted from *Dunaliella* and *Spirulina* and residual biomass, which are widely useful as animal feed stocks and a source of fertilizer. These outlined microalgal connected benefits for power plants waste CO<sub>2</sub> utilisation, bioremediation of wastewater and its potential for biofuel production justifies the need for extended research and development effort in microalgae biofuel technology (Brennan and Owende, 2010; Kumar et al., 2010; Courchesne et al., 2009).

However, in spite of its huge potentials, microalgae biomass cultivation process, incorporation of production system for power plants waste flue gas utilisation, gas transfer and mixing, wastewater utilisation options, algal harvesting and oil extraction, and biomass conversion techniques have many challenges which limit the development of microalgae biofuel technology. As a result, these processes require specific enquiry to allow for commercial full-scale production and viable utilisation of microalgal biomass for biofuel processing.

## 1.2 Aim and Objectives

This research project aims at developing a sustainable process model of producing biofuel from microalgae biomass utilising CO<sub>2</sub> from gas turbine power plant flue gas. The study intends to achieve this through analysing and developing sustainable model process route for biofuel production using microalgae biomass to sequester power plants waste CO<sub>2</sub>. The research project, unlike previous studies is distinctive in the sense that it integrates several processes of microalgal production, exploring prospects of alternative design of microalgae production systems aimed at sustainable microalgae biofuel production.

In more details, the objectives of this project are:

- To develop biofuel production process models and analyse the models to identify possible energy and material input savings alternatives for each process route.
- To evaluate the technical/environmental impact considerations of all energy inputs, materials inputs as well as energy output and environmental emissions for each developed alternative process models using Life Cycle Assessment (LCA) methodology.
- Finally, to carry out sensitivity analysis of the different considerations using Eco-indicator 99 methods in SimaPro Software to identify the most suitable process of microalgae biofuel production.

### 1.3 Thesis Structure

This thesis is structured into five (5) chapters. Following this general introduction is Chapter 2, which reviews relevant literatures from published sources in line with the set objectives. This includes the discussion of the different technological components associated with the utilization of microalgae biomass for biofuel production. Also, microalgae characterisation, cultivation techniques, light, CO<sub>2</sub> from gas turbine power plant waste flue gas and nutrient inputs requirements are examined. The review also examines microalgae harvesting and dewatering techniques, biomass conversion/biofuel process route options and the resultant biofuel products. And finally an evaluation of previous comparative LCA studies of microalgal based biofuel production systems. In chapter 3, the LCA research methodology adopted for this study is discussed and justified, stating the reason for the choice, and how the LCA has been carried out. It provides practical information about the backgrounds of the LCA methodology.

Chapter 4 is the data analysis component of this research. It includes the gathering of data from LCA databases and analysis of the full life cycle inventory for each unit process and the assessment and evaluation of the likely impacts for each process model using eco-indicator '99 method. Sensitivity analysis of the weights is determined using Monte Carlo function in SimaPro to establish the relative effects of the most important assumptions on the overall results. And also to establish if significant differences exist to fulfil the goal and scope of the LCA study. This is aimed at providing clearer understanding of the results, and justification as to the conditions for which a particular result or conclusion is valid. In Chapter 5, conclusions are made with recommendations for future research work based on the findings and limitations that are encountered in the process of carrying out this research study.



# CHAPTER TWO

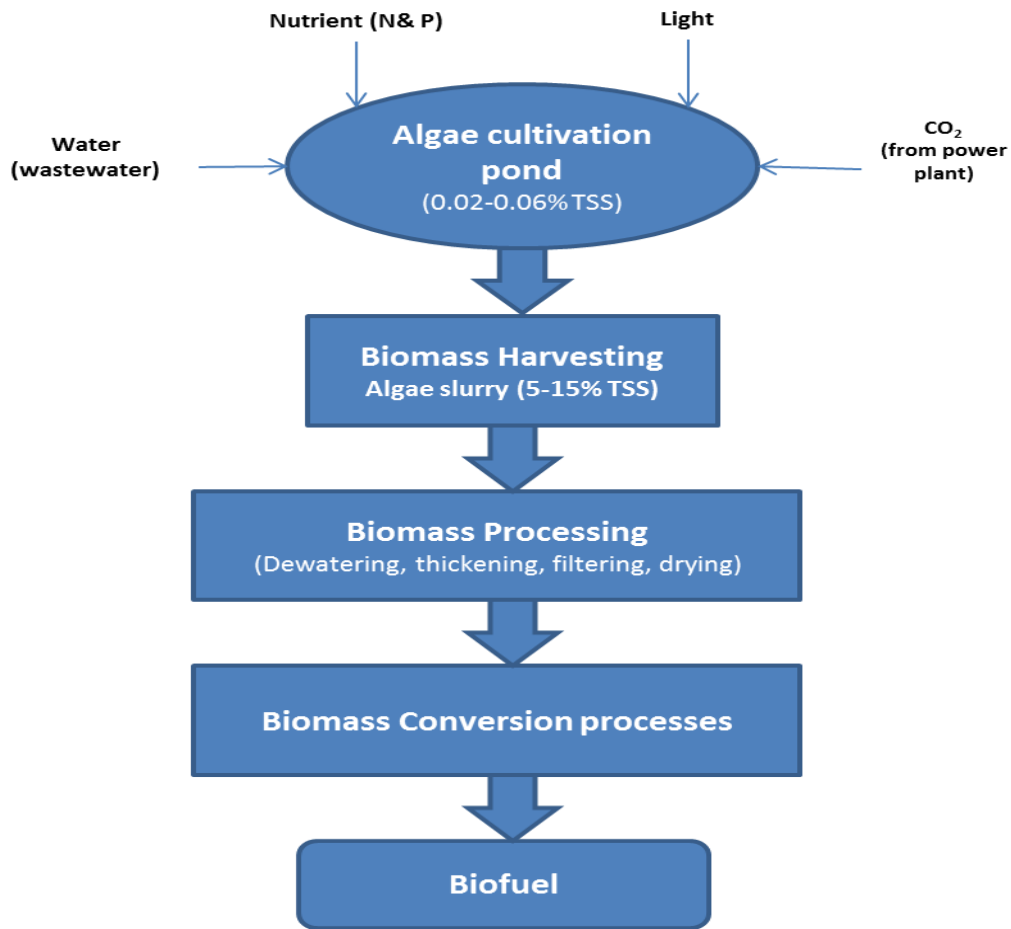
## LITERATURE REVIEW

### 2.1 Overview

Microalgae biofuel production technology is considered as a strategically important sustainable fuel derivable resource as it offers the possibility of limiting GHG emissions by reducing fossil fuel use. It is renewable and can be a source of biological sequestration of waste CO<sub>2</sub> and other GHG's emitted from fossil fuel power plants, and also yields a variety of liquid and gaseous fuels. Microalgae biofuel production would potentially offer a new window of opportunities as an alternative biofuel supply source (Mata et al., 2010). This is particularly, in comparison with conventional first generation biofuels which are predominantly produced from food crops and oil crops. The latter biofuel production technology is relatively well developed, but has come under heavy criticism with regards to its sustainability and potential contribution to climate change mitigation (Brennan and Owende, 2010). These concerns have propelled research interest in advancing the prospects of utilising non-food resources for biofuel production. Microalgae appear to be the most interesting option for biofuel production amongst the various possibilities sources being investigated at various stages of advancement.

However, microalgae biofuel production technology is still at early stages of development as it is not yet operationally cost effective to compete with fossil-derived fuels without government subsidies (Mata et al., 2010). Consequently, research is being intensified in both the academic circles and industry targeted at developing strategies to make all stages of the algae biofuel production value chain technically and economically viable.

Therefore, the literature review will focus on the existing status of microalgae biomass cultivation, with incorporation of the production system for power plants waste flue gas utilisation, gas transfer and mixing, wastewater utilisation strategy, algal harvesting, biomass oil extraction, and biomass conversion techniques into biofuels as illustrated in Figure 2.



**Figure 2 Generic Microalgae biofuel process chain (adapted from Mata et al., 2010)**

## 2.2 Types and classes of Microalgae

Microalgae are unicellular or simple multicellular photosynthetic microorganisms that can utilise solar energy in converting simple inorganic salts, nitrogen source and CO<sub>2</sub> for rapid cell growth only (Brennan and Owende, 2010; Khan et al., 2009). They can thrive in different environmental conditions such as marine, freshwater, deserts, hot springs and Antarctica, due to their unicellular or simple multicellular make-up (lacking roots, stems and leaves) (Mata et al., 2010). Microalgae cells could be prokaryotic or eukaryotic in composition (Brennan and Owende, 2010; Kumar et al., 2010).



Prokaryotic microalgae (cyanobacteria) are more like bacteria (Brennan and Owende, 2010) as they lack membrane structures (nuclei, mitochondria, plastids, flagella, and Golgi bodies) which regulate cellular functions. While, eukaryotic microalgae possess membrane organelles and consist of many different types of widespread algae, that are well researched and used for biofuel production (Guschina and Harwood, 2006).

Current knowledge of prokaryotic microalgae (cyanobacteria) is centred on their blooms-forming ability in aquatic systems, which is mainly attributed to global warming (Kahru et al., 2000). Similarly, most cyanobacteria species produce toxins, such as microcystins which are of grave concerns to human health due to their toxic nature (Sellner et al., 2003). Also, their relative inability to accumulate considerable amounts of desirable neutral lipids or Triacylglycerol (TAG's) has limited their use as feedstock for biofuel production (Qiang et al., 1997). As a result, there is limited information of their exploitation for biofuel production in current literatures.

The term microalgae for this research refer to eukaryotic cell algae. Eukaryotic microalgae are particularly important to biofuel production due to their unique ability to manipulate their biomass and lipid composition to amass a range of desirable energy yielding concentrated lipids TAG's in mass culture in response to nutrient limitation (Qiang et al., 1997; Richmond, 2004). The most often reported eukaryotic microalgae, noted for having desirable attributes for resourceful and economic combination of CO<sub>2</sub> utilisation, wastewater application and lipid synthesis for biodiesel processing are; green algae (*Chlorophyceae*) and diatoms (*Bacillariophyceae*) (Brennan and Owende, 2010; Kumar et al., 2010). A more detailed description of microalgae is presented by Richmond (2004).

### **2.2.1 Green algae (Chlorophyceae)**

The *Chlorophyceae* or green algae are a large group of organisms that exist in different forms, ranging from microscopic to macroscopic form. Their primary storage product is starch composed of amylose and amylopectin and TAGs formed within the chloroplast (Richmond, 2004). They occur primarily in freshwater, but also in marine terrestrial and sub-terrestrial settings. Some of the commercially exploited microscopic green algae of the *Chlorophyceae* class are *Chlorella sp.*, *Dunaliella sp.* and *Haematococcus sp.* (Richmond, 2004). According to Kojima and Zhang (1999), *Botryococcus braunii* was proposed and cultivated as a renewable source of liquid fuel owing to its remarkable ability to yield high desirable levels of liquid hydrocarbons called *botryococcenes* which can readily be processed into biofuel.

### **2.2.2 Diatoms (Bacillariophyceae)**

Diatoms are unicellular eukaryotic microalgae that are generally predominant in freshwater and marine environments, with the primary function of sustaining the marine food chain (d'Ippolito et al., 2004). Since the primary storage material in this class of microalgae is lipids, they are of potential value to the biodiesel and biotechnology industry for lipid production and specifically, polyunsaturated fatty acids (PUFA) (Richmond, 2004).

However, the commercial use of the cells of diatom is mainly related to aquacultural practices, since they are a source of significant amounts of PUFAs (Richmond, 2004). TAG's are predominantly the most common storage lipids constituting up to 80% of the total lipid fraction in eukaryotic algae (Brennan and Owende, 2010; Richmond, 2004).

## **2.3 Algae biochemical composition**

All algae primarily consist of proteins, carbohydrate, fats and nucleic acids (lipids) in varying proportions (Brown et al., 1997). Though, the fractional percentage of these constituents varies with the type of algae or microalgae.

However, a few microalgae have the unique ability to synthesize fatty acids in the form of TAGs which are considered as very useful feedstock for the production of biofuels, especially biodiesel (Khan et al., 2009).

The biochemical component of algae according to current understanding differs according to species based on genetic disparities which are sometimes attributed to culture conditions (Sergio et al., 2002; Renaud et al., 2002). It is also influenced by growth stage and nutrients or culture media composition (Renaud et al., 2002). Abiotic environmental factors such as light intensities and temperature are key considerations (Richmond, 2004).

In spite of the influence of these parameters, there seems to be a common similarity regarding fractions of the gross biochemical constituent of microalgae. The main organic constituents are protein (30-40% of total dry weight), followed by lipid (10-20% of total dry weight) in addition to carbohydrate (5-15% of total dry weight) (Brown et al., 1997). Studies by Brown et al., (1998), Thinh et al., (1999) corroborate the work of Brown et al., (1997) in this regard. Indeed, under optimal growth conditions, most microalgae cells, although with few exceptions tend to be similar to each other by the relative amounts of protein, lipids, and carbohydrate they store.

However, Qiang and Ben-Amotz in Richmond (2004) reported how *Chlorella* sp., *Botryococcus braunii*, and *Dunaliella salina*, all representatives of the green algae, show distinctive biochemical composition of, 30-50% proteins, 20-40% carbohydrate and 8-15% lipids under adjusted ecological conditions. These clearly demonstrate how environmental factors, mainly light, temperature, nutrient grade, CO<sub>2</sub> supply and pH, affects the productivity of microalgae cell biomass by influencing the cellular metabolic pathway.

## **2.4 Microalgae Growth (Nutritional) and Cultivation Methods**

During the past decades, there has been extensive interest and attempts to utilize microalgae on an industrial scale as a source of renewable energy, food, feed, lipids, vitamins, pigments, fertilizers, pharmaceuticals etc. (Brennan and

Owende, 2010; Richmond, 2004). This interest is due to the need for additional food supplies, growing environmental concerns, and depletion of natural and energy resources (Mata et al., 2010).

It is predictable that with the advancement of detailed culture and processing techniques, microalgae biotechnology can meet the high demands of food, energy and pharmaceutical industries (Mata et al., 2010; Richmond, 2004).

## **2.5 Microalgae Growth (Nutritional) modes**

The importance of mineral nutrients for microalgae (aquatic plant) growth and nutrition is credited to Justus von Liebig (1803-1873) (Richmond, 2004). Thus, the need to supply adequate mineral nutrient and other growth needs to microalgae has been known for a long time. Nevertheless, microalgae unlike terrestrial plants are adapted to scavenge their environment for resources, to store them, or increase their efficiency in supply utilization. Such resources are generally used for biomass development (consisting of 40-50%), which are dependent on adequate supply of carbon source (CO<sub>2</sub>) and light to enable photosynthesis (Mata et al., 2010; Richmond, 2004).

Consequently, algae may undertake many types of nutrition pattern and are equally capable of nutritional modification as a response to changes in environmental settings. Some of the nutritional or growth pattern exhibited by microalgae includes; (1) photoautotrophic, (2) heterotrophic, and (3) mixotrophic growth modes.

### **2.5.1 Photoautotrophic**

Phototrophic algae under normal growth conditions obtain their energy by absorbing sunlight, for the reduction of assimilated CO<sub>2</sub> from the air and nutrient (N & P) from their surrounding environment by photosynthesis (Mata et al., 2010). Consequently, the principal focus of most artificially deployed production

systems is aimed at replicating and improving these optimal natural growth conditions.

Photoautotrophic algae production comes with the benefit of utilizing sunlight, a free natural resource (Janssen et al., 2003). However, the commercial application of this production process may be constrained in regions with low solar radiation due to diurnal cycles and seasonal changes. Artificial lighting using fluorescent lamps which enables continuous cell division is employed as an alternative source of light, and this comes with additional cost and environmental burden (Muller-Feuga et al., 1998).

### **2.5.2 Heterotrophic**

In this form of nutrition, microalgae derive their material and energy needs from organic composites formed by other organisms (Brennan and Owende, 2010). Certain algae can be grown exclusively on organic substrates and this has become a viable option in conventional Photobioreactor (PBR) production systems for biomass and bio compounds produced by certain microalgae species under specific culture conditions (Richmond, 2004).

### **2.5.3 Mixotrophic**

This is a combination of autotrophic and heterotrophic mode of nutrition in the production system, a scenario requiring both organic compounds and CO<sub>2</sub> are essential growth elements. It implies carrying out photosynthesis as the core energy source, with CO<sub>2</sub> and organic substrates needed as supplements subject to the concentration of the culture medium and existing light intensity (Mata et al., 2010).

Photoautotrophic mode of production is the most ideally and economically viable technique for industrial scale production of microalgal biomass amongst the three distinct production method (Borowitzka, 1997). This is because it follows the natural growth processes of algae. Therefore, this study would be considering this mode of production for our culture system. However, the

appropriateness of each scenario is dictated by the strain of microalgae, nutrient concentration, CO<sub>2</sub>, pH, as well as climatic factors (Mata et al., 2010).

## **2.6 Microalgae Cultivation Methods**

A number of studies have been made to examine the techniques, procedures and methods of producing commercial quantities of microalgae biomass (Spolaore et al., 2006; Huesemann and Benemann, 2009). Open raceway ponds and closed PBR systems are the two most common microalgae cultivation techniques.

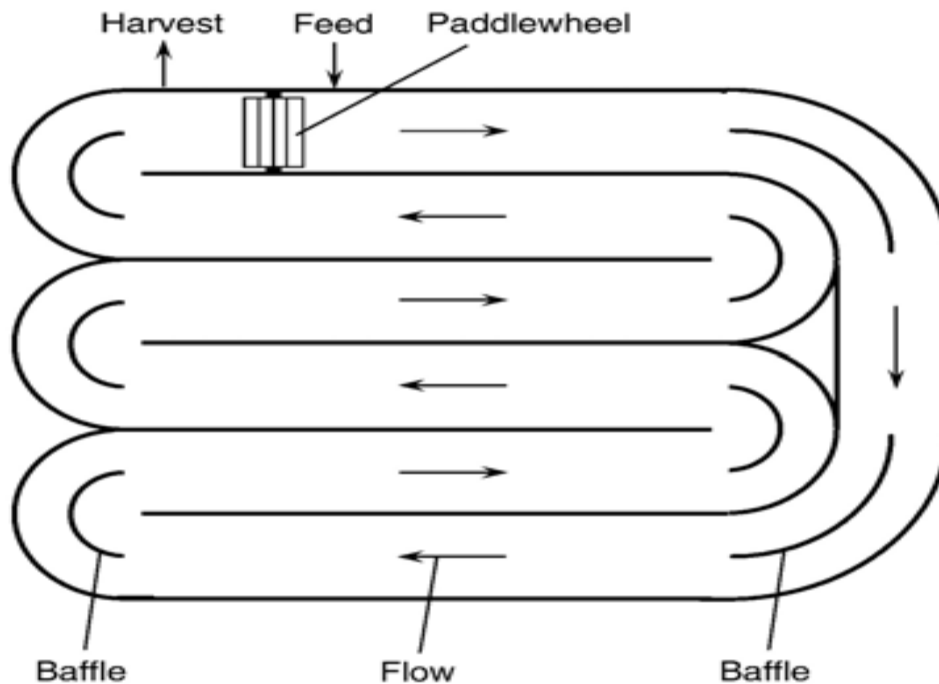
The open raceway ponds scenario is less favourable due to limitations in preventing contaminations, while the PBR provides a relatively easy system of controlling nutrient requirements for growth, as well as other cultivation factors such as temperature, dissolved CO<sub>2</sub>, and pH, and prevents contamination (Brennan and Owende, 2010; Mata et al., 2010; Knuckey et al., 2006). However, PBR come with a comparatively high initial cost, although they are precisely specific to the physiology of the algae strain being grown (Harun et al., 2010). Consequently, the choice of the production facility is of high priority for the techno-economic production of specific microalgae specie.

Therefore, in order to maximise microalgae biomass production for biofuel production at low cost, it is vital to understand the different techniques and methods of microalgae cultivation.

### **2.6.1 Open pond cultivation system**

The open pond production scenario of growing microalgae is quite common and had been used since the 1950s (Brennan and Owende, 2010). Such ponds could be natural waters (lakes, lagoons and ponds) or man-made ponds which come in different sizes, shapes and forms, each having certain advantages and drawbacks. Some of the artificial ponds currently used for research and industrial purposes include;

- Raceway ponds (see Figure 3)
- Shallow big ponds
- Circular ponds and
- Closed ponds



**Figure 3 Aerial view of a raceway pond (Y. Chisty 2007)**

The microalgae strain, the amount of light for photosynthesis and local climate in which the raceway is located are very critical factors in selecting the type of pond (Harun et al., 2010). Open pond systems are associated with large production capacity and are relatively less expensive to build and operate, and more durable than closed PBRs system (Brennan and Owende, 2010; Mata et al., 2010). Open pond systems offer a relatively cost-effective dimension to large scale microalgae production. According to Richmond (2004), pond systems of algae are constrained by several limitations such as:

- They cannot be operated at water levels lower than 15cm due to the likelihood of severe turbulence occurring afterwards,
- Excessive evaporative losses,

- A lack of temperature control which enables contamination and
- The requirement for more energy to homogenize nutrients.

Although, they may produce large quantities of microalgae biomass, they are thought to occupy more extensive land area (Mata et al., 2010). Moreover, since atmosphere only contains 0.03-0.06% CO<sub>2</sub>, it is expected that mass transfer challenges could slow down cell metabolism of microalgae. Light penetration into the pond could be an issue but this is dependent on biomass concentration in the pond. Consequently, biomass outputs in raceway pond production scenarios are less effective relative to closed system PBRs (Brennan and Owende, 2010).

Over the years, there have been several researches to assess the viability of growing microalgae using open pond system. After more than 50 years of repeated attempts, only a small amount of resistant microalgae species (*Dunaliella*-high salinity, *Spirulina*-high alkalinity, and *Chlorella*-high nutrition), can be cultured in open ponds due to tough growth medium culture environment in open systems (Harun et al., 2010; Huntley and Redalje, 2006). The secretion of carotenoids by *Dunaliella salina* is reported to offer it protection against the high saline condition, making it the most successful species grown in open pond system (Borowitzka, 1999).

### **2.6.2 Photobioreactor (PBR) cultivation system**

Algae production in closed PBR production systems is intended to overcome some of the major drawbacks linked with the open pond cultivation system. PBRs are scalable devices which can be optimized in line with the genetic, biological and physiological features of the microalgae species being cultivated, thereby enabling the cultivation of species that cannot be grown in open ponds (Mata et al., 2010). Hence, PBRs offer better control on most of the essential parameters of pH, temperature, nutrients and light relative to open pond cultivation system (Huntley and Redalje, 2006; Molina Grima et al., 1999).

In a PBR production system, a great proportion of the light does not impinge directly on the culture surface but has to cross the transparent reactor walls



(Richmond, 2004). Also, direct exchange of gases and potential contaminants (e.g. dust, microorganisms) between biomass culture and the atmosphere is limited or not allowed by the reactor wall (Huntley and Redalje, 2006).

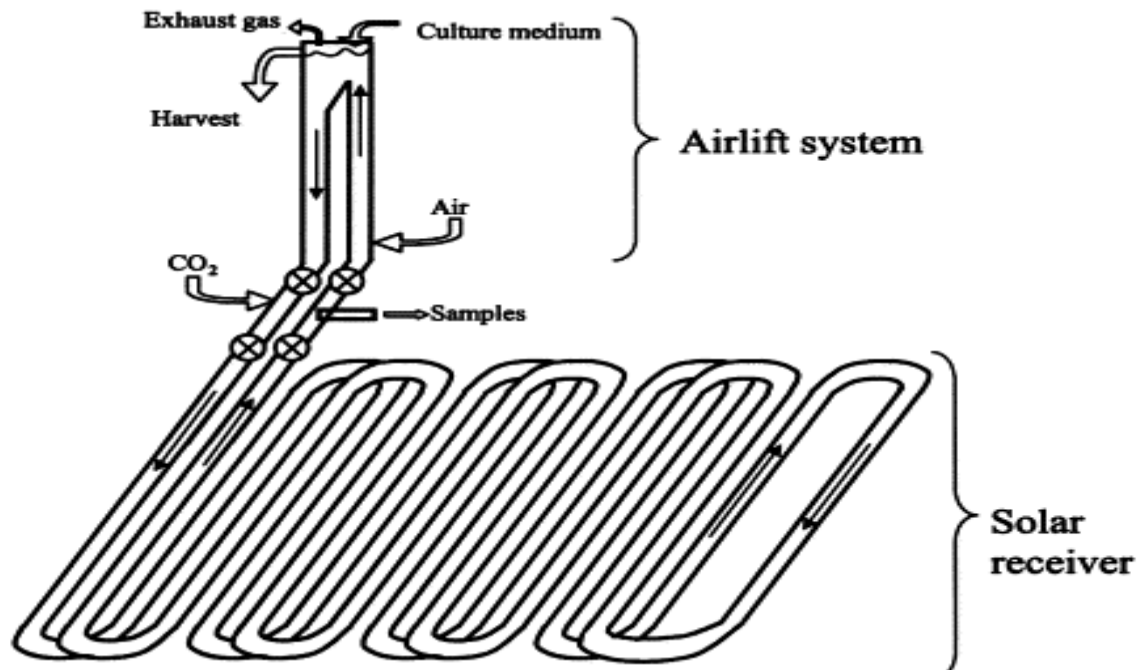
PBRs are considered to have several advantages over open ponds depending on their shape or design. Some of these advantages include:

1. Offer control over culture environment and growth parameters (pH, temperature, mixing, CO<sub>2</sub> and O<sub>2</sub>).
2. Check evaporation and reduces CO<sub>2</sub> losses.
3. Offer higher productivity by promoting higher biomass concentration.
4. Provides a safe culture environment by isolating contamination and invasion by competing microbes.

Despite these enhancements, PBRs suffer from several limitations that need to be considered and resolved. Some of these drawbacks as reported by Carvalho et al., (2006), include:

- High initial cost of building, operating and biomass production
- Overheating
- Fouling
- Oxygen accumulation
- Cell damage due to shear stress and deterioration of PBRs materials input.

PBRs can be classified on the basis of both design and their mode of operation. In design terms, they may come in tubular or plate design. Tubular PBRs are considered more suitable for outdoor cultivation in comparison with other PBRs as they offer large illumination surface area. In terms of operational mode, PBRs could be [1] Air or pumped mixed and [2] single or two-phased reactors – filled with media, with gas exchange taking place in separate gas exchanger and media with both gas and liquid present with continuous gas mass transfer taking place simultaneously in the reactor, respectively.



**Figure 4 the Photobioreactor (adopted from Molina et al., 2001)**

The tubing could be assembled in a straight line and coiled tubing formation as shown in Figure 4 depending on the design requirement of the system. Some of the common tubular reactors specifications are vertical, horizontal and inclined plane. The essential feature between the configurations is that the vertical design allows greater mass transfer and cut in energy utilization, while the horizontal reactor is more scalable, but entails a relatively larger area of land (Ugwu et al., 2008).

Quite a number of studies have examined the application of tubular PBRs for algae cultivation. In general, it has been established that the performance of the culture (in terms of biomass productivity) is critically dependent on attaining an optimal design specification which enables the required flow and gaseous exchange within the PBR system. Thus, the geometry of the PBR should be customized towards maximizing sunlight capture while also minimising the entire land area occupied (Molina et al., 2001).

### **2.6.3 Hybrid or coupled cultivation systems**

The Hybrid cultivation system is a two-stage production scenario that integrates the unique growth phases in the PBRs and the raceway pond production

system. The initial phase in the PBR favours continuous biomass cell division under controlled culture conditions which minimises contamination and competition from invasive microorganisms (Huntley and Redalje, 2006). The later or second stage which involves the relocation of the culture broth from the PBR into the raceway pond is intended at exposing the microalgae cells to nutrient deprivation, which stimulates as rapidly as possible the biosynthesis of desired energy yielding concentrated lipid products (Brennan and Owende, 2010; Richmond, 2004). It is believed that the change in culture setting induces a natural stress on the algae culture when they are transferred from the PBRs into the open pond.

Table 1 below makes a comparison between open ponds and PBR cultivation systems for several culture and growth parameters.

**Table 1 Comparison of microalgae production methods (adapted from Harun et al., 2010)**

Factor	Open ponds	Photobioreactor (PBR)
Space required	High	Low
Water loss	Very high	Low
CO <sub>2</sub> – loss	High, depending on pond depth	Low
Oxygen concentration	Low due to continuous spontaneous outgassing	Build-up occurred require gas exchange device
Temperature	Highly variable	Required cooling/heating
Shear	Low	High
Cleaning	None	Required due to wall growth and dirt
Contamination	High	Little
Evaporation	High	No evaporation
Biomass quality	Variable	Reproducible
Harvesting cost	High	Low
Automatic cooling system	None	Built in
Automatic heating system	None	Built in
Air pump	Built in	Built in
Energy requirement (W/h)	4000	1800

## 2.7 Factors affecting Microalgae Cultivation for biofuel production

Microalgae biomass cell growth rate and eventual biochemical composition are the two key characteristic factors which must be optimized for efficient microalgae biomass cultivation for biofuel production. It appears that these factors are governed by the algae specie (strain) and the growth culture medium (environmental factors). As, according to de Castro Araújo and Garcia (2005), microalgae growth and chemical composition are controlled by environmental factors of light, nutrients, temperature, and pH and in some instances salinity.

### 2.7.1 Impact of strain

In the perspective of this study, the following are key consideration in selecting the most appropriate microalgae strain for successful biofuel production. The strain should: (1) Be robust and have high lipid productivity; (2) Dominate intrusive strains in open pond facility and tolerant to a wide range of temperature; (3) Have limited nutrient requirements and high CO<sub>2</sub> utilization capacity; (4) Deliver high value biomass co-products and display auto-flocculation features.

Presently, no microalgae strain is able to deliver all these necessities or attributes (Brennan and Owende, 2010). Therefore, site specific adaptation is thought to be a useful consideration as it allows for the choice of microalgae specie that are conversant to the prevailing culture environmental conditions (Dismukes et al., 2008).

Similarly, Yoo et al., (2010) after comparing three microalgae species (*Chlorella vulgaris*, *Botryococcus braunii*, and *Scenedesmus sp.*) under high level CO<sub>2</sub> culture medium for biodiesel processing, demonstrated that functional characteristics is a key consideration in specie selection. Also, amongst most common microalgae (*Chlorella*, *Dunaliella*, *Porphyridium*, *Nannochloropsis*, *Isochrysis*, *Tetraselmis*, etc.), *Chlorella* appears to be a good candidate for biofuel production as it is very robust in wide-ranging applications (Mata et al.,

2010; Richmond, 2004), and has available quantitative and qualitative production data. However, there is need for further research in the isolation of local strains of microalgae for biofuel production.

### **2.7.2 Environmental factors**

Microalgae, just like most living things, respond to changes in their environment as a characteristic of living organisms. Changes in environmental factors, particularly light regime, temperature and nutrient grade and in some instance pH, not only affects photosynthesis and cell biomass, but likewise effects the arrangement, pathway and ultimate microalgae biochemical cell composition (Richmond, 2004).

### **2.7.3 Light regime**

Microalgae cells undergo energetic variations in cell composition, in response to alteration in light intensity and quality to enhance photosynthesis and ultimate biomass growth (Molina Grima et al., 1999; Jacob-Lopes et al., 2009). Photoacclimation – the role of incident light as an important growth factor is principally what modulates the effects of light on the biochemical constituent of photosynthetic microalgae (Richmond, 2004).

The mechanism of Photoacclimation process has been widely investigated in terms of photo flux density (PFD). Based on current understanding, low PFD has been linked with increased protein accumulation and greater extracellular polysaccharide synthesis at higher PFD (You and Barnett, 2004). In addition, the fatty acid (lipids) levels of cells are equally influenced with alteration of PFD.

But more recent studies by (Carvalho et al., 2009) reported evidence that some species could modify their photoacclimation preference away from conventional linear and independent behaviours in response to interactions between different factors. Since according to this report, irradiance and temperature effects were observed to play a combined role in tempering the biochemical composition of microalgae cells.

#### **2.7.4 Temperature**

Temperature is one of the most key environmental conditions controlling the biochemical composition of microalgae and is also extensively researched (Richmond, 2004; Thompson et al., 1992). A decrease in optimal temperature generally increases the extent of unsaturation of lipids in membrane system. Microalgae boost cellular membrane stability and fluidity through increased intensities of unsaturated fatty acids in tissue lipids at lower temperatures in order to protect their photosynthetic mechanisms from photo inhibition (Nishida and Murata, 1996).

Yet, other studies have reported that microalgae biochemical composition variations due to low and high temperatures vary from species to species (Renaud et al., 2002). High growth temperature has been attributed to increase in protein fraction and decrease in carbohydrate and lipids in some microalgae species. However, according to the reports (Thompson et al., 1992; Sánchez et al., 1995), there was no established regular trend in biochemical composition for all the over eight species of microalgae examined over temperatures (20, 25, and 30°C).

#### **2.7.5 Salinity**

Algae could either be described as halophilic (salt requirement for optimal growth) and or halotolerant (having response system that permits their survival in saline environment), based on their extent of tolerance and adaptability to different salinities (% sodium chloride NaCl (w/v)) (Rao et al., 2007). A few algae are capable of producing osmoregulatory metabolites (osmoticants) in response to an increase in salinity or osmotic pressure to protect their cells from salt injury (Richmond, 2004; Rao et al., 2007; de Castro Araújo and Garcia, 2005).

The fractional composition of carbohydrates, protein and lipids seem to be marginally affected by an extensive range of salinity for most algae types. However, Richmond (2004) observed that increased salinity could lead to slightly amplified fraction of total lipid yield in microalgae, as observed in

cultures of (*Dunaliella spp.* and *Monodus subterraneus*), with decrease in the proportion of unsaturated fatty acids of (*M. subterraneus* and *Nannochloropsis aculata*) in the same salinity condition. Similarly, Febregas et al., (1985) reported a decline in protein fraction with upsurge in salinity in the microalgae *Isochrysis galbana*, under 56 different nutrient/salinity concentrations.

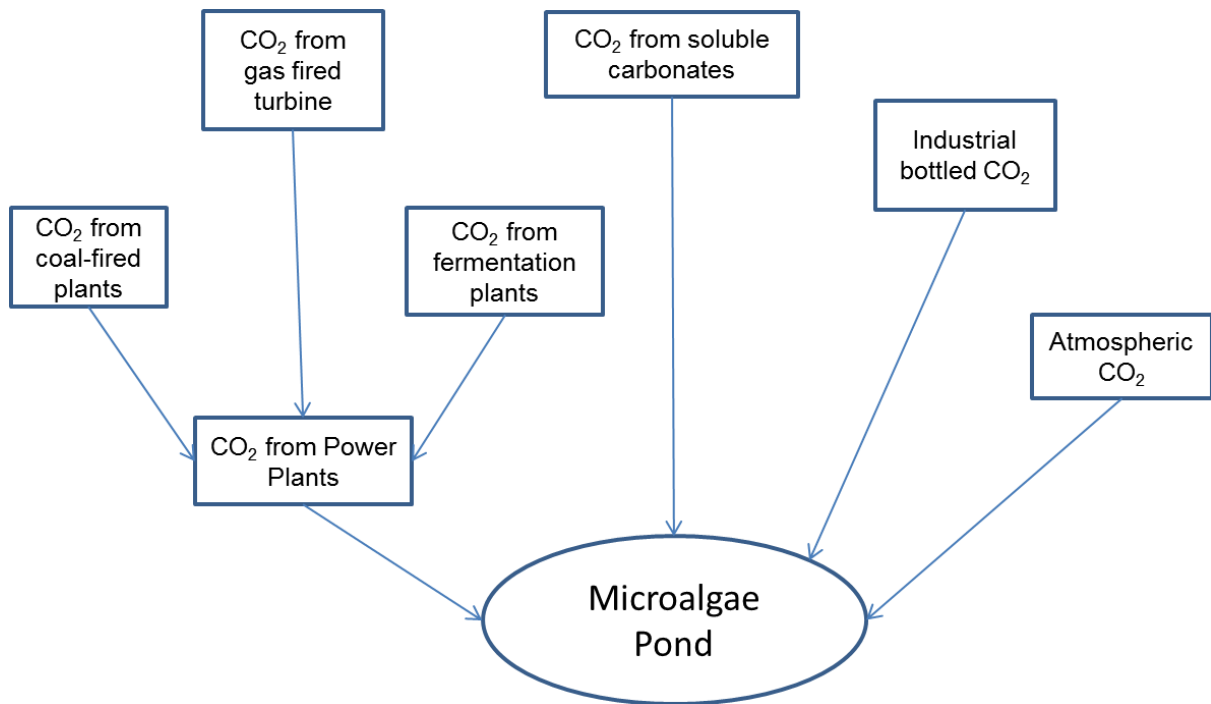
## **2.8 Nutrient requirements**

Apart from the effects of environmental factors of light, temperature and salinity, microalgae require nutrients to grow. The most essential nutrients are being carbon (CO<sub>2</sub>), nitrogen (N), and phosphorous (P) (Richmond, 2004). These nutrients can be supplied in the form of CO<sub>2</sub> from (atmosphere, bottled CO<sub>2</sub> and flue gas sources), with N and P sourced from agricultural fertilizers, and wastewater application (Moheimani and Borowitzka, 2007; Chisty, 2008).

### **2.8.1 Microalgae CO<sub>2</sub> requirement**

Microalgae like all other heterotrophs require CO<sub>2</sub> as a carbon source, for optimum growth. Approximately 1.6 to 1.8 kg of CO<sub>2</sub> is required to grow 1kg of algae biomass, based on the average chemical composition of microalgae (Khan et al., 2009; Patil et al., 2008). Microalgae can derive or be used to capture CO<sub>2</sub> from different sources (Wang et al., 2008), such as (See figure 5);

- (1) Atmospheric CO<sub>2</sub>
- (2) CO<sub>2</sub> from soluble carbonate salts (Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub>)
- (3) Industrial bottled CO<sub>2</sub> and
- (4) CO<sub>2</sub> emissions from industrial power plants



**Figure 5 Sources of CO<sub>2</sub> for microalgae growth**

Atmospheric CO<sub>2</sub> is the most basic means algae derive CO<sub>2</sub> through photosynthesis for cellular growth (Wang et al., 2008). However, atmospheric CO<sub>2</sub> is limited due to low CO<sub>2</sub> concentration in the air of approximately 360 ppm (0.03% - 0.06% CO<sub>2</sub>), all of the CO<sub>2</sub> in about 37,000 m<sup>3</sup> air is thus required to produce 1 tonne of dry algae. This makes it economically infeasible (Stepan et al., 2002).

However, certain algae such as *Chlamydomonas reinhardtii* and other aquatic photosynthetic organisms have developed CO<sub>2</sub> – concentration mechanisms (CCM) to detect changes in their environment, which enable them, adjust their metabolism and physiology, with rapid acclimation to Inorganic Carbon (Ci) supply to survive the usually large Ci concentration fluctuations (Spalding, 2009; Spalding et al., 2002).

According to Spalding, (2009), the activation of the CCM of *C. reinhardtii* and other algae species is only triggered when CO<sub>2</sub> supply is limited, leading to spontaneous changes in gene expression. This is believed to be controlled by transduction pathway not yet fully explored. Consequently, a lot of research is



directed at understanding the CCM and microalgae acclimation to limiting CO<sub>2</sub> with specific focus on two physiological states:

- Limiting CO<sub>2</sub> (typically air levels of CO<sub>2</sub>, 0.003%, or below; CCM induced) and
- Elevated CO<sub>2</sub> (typically 1–5% CO<sub>2</sub> in air; no CCM induced).

Apart from these well characterized two physiological states, Van et al., (2002) showed how *C. reinhardtii* grows in 5% CO<sub>2</sub>, dies in air levels of CO<sub>2</sub>, and yet grows as Wild-Type (WT) cells in limited conditions (less than 0.01% CO<sub>2</sub>).

The following reports (Wang et al., 2008; Spalding, 2009; Emma et al., 2000) demonstrated that certain microalgae (*C. reinhardtii*, *Nannochloris maculate*, and *Porphyridium cruentum*) are able to uptake CO<sub>2</sub> from soluble carbonates such as Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> for growth purposes. Emma et al., (2000) attributed high extracellular carboanhydrase activities as being responsible for the conversion of carbonate to free CO<sub>2</sub> to facilitate CO<sub>2</sub> assimilation. While according to (Spalding, 2009; Van et al., 2002), certain algae species utilize bicarbonate as a source of substrate for photosynthesis via active transport system.

This capability can be used to control evasive species as only a few numbers of microalgae can survive using soluble carbonate salts as CO<sub>2</sub> source due to the relative pH values (in the range of 9.0 to 11) of such culture media (Wang et al., 2008; Spalding, 2009). Therefore, this study would not consider soluble carbonate salts as an option for the supply of CO<sub>2</sub> to microalgae.

In comparison, CO<sub>2</sub> capture from waste flue gas emission from fossil fuel power plants showed better recovery due to greater CO<sub>2</sub> concentration of the range of 5-15% coupled with its adaptability for individual PBR and raceway pond systems of algae cultivation (Wang et al., 2008; Doucha et al., 2005). Microalgae offer the benefit of CO<sub>2</sub> bio-mitigation in this respect when compared with terrestrial plants, which typically absorb CO<sub>2</sub> from the atmosphere holding only 0.03% - 0.06% CO<sub>2</sub> (Wang et al., 2008). However, the presence of high levels of nitrogen oxides (NO<sub>x</sub>) and sulphur oxides (SO<sub>x</sub>) in raw flue gas (see

Table 2) could pose some problems as only a limited number of algae are tolerant to high doses of NO<sub>x</sub> and SO<sub>x</sub>.

**Table 2 Typical concentrations of emissions of flue gases from power generating plants using different types of fuel (Ramachandra et al., 2002)**

<b>Emissions</b>	<b>Natural gas</b>	<b>Fuel oil</b>	<b>Coal</b>
NO <sub>x</sub> (ppm)	25-160	100-600	150-1000
SO <sub>x</sub> (ppm)	≤0.5-20	200-2000	200-2000
CO <sub>2</sub> (%)	5-12	12-14	10-15
O <sub>2</sub> (%)	3-18	2-5	3-5
H <sub>2</sub> O (%)	8-19	9-12	7-10
N <sub>2</sub>	balance	balance	balance

Consequently, if flue gas is to be the prime CO<sub>2</sub> source, there is an obligation to choose viable algae species assuming there is little or no requirement for gas purification. Otherwise, there is a need to deploy appropriate flue gas pre—treatment procedures for optimum culture media (Wang et al., 2008; Ju-No et al., 2000). Furthermore, the choice of microalgae that is tolerant to high temperatures associated with flue gas feed from industrial gas turbine power plants (Wang et al., 2008).

### **2.8.2 Fertilizer requirement option**

It has become necessary to explore the use of cheaper and simpler sources of nutrient (e.g. agricultural fertilizers) for large-scale cultures of microalgae production to reduce the burden of the cost of production (Schenk et al., 2008; Simental and Sánchez-Saavedra, 2003). A few studies have considered the use of certain formulated agricultural fertilizers as cheap nutrient source for algae culture, particularly common in Aquacultural practices.

Park et al., (2011) proposed the use of fertilizer in a commercial hypothetical High Rate Algae Pond (HRAP) to avoid nutrient limitation for algae growth,

assuming typical algae composition of (C<sub>106</sub>H<sub>181</sub>O<sub>45</sub>N<sub>16</sub>P), a fertilizer with N:P formulation of 16N:P (i.e. 7.3gN:1gP) would essentially be required.

Although the use of agricultural fertilizer as a nutrient source for microalgae production is not conventional (Simental and Sánchez-Saavedra, 2003), none the less, it cost less than 1/8 of the cost of providing nutrient in a standard conventional medium. However, there is no remarkable difference in terms of the growth rate and biomass concentration between the microalgae (diatoms) cultured with agricultural fertilizers compared with the control standard medium.

Furthermore, using cheap agricultural grade fertilizers (e.g. Urea 46) may be economically advantageous but the source of heavy metal contamination that can limit the growth of sensitive algae strains while posing considerable burden on the net energy balance (NEB) and sustainability of the entire production process, factoring in the energetic costs of fertilizer production (Schenk et al., 2008).

### **2.8.3 Wastewater application option**

Microalgae water requirement is estimated to be as high as 11-13 million L/ha/year for open pond production system (Chinnasamy et al., 2010). Therefore, the potential of algae to grow in industrial, municipal and agricultural wastewater would not only allow the minimization of the use of freshwater but also provide treated water for other applications while at the same time proffering a cost-effective and sustainable means of microalgal cultivation for biofuel (Chinnasamy et al., 2010; Pittman et al., 2011; Li et al., 2008).

Oswald and Golueke (1960) were the first to propose using wastewater application for large-scale production of microalgae for biofuel (Park et al., 2011). Consequently, a number of research have been piloted to explore the potentials of using microalgae for low-cost and environmentally friendly wastewater treatment, particularly for the exploitation of N and P from wastewater effluents as nutrients for algae biomass growth (Aslan and Kapdan, 2006; Martínez et al., 2000).

A review of studies of utilization of wastewater nutrient resources for cost effective biofuel production have reported high biomass yields and in some cases high lipid outputs. However, most of the reports were from small scale laboratory-based experimental studies (Pittman et al., 2011). Furthermore, the efficient utilisation of wastewater for algae cultivation and growth depends on a variety of constraining parameters that includes:

(1) High concentration of nutrients in wastewaters, such as N and P and high pH values which can restrain microalgae growth (Pittman et al., 2011; Zimmo et al., 2003). With typical total N and P concentrations found to be within these ranges, 10-100mg/L in municipal wastewater and >1000mg/L in agricultural effluent wastewater (Pittman et al., 2011).

(2) Presence of toxic heavy metals such as cadmium, mercury, zinc, or organic chemicals that require special and expensive chemical treatments to remove during wastewater treatment (Gasperi et al., 2008; Perales-Vela et al., 2006).

(3) Prevalence of pathogenic bacteria and predatory zooplankton which may cause contamination or out-compete the microalgae for essential nutrients (Richmond, 2004; Park et al., 2011).

Apart from these basic factors, in open system, it is difficult to control algae species that would dominate the culture, frustrating the target of maintaining stable product quality. Harvesting is very costly as centrifugation of wastewater is prohibitive, with filtration being impracticable leaving flocculation flotation as the preference method. Similarly, microalgae have different nutritional requirements and different harvesting conditions which are all of considerable concern to biofuel production (Richmond, 2004; Pittman et al., 2011; Park et al., 2011).

## **2.9 Biotechnological approaches to control cell composition**

Control of environmental conditions by PBR cultivation systems and through the use of hybrid (multistage) coupled cultivation system, have been shown to readily optimize microalgae cell composition (Brennan and Owende, 2010; Yusuf, 2007; Richmond, 2004; Carvalho et al., 2006; Pulz, 2001; Huang et al., 2010). Light regime which average single cell in the culture medium is exposed to (in PBR) has been identified as the most critical factor, as photoacclimation of microalgae to specific light intensity often results in changes in biochemical composition (Richmond, 2004; Jacob-Lopes et al., 2009; Carvalho et al., 2009).

Amongst the operational factors, cell concentration (population density) is the most effective biological factor affecting microalgae biochemical composition (Zou et al., 2000). Similar findings were previously reported by Qiang et al., (1997), which demonstrates how control over cell concentration of the culture would essentially allow other parameters to function at their optimum.

However, the aim of inducing the synthesis of high content of specific product by factoring in salinity, nutrient limitation, and temperature variations to the microalgae culture may not only reduce the overall biomass productivity but also affect the potential stability of the culture (Courchesne et al., 2009; Richmond, 2004).

Therefore, the concept of the hybrid or multistage coupled strategy ensures biomass productivity under controlled conditions in one stage and maximum induction and accumulation of desired energy yielding concentrated products in the other stage (Brennan and Owende, 2010; Huntley and Redalje, 2006; Rodolfi et al., 2009). It is upon such practical basis that biotechnological (genetic and metabolic engineering) advances are being developed as an integral and active path for industrial microalgae based biofuel production (Yusuf, 2007).

### 2.9.1 Biochemical (metabolic) engineering approach

These are strategies (nutrient-limiting) aimed at increasing lipid accumulation in microalgae through altered metabolism to induce lipid synthesis (Courchesne et al., 2009). N is the most commonly reported nutrient limiting factor, although there have been reports of P and Fe deficiency being able to cause cell growth respond to accumulation of lipids/fatty acids. Metabolic engineering permits the control of microalgal cellular mechanism through the alteration of gene or mutagenesis to induce desired fluxes in metabolism (Huang et al., 2010).

Interestingly, N limitation has been reported to enhance the accumulation of astaxanthin (the oxidant pigment) in green algae *Haematococcus pluvialis* (Boussiba, 2000). Similarly, the green algae *Chlorella* sp. (*C. emersonii*, *C. minuteissima*, *C. vulgaris*, and *C. pyrenoidosa*) whose main storage product is starch, were reported to accumulate lipids of up to 63%, 57%, 40% and 23% respectively on dry weight (DW) basis (Illman et al., 2000), under low-N culture medium. Both of these instances show algae cell adaptive response to ensure their survival during periods of nutrient stress, with astaxanthin offering protection against invasion by indigenous oxygen microbes while lipids serves as energy storage (Boussiba, 2000).

Phosphate limitation has also been recently reported to trigger high lipid biosynthesis in *Monodus subterraneus* by decreasing P concentration to 52.5, 17.5 and 0 $\mu$ M ( $K_2HPO_4$ ) from 175  $\mu$ M. This remarkably increase in fractional cell lipid content was observed due to the absence of phosphate (Courchesne et al., 2009). According to the report, total phospholipids dropped from 8.3% to 1.4% of total lipids due to the absence of P, with complimentary TAG increase from 6.5% up to 39.3% of total fractional cell lipids. In addition Fe deficiency according to Liu et al., (2008) in a similar scenario, stimulated 56.6% lipid accumulation in *Chlorella vulgaris* on DW basis of biomass under optimal culture conditions.

Other stress conditions such as salinity (Takagi et al., 2006) have been shown to increase TAG lipids accumulation from 60% to 67-70% in marine microalgae *Dunaliella*, under high salinity culture conditions. Therefore, the understanding

of the mechanisms of controlling fatty acids and lipids simulation in microalgae cells for storage of polyunsaturated fatty acids (PUFA) is of particular industrial importance in biodiesel production from TAGs (Rosenberg et al., 2008).

### **2.9.2 Genetic engineering approach**

Although much of earlier efforts of algae production have been devoted on cultivation techniques and species selection, innovative microalgae applications are presently being accomplished with the help of genetic engineering (Rosenberg et al., 2008). Genetic engineering of microalgae cells allows the isolation and exploitation of key genes of particular relevance for genetic transformation (Courchesne et al., 2009). It involves the successful incorporation of a DNA fragment (gene) into a temporary permeable nucleus or chloroplast organelle of microalgal cell (Rosenberg et al., 2008).

Quite a lot of classical techniques are currently available for genetic transformation of microalgae. With cell-cell mixture in acid washed glass bead and DNA molecules in polyethylene glycol being brought to collision at velocities sufficient enough to perforate the cell membrane and generate an incorporated nuclear transformant (genome) as the commonest method (Kindle, 1990). This technique is only applicable to microalgae that lack definite cell wall, either naturally or as a consequence of enzymatic dilapidation (Rosenberg et al., 2008). Other options include the use of micron-long silicon carbide whiskers in place of glass beads to perforate cell walls (Dunahay, 1993; León-Bañares et al., 2004). Also, the use of electric current (electroporation) to achieve temporal permeabilization of cell membrane has been reported to be more successful with cell wall deficient microalgae (Sun et al., 2005; Shimogawara et al., 1998).

Similar, effective transformations have also been achieved by propelling micro-particles of DNA – coated gold or tungsten at microalgal cells (Coll, 2006). However, due to the impact and forceful nature of the bombardment, most cells die as a result of cell membrane rupture (Rosenberg et al., 2008; Coll, 2006). Consequently, a less aggressive approach involving the utilization of the

microbe *Agrobacterium tumefaciens* which induces tumours in plants, and proves to be a suitable biological vector that allows the isolation of useful gene constructs that can be initiated into microalgae (Kumar et al., 2004).

The use of genetically engineered (transgenic) microalgae for industrial application has advanced significantly over the past decades (Rosenberg et al., 2008). Although, complete genetic transformation has only been achieved in a few green algal species (*Chlamydomonas*, *Volvox*, *Chlorella*, *Dunaliella* and *Haematococcus*) (Rosenberg et al., 2008; Walker et al., 2005), the number of sequenced plastid, mitochondrial and nucleomorph genome continues to grow. Courchesne et al., (2009) reported complete genome sequence of the red alga *Cyanidioschyzon merolae*, the diatoms *Thalassiosira pseudonana*, *Phaeodactylum tricornutum* and the unicellular green microalgae *Ostreococcus tauri*.

However, maximum yields of eukaryotic microalgae transformant obtained are 10-100 folds lower than figures obtained in genetically engineered animal cells (Coll, 2006). Also, there has not been any successful report of lipid overproduction of microalgae using genetic engineering approach to date, although extensive research are on-going in different species aimed at enhancing lipids synthesis using genetic engineering techniques (Courchesne et al., 2009).

Nevertheless, the ability to select desired traits and improving the stability of transformed cells is hinged on improving traditional genetic transformation methods as well as developing new techniques.



## 2.10 Microalgae harvesting and biomass concentration processes

Harvesting of microalgae biomass which usually entails one or more solid-liquid separation methods is demanding and accounts for 20-30% of the total cost of biomass production (Brennan and Owende, 2010; Mata et al., 2010; Molina Grima et al., 2003). Biomass recovery is significantly challenged by; low cell density, typically  $0.3 - 5.0\text{gl}^{-1}$  ( $< 0.5\text{kgm}^{-3}$ ) dry biomass in some commercial production systems, and small size of microalgae cells (2-40  $\mu\text{m}$  diameter) (Brennan and Owende, 2010; Wang et al., 2008; Molina Grima et al., 2003). Consequently, the harvesting process may involve one or more physical, chemical, or biological methods in achieving the desired solid-liquid separation (Mata et al., 2010), as there is no collective harvesting method at the moment.

Some of the most common harvesting processes, which are usually energy intensive, consist of flocculation, filtration, flotation and sedimentation using centrifuges (Brennan and Owende, 2010; Mata et al., 2010).

## 2.11 Harvesting methods

The choice of harvesting method is dependent on the size of the microalgae cells, cell density and the value of the target product (Richmond, 2004; Molina Grima et al., 2003; Olaizola, 2003). Yet biomass recovery is commonly a two stage process (Brennan and Owende, 2010; Wang et al., 2008), viz:

- Bulk harvesting – which is dependent on the initial cell density of the culture broth and the use of biomass concentration techniques like, flocculation, flotation and centrifugal sedimentation.
- Biomass concentration (thickening) – entails procedures such as centrifugation, filtration and the use of ultrasound for aggregation aimed at concentrating the biomass slurry.

However, the choice of harvesting technology is crucial as a high volume moisture in the processed biomass can significantly affect the economics of

downstream product recovery processes (Brennan and Owende, 2010; Molina Grima et al., 2003).

### **2.11.1 Flocculation**

Flocculation is the use of flocculants to amass or aggregate cells of microalgae biomass to increase the effective particle size, by coalescence of finely divided suspended cells into large loosely attached conglomerates, which slowly sink to the bottom of the culture medium and significantly enhances the ease of further processing (Molina Grima et al., 2003). Aggregation is enhanced through a process called bridging, by the addition of chemical, polymer, or organic/biological based flocculants. A mechanism aimed at reducing the negative charge on microalgal cellular surface, which hinders normal cell aggregation in suspension (Harun et al., 2010; Wang et al., 2008), without affecting the composition and toxicity of the biomass products.

Some of the flocculants commonly used are multivalent metal salts such as (ferric chloride ( $\text{FeCl}_3$ ), aluminium sulphate ( $\text{Al}(\text{SO}_4)_3$ ) and ferric sulphate ( $\text{Fe}(\text{SO}_4)_3$ )), and cationic polymers (Wang et al., 2008; Molina Grima et al., 2003). Their efficiency is usually measured by the concentration required to induce rapid coagulation. Alum has been reported as an effective flocculants for *Scenedesmus* and *Chlorella*, even though the use of metal salts may be deplorable for biomass recovery of high-value products, such as for food and Aquacultural applications (Mata et al., 2010; Molina Grima et al., 2003).

Recently, Knuckey et al., (2006), demonstrated another mechanism of flocculation, by adjusting the pH of the microalgae growth medium within the ranges of 10 and 10.6 using NaOH and subsequent addition of non-ionic polymer Magnafloc LT-25 to neutralise the negative charges on cell surface, at a finishing concentration of  $0.5\text{mg l}^{-1}$ . A resultant flocculate biomass concentration of  $6\text{-}7\text{ g l}^{-1}$  was attained upon draining off surface water after settling phase and subsequent neutralization. This process is widely reported as being successfully applied for harvesting a range of microalgae species with

efficiencies > 80% (Brennan and Owende, 2010; Wang et al., 2008). Likewise, it has been shown that flocculation could be achieved using a bio-flocculant Chitosan (a polymer of acetylglucosamine) within pH range of 4 to 9, recording maximum flocculation at pH 7.0 for freshwater species, and lesser for marine species (Brennan and Owende, 2010; Harun et al., 2010). However, its flocculation power is reduced in salt water (Danquah et al., 2009).

Auto flocculation which is induced by the effect of the modification of culture medium (e.g. interruption of CO<sub>2</sub> supply), causing algae to flocculate on its own has been investigated for both fresh and marine culture systems (Harun et al., 2010; Molina Grima et al., 2003). In addition, Oh et al., (2001) showed how the non-microalgal microbe *Paenibacillus sp. AM49* can be used to effectively (83% efficiency) harvest *C. vulgaris* from large-scale cultures.

Similarly, there has been reports (Brennan and Owende, 2010) of using ultrasound to acoustically induce flocculation (92% aggregation efficiency), followed by enhanced sedimentation, with a flocculate 20 times the concentration of the original biomass. The potential benefit of this technique, are that it occupies minimal space, can continuously be accomplished without prompting shear stress on the biomass, which could destroy high-value metabolites and it is a non-fouling technique.

### **2.11.2 Flotation**

Flotation harvesting method, unlike flocculation does not involve the addition of any chemical, as it is based on trapping microalgal cells by dispersed micro-air bubbles (Brennan and Owende, 2010; Wang et al., 2008), thereby ensuing in a very clean slurry. However, large scale flotation engineering comes with a lot of challenge coupled with very limited data of its techno-economic feasibility (Brennan and Owende, 2010).

### **2.11.3 Filtration**

Biomass recovery by filtration methods (pressure filtration, vacuum filtration, dead end filtration, microfiltration, ultra filtration, and tangential flow filtration (TFF)), is the most competitive compared to other harvesting process options (Harun et al., 2010). Filtration harvesting, which involves running the microalgae broth continually through the filter medium operating under pressure or vacuum, is suitable for harvesting relatively large filamentous microalgae ( $> 70\mu\text{m}$ ) such as *Coelastrum proboscideum* and *Spirulina platensis* as it cannot be used to recover algae with smaller dimension ( $< 30\mu\text{m}$ ) such as *Scenedesmus*, *Dunaliella* or *Chlorella* (Brennan and Owende, 2010; Mata et al., 2010; Molina Grima et al., 2003).

Consequently, membrane microfiltration and ultra-filtration have been demonstrated to be technically viable alternative to conventional filtration, for the recovery of smaller microalgal cells ( $< 30\mu\text{m}$ ) (Wang et al., 2008; Molina Grima et al., 2003). But due to high-cost consideration factor and constant membrane replacement, modern large-scale microalgal biomass production facilities do not commonly use membrane filtration process units' option (Harun et al., 2010; Wang et al., 2008).

However, Danquah et al., (2009) examined data on the concentration factor and energy consumption of specific filtration units, showing tangential flow filtration (TFF) and pressure filtration as energy efficient biomass recovery methods. These are thought to consume adequate amount of energy, when the output and initial concentration bulk of the feedstock are considered.

### **2.11.4 Centrifugal sedimentation**

Centrifugal sedimentation which involves the application of centripetal acceleration to separate the algae broth into layers of greater density, is preferred for the recovery of high-value products (Brennan and Owende, 2010; Wang et al., 2008). This is because it can process large volumes relatively rapidly. Hence, it is highly efficient, depending on the settling characteristics of

the cells, slurry residence time, and the settling depth in the centrifuge (Richmond, 2004; Molina Grima et al., 2003).

Harun et al., (2010), reported 88-100% cell viability and harvesting efficiency of around 95-100% using centrifugation, however, the process is highly energy intensive and it is thought to have a potentially high maintenance requirement due to its mechanical parts (Brennan and Owende, 2010).

## **2.12 Biomass drying processes**

Biomass drying processes, which is usually a combination of mechanical and thermal separation techniques such as (sun drying, spray drying, drum drying and freeze-drying), is commonly aimed at extending the shelf-life of the biomass by reducing the water content for the formulation of food, feed (fisheries) and biofuels, especially if biomass is the final products (Brennan and Owende, 2010; Molina Grima et al., 2003). This is because most harvested commercial biomass slurry is usually dilute (5-15 % i.e.  $< 0.5 \text{ kgm}^{-3}$  of dry biomass) and can decline in value in few hours in hot climate due to biochemical, chemical and microbial deterioration (Brennan and Owende, 2010; Jacob Lopes et al., 2007).

Sun drying is considered to be the cheapest drying technique that has been employed for microalgal biomass processing but not a very effective option due to long drying period because of the high water content of algal biomass, a condition for vast drying surface area and the likelihood of material loss (Brennan and Owende, 2010; Mata et al., 2010).

Spray drying with drum drying is the choice option for recovery of high worth products ( $\beta$ -carotene, polysaccharides), however it is comparatively expensive for producing low cost commodities (feeds, food and biofuels) (Brennan and Owende, 2010; Mata et al., 2010; Molina Grima et al., 2003). Freeze drying is thought to facilitate the recovery of intracellular elements such as lipids and oil which are challenging to extract from wet biomass without cell disruption using solvents, nevertheless it is also too expensive for large-scale industrial recovery of microalgal products (Brennan and Owende, 2010; Molina Grima et al., 2003).

Grima et al., (1994), demonstrated lipid extraction directly from freeze-dried biomass of *Isochrysis galbana*. However, biomass products may be susceptible to adverse colour/quality deterioration, especially of carotenoids and chlorophyll due to exposure to high temperature during thermal processing (Olaizola, 2003). Consequently, the choice of postharvest drying process is considered to depend strongly on the desired biomass product (Mata et al., 2010; Molina Grima et al., 2003).

### **2.12.1 Biomass extraction and purification processes**

The efficiency of the drying process and cost-effectiveness of extracting biofuels from microalgal biomass are important considerations that need to be determined in order to maximise the energy output of the resultants biofuels (Harun et al., 2010; Li et al., 2008). As it has been shown, that temperature affects the lipid yield as well as lipid composition of algal biomass during lipid extraction (Brennan and Owende, 2010; Widjaja et al., 2009). An example of this scenario is the report by Widjaja et al., (2009), which indicates that drying at 60°C showed only slight decrease in lipid yield while retaining a high concentration of TAG (but at 80°C or higher temperature) results in significantly reduced yields.

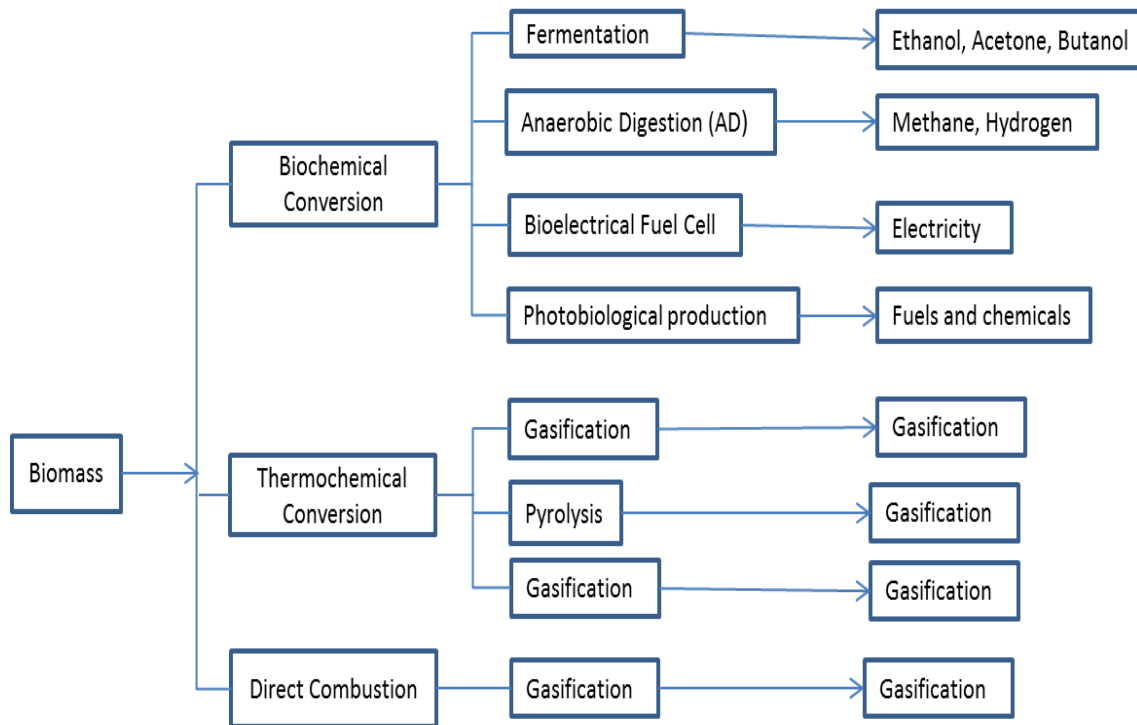
Wet extraction process, a combination of ultrasound and electromagnetic pulse induction has recently been developed as an alternative extraction method by a Los Angeles based biofuel company (OriginOil) (Brennan and Owende, 2010). Other metabolites extraction and purification schemes include the use of solvents which enhances the drift of globules towards the outside of the cell due to alterations of the cell membranes have been used to extract metabolites such as astaxanthin,  $\beta$ -carotene and fatty acids from algal biomass (Molina Grima et al., 2003). However, cell membrane properties define the effectiveness of this process, as presence of cell wall may impede the efficacy of solvent extraction (Wijffels and Barbosa, 2010).

Usually, most cell disruption techniques applicable to microalgae are modified from applications developed for use on intracellular non-photosynthetic microbes as a prerequisite for recovering desirable intracellular product from microalgal biomass (Brennan and Owende, 2010). Autoclaving, high-pressure homogenisers and the use of HCl, NaOH, or alkaline lysing of cell wall are methods that have been used successfully (Molina Grima et al., 2003).

### **2.13 Microalgae biofuel energy production technologies**

Microalgae biochemical products, such as pigments, antioxidants,  $\beta$ -carotenes, polysaccharides, TAGs, fatty acids, vitamins and biomass may be extracted as source of bulk raw material for various industrial applications (e.g. pharmaceuticals, cosmetics, nutraceuticals, foods and biofuels) depending on the algae species (Brennan and Owende, 2010; Mata et al., 2010). This section is set to examine technically viable microalgae based (thermochemical and biochemical conversion processes) biofuel energy conversion technology, in line with the focus of this study.

Consequently, the different conversion process options considered are based on different existing biomass-to-energy conversion processes (see Figure 6) used for terrestrial biomass, which depends largely on the type and sources of biomass, specific conversion technology and the target end use products (Brennan and Owende, 2010; McKendry, 2002a).



**Figure 6 Energy Conversion Processes from Biomass (Tsukahara and Sawayama, 2005)**

### **2.13.1 Anaerobic Digestion (AD) conversion processes**

Anaerobic Digestion (AD) and alcoholic fermentation are the two main bioconversion technologies of converting biomass into bio-energy carriers (McKendry, 2002a), with photobiological hydrogen production being a less commonly used process (Brennan and Owende, 2010). These conversion technologies are based on microbial and enzymatic processes, coupled with chemical hydrolysis for the conversion of starch and cellulosic components of the biomass fraction into alcohol and other solvents (biofuels) of interest (Naik et al., 2010).

Anaerobic digestion (AD) transformation of biomass has been applied and demonstrated to be commercially successful in the conversion of organic biomass directly into biogas, a mixture of (CH<sub>4</sub> (60-70%) and CO<sub>2</sub> (30-40%)) in several situations for a range of feedstocks such as organic wastes and organic biomass (Brennan and Owende, 2010; McKendry, 2002a). It entails the conversion of the carbon constituent in organic biomass by subsequent



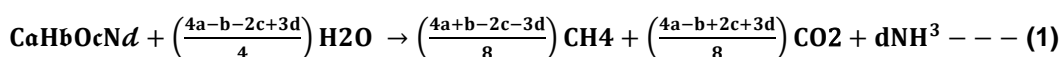
oxidation and reduction to its most oxidized state (CO<sub>2</sub>), and its most reduced state (CH<sub>4</sub>) respectively under microorganisms induced catalysis in the absence of oxygen (Cantrell et al., 2008). AD can be useful for conversion of wet microalgal biomass (Brennan and Owende, 2010), as it is thought to be particularly suitable for high moisture content (80-90% moisture) organic waste/wet biomass materials (McKendry, 2002a).

The three main unit operations of AD process technology according to existing literatures (Brennan and Owende, 2010; Naik et al., 2010; Cantrell et al., 2008) are:

- Hydrolysis – breakdown of complex compounds into soluble sugars,
- Fermentation – conversion of sugars into alcohols, acetic acid, volatile fatty acids (VFA) and off-gas, a mixture of H<sub>2</sub> and CO<sub>2</sub> by fermentative anaerobic microbes and
- Methanogenesis – metabolism of off-gas into primarily CH<sub>4</sub> (60-70%), CO<sub>2</sub> (30-40%) and other associated gases by methanogens.

AD of microalgal biomass into CH<sub>4</sub> has been estimated to recover as much energy as that accomplished from the extraction of cell lipids (Brennan and Owende, 2010; Sialve et al., 2009). This gives a biomass substrate product that can be further processed into other biofuel derivatives by thermochemical processes which can potentially lead to an energetic balance of microalgal-based biofuel production process (Sialve et al., 2009).

Theoretically, the potential methane yield of microalgae biomass can be estimated with regards to the gross fractional composition of biochemical constituents of the biomass from the AD process (Sialve et al., 2009), based on a formula (below) adapted from Symons and Buswell (1993).



In equation (1), the organic biomass matter is converted to methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and ammonia (NH<sub>3</sub>).

The specific methane yield expressed in litres of CH<sub>4</sub> per gram of VS can be calculated according to (Sialve et al., 2009), as given in equation 2.

$$Bo = \frac{4a+b-2c-3d}{12a+b+16c+14d} * V_m \text{ --- (2)}$$

Where  $V_m$  is the normal molar volume of CH<sub>4</sub>

Certain studies have linked the prospects of higher potential methane yield by AD of organic biomass with very high lipid fractional content biomass substrate (Cirne et al., 2007; Li et al., 2002). However, lipid hydrolysis is well thought-out to be slower than protein and carbohydrate hydrolysis (Sialve et al., 2009). This is based on reported values of minimum limiting generation time (in days) for anaerobic digestion of various substrates of carbohydrates (0.18 days), proteins (0.43 days) and lipids (3.2 days) respectively (Pavlostathis and Giraldo-Gomez, 1991; Christ et al., 2000).

Furthermore, methanogenic biogas production rate by AD of organic biomass is thought to be sensitive to changes in the following variable (Cantrell et al., 2008; Sialve et al., 2009):

- i. Operating condition – which depends on the species and culture conditions of the microalgal biomass, i.e. multispecific (Yen and Brune, 2007) or monospecific cultured biomass in either raceway ponds or PBR's,
- ii. Temperature – remarkable increase in CH<sub>4</sub> production was reported with increase in temperature over temperature range of 4-25°C. Consequently, there are three common temperature ranges for AD (1) low or psychrophilic temperature ranges (< 20°C), (2) digestive or mesophilic temperatures (within 20-45 °C) and (3) thermophilic temperature ranges (45-60 °C),
- iii. pH – influences the activity of hydrolytic enzymes and microbes, thus, a balance between the acidogens/acetogens (VFA) and methanogens is

vital for effective AD for biogas production, as methanogenic reduction activities are weakened when pH falls below 6.3,

- iv. Organic loading rate (OLR) – this is the ratio of the amount of volatile solids (VS) or chemical oxygen demand (COD) constituents fed per day per unit digester volume. This implies that, enough time should be permitted for the microbes to breakdown the organic material and convert it to gas, as higher feed rates can strain and ultimately damage the digestion process, and
- v. Hydraulic retention time (HRT) – is a value calculated as the ratio of the digester volume to the effluents volumetric flow rate, and it expresses the average time the liquid is housed in the digestion process unit. It has been shown that methane yield is constant and maximal when the process is operated at low loading rate and high HRT, while the converse is the case when the maximal loading rate or minimum HRT is sustained. This often results in decrease in yield.

Due to high protein content of microalgae, high volume of  $\text{NH}_3$  is produced upon microbial protein hydrolysis leading to low C/N ratio which inhibits the activities of anaerobic microbes in the AD process (Brennan and Owende, 2010). However, Yen and Brune (2007) revealed that co-digestion of microalgal biomass with a high C/N ratio product (e.g. waste paper), i.e. 50/50 waste paper/algae biomass could significantly increase (double)  $\text{CH}_4$  production rate from  $0.57 \text{ ml l}^{-1}$  to  $1.17 \text{ ml l}^{-1}$  compared to AD of pure microalgal biomass.

Consequently, pre-treatment and co-digestion are strategies that can increase the  $\text{CH}_4$  yield potential of the AD of microalgal organic biomass both significantly and efficiently (Sialve et al., 2009). Hitherto, microalgae have received far less attention than other organic biomass substrates in terms of studies dealing with AD (Sialve et al., 2009).

Alcohol fermentation is another process used to convert organic biomass materials containing sugars, starch or cellulose into ethanol (Brennan and Owende, 2010; McKendry, 2002a). It entails the enzymatic breakdown of the fractional starch component in well ground biomass into sugars, with

subsequent conversion of these sugars into ethanol by yeast in fermentation tanks (Demirbaş, 2001). It also, involves the purification of ethanol by distillation (energy intensive process) aimed at concentrating the initial diluted alcohol product (10-15% ethanol) of water and other impurities to a concentrated (95% by volume) ethanol (Brennan and Owende, 2010; Demirbaş, 2001).

The distillate is usually abridged into liquid form which can serve as a supplement or substitute for petrol in cars (Demirbaş, 2001). The resultant solid residue from the biomass fermentation process can be used as cattle feed or for subsequent gasification (Brennan and Owende, 2010; McKendry, 2002a).

Hirano et al., (1997) reported a 65% ethanol conversion efficiency production with intracellular fermentation of *Chlorella vulgaris*, a high starch content alga (37% dry wt.). Similarly, Ueno et al., (1998) demonstrated ethanol production using marine green alga, by the catabolism of endogenous carbohydrates via fermentation under dark anaerobic conditions, recording maximum ethanol productivity of 450  $\mu\text{mol g}^{-1}$  dry wt. at 30 °C. Hence, it appears that ethanol production from microalgal biomass fermentation is not only technically feasible, but may be a viable option (Brennan and Owende, 2010).

### **2.13.2 Transesterification**

Transesterification or alcoholysis is the reaction of TAG's with a primary or secondary monohydric aliphatic alcohol such as; methanol, ethanol, propanol, butanol and amyl alcohol (methanol is more commonly applied because of its low-cost and physical advantage) to produce biodiesel or fatty acids methyl esters (FAME) and glycerol (Brennan and Owende, 2010; Yusuf, 2007; Huang et al., 2010; Demirbas, 2009). The reaction process is often catalysed by acids, alkalis, lipase enzymes or supercritical methanol (See Table 3 below) to improve the reaction rate and yield (Yusuf, 2007; Huang et al., 2010).

**Table 3 Merits and demerits of different transesterification processes (adapted from Huang et al., 2010)**

Type of transesterification	Advantages	Disadvantages
Chemical catalysis	<ul style="list-style-type: none"> <li>(a) Reaction condition can be well controlled</li> <li>(b) Large scale production</li> <li>(c) The cost of production process is cheap</li> <li>(d) The methanol produced in the process can be recycled</li> <li>(e) High conversion of the production</li> </ul>	<ul style="list-style-type: none"> <li>(a) Reaction temperature is relatively high and the process is complex</li> <li>(b) The later disposal process is complex</li> <li>(c) The process requires much energy</li> <li>(d) Need an installation for methanol recycle</li> <li>(e) The waste water pollutes the environment</li> </ul>
Enzymatic catalysis	<ul style="list-style-type: none"> <li>(a) Moderate reaction condition</li> <li>(b) Small amount of methanol required in reaction</li> <li>(c) Have no pollution to natural environment</li> </ul>	<ul style="list-style-type: none"> <li>(a) Limitation of enzyme in the conversion of short chain of fatty acids</li> <li>(b) Chemicals exist in the process of production are poisonous to enzyme</li> </ul>
Supercritical fluid techniques	<ul style="list-style-type: none"> <li>(a) Easy to be controlled</li> <li>(b) It is safe and fast</li> <li>(c) Friendly to environment</li> </ul>	<ul style="list-style-type: none"> <li>(a) High temperature and high pressure in the reaction condition leads to high cost of production and waste of energy</li> </ul>

Alkali-catalysed transesterification is about 4000 times faster than acid catalysed reaction for triglyceride transesterification due to higher reaction and

conversion rates (Yusuf, 2007; Huang et al., 2010). Consequently, alkali such as NaOH and KOH are the most commonly used commercial catalysts.

However, the free fatty acid (FFA) may react with the alkali catalyst to form soap and water (Figure 7) when the FFA level exceeds 5%, leading to loss of alkali catalyst with the resultant soap restraining separation of the biodiesel (or FAME) and glycerol (Huang et al., 2010; Demirbas, 2009).

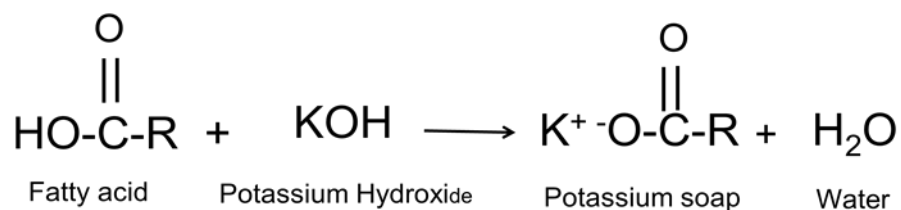


Figure 7 Transesterification with alkali catalyst

Consequently, with an alkali catalyst to convert triglycerides to methyl esters, it is essential to first convert FFA's to methyl ester (Huang et al., 2010), in order to reduce the content of FFA's (see Figure 8).

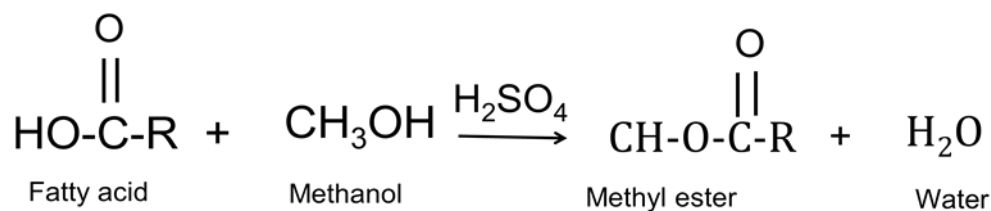


Figure 8 Transesterification by acid catalyst

Hence, the use of acid catalyst is useful for the conversion of FFA feedstock to alkyl esters although the reaction rates for converting triglycerides to methyl esters are reported to be slower than alkali catalysts (Huang et al., 2010; Gerpen, 2005). In contrast, enzymatic catalysts are more tolerant to higher FFA's feedstocks, but are costly and not able to offer the grade of reaction completion to meet the American Society for Testing and Materials (ASTM) fuel

specification (Huang et al., 2010). The use of supercritical transesterification process (>240°C and >8 MPa, respectively in the absence of a catalyst) option for microalgae biodiesel production is rare and also restricted due to safety concerns and related high cost of the reaction condition (Ehimen et al., 2010).

Some important variables that influence the production of biodiesel from microalgal lipids, by transesterification process include, temperature, reaction time, molar ratio of alcohol to glycerides, moisture content in biomass, and FFA's content (Huang et al., 2010; Demirbas, 2009; Gerpen, 2005; Ehimen et al., 2010). The molar ratio of 6:1 is commonly used to bring the reaction process to completion, even though the theoretically prescribed molar ratio is 3:1, with anticipated theoretical feedstock input and biodiesel output yield ratio of about 1:1 (Mata et al., 2010).

Also, equilibrium biodiesel conversion were reported after 2 and 4 hours reaction time for temperatures of 60 and 90°C (Ehimen et al., 2010), with 60°C reaction temperature recommended as more beneficial relative to the total energy consumption and operating cost of the entire biodiesel conversion process. Likewise, transesterification process would be inhibited in microalgal biomass samples with moisture levels greater than 115% of the reacting oil weight. Hence a 73% removal of water from the freshly harvested sample is recommended for in situ transesterification (Ehimen et al., 2010).

In summation, the production of biodiesel from microalgal biomass via transesterification process option is still at laboratory scale research and development stages unlike feedstock such as terrestrial oil-plants and vegetable oil which are well developed and documented (Huang et al., 2010). Therefore, there is a need for further unit process (such as biomass drying, filtration, evaporation, extraction, adsorption) design, optimisation and integration to optimise the utilisation of microalgal biomass feedstock (Huang et al., 2010; Ehimen et al., 2010).

## 2.14 Thermochemical conversion processes

Thermochemical conversion processes of biomass encompass the application of classical methods such as direct biomass combustion, pyrolysis, gasification, and thermochemical liquefaction to breakdown the organic components in biomass (Brennan and Owende, 2010; McKendry, 2002a; Balat et al., 2009), in order to yield fuel product (see **Figure 9**). It defines the thermal decay and chemical transformation process of biomass by essentially heating the biomass in various concentration of oxygen (McKendry, 2002a). It has the unique advantage of essentially converting all the organic fractions of biomass, compared with biochemical process options which mostly focus on the polysaccharides (Brennan and Owende, 2010).

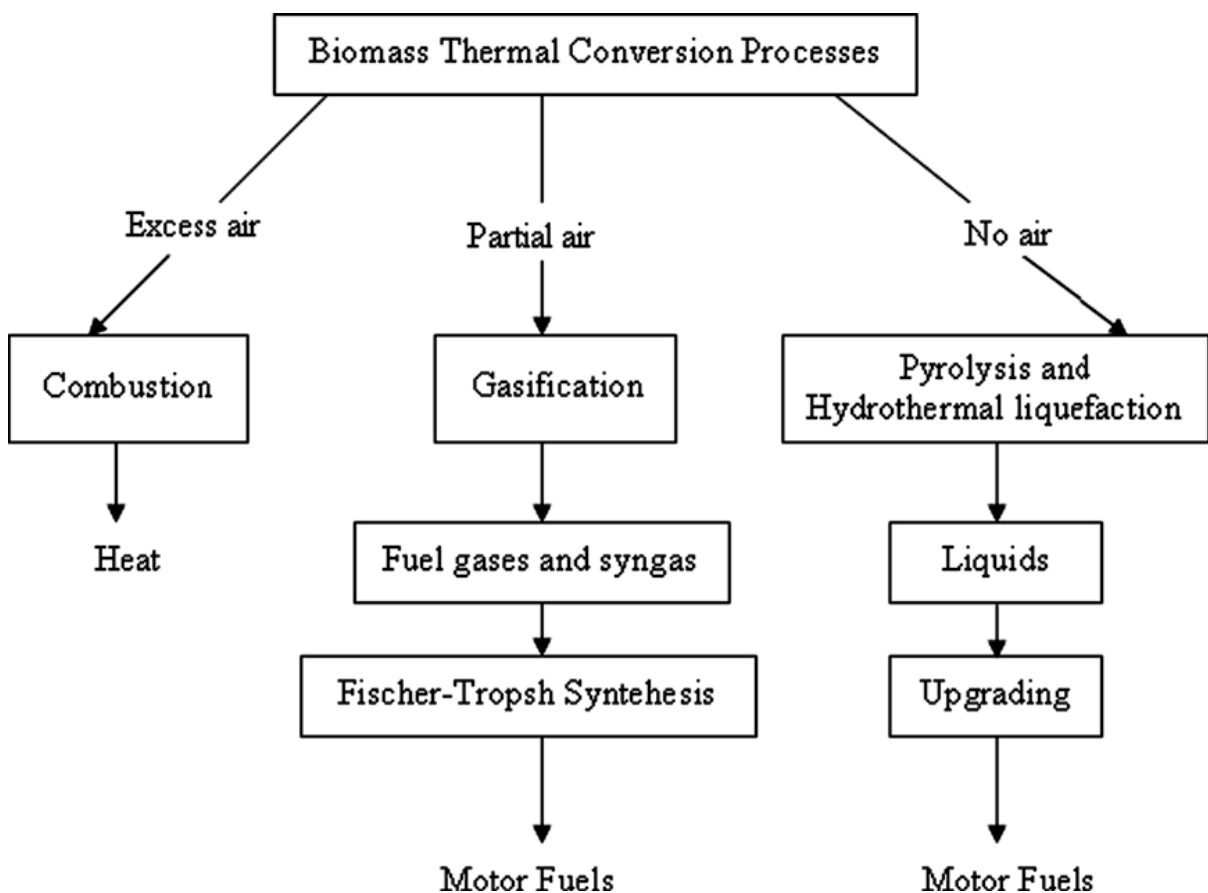


Figure 9 Biomass thermochemical conversion processes (Balat et al., 2009)



### **2.14.1 Combustion**

This is the direct burning of biomass in air over a wide range of temperatures (around 800-1000°C) utilising process apparatus like (e.g. furnace, boiler, steam turbines, turbo-generators, etc.) to convert the chemical energy stored in microalgae biomass into various gases (McKendry, 2002a), for other applications (heat, mechanical power, or electricity). Currently, the scale of combustion plants available range from very small scale apparatus (domestic space and water heating) up to large-scale industrial systems in the range of 100-3000MW (Brennan and Owende, 2010; McKendry, 2002a). In practice though it is possible to combust most types of biomass, however combustion is thought to be realistic only for biomass with moisture content <50% with the exception of pre-dried biomass (Brennan and Owende, 2010; Goyal et al., 2008). As biological conversion process options are better suited for high moisture content biomass (McKendry, 2002a).

Direct biomass conversion into bio-energy by combustion has the disadvantage of incurring supplementary energy demand and cost due to the requirement for pre-treatment process options such as drying, cutting and grinding (Brennan and Owende, 2010). Consequently, the traditional net conversion efficiencies of typical biomass combustion plants vary from 20% to 40%, with higher efficiencies obtained in bigger plants (>100MW) or when biomass is co-fired in coal-fired power plants. Hence, it is conventional to generate combined heat and power (CHP) in order to increase on the total system efficiency.

However, apart from a report by Kadam (2002), suggesting possible environmental benefits inherent from electric power generation via coal-microalgae cofiring, using LCA tool, there is limited information on evidence in current literature of technically feasible exploitation of microalgal biomass through direct combustion (Brennan and Owende, 2010).

### 2.14.2 Pyrolysis

Pyrolysis is the process of converting biomass directly into solid (charcoal), liquid (bio-oil), and gaseous fuel products by thermal decomposition of biomass in the absence of oxygen (Naik et al., 2010; Goyal et al., 2008; Kadam, 2002), or partially combusted in a limited oxygen supply (Balat et al., 2009). Biomass pyrolysis into bio-liquids and other products is a significantly researched central thermochemical process for converting biomass into more useful fuel (Balat et al., 2009; Goyal et al., 2008). Commercial production of a wide range of fuels and chemicals from biomass feedstock with pyrolysis has been successful (Balat et al., 2009). Pyrolysis processes can be divided into three divisions (see Table 4), depending on the operating conditions (McKendry, 2002a; Goyal et al., 2008).

**Table 4 Operating conditions and expected products yields for pyrolysis (Brennan and Owende 2010)**

Mode	Conditions	Liquid (%)	Char (%)	Gas %
Flash pyrolysis	Moderate temperature (500°C), short hot vapour residence time (about 1 s)	75	2	13
Fast pyrolysis	Moderate temperature (500°C), moderate hot vapour residence time (about 10-20 s)	50	20	30
Slow pyrolysis	Low temperature (400°C), very long solids residence time	30	35	35

In Conventional (slow) pyrolysis, biomass is subjected to slow heating rates (5-7 K/min), leading to more of solid (charcoal) production with less liquid and gaseous products (Naik et al., 2010; Balat et al., 2009; Goyal et al., 2008). The first stage is biomass internal rearrangement which occurs between 550K and 950K, followed by the formation of pyrolysis products (char), and the final stage

of char decomposition at slow rate yielding carbon rich solid residues (charcoal) (Naik et al., 2010).

Fast pyrolysis occurs when fine particle biomass (<1mm) is subjected to conditions of high temperature range of 850-1250 K with fast heating rate (10-200 K/s) under a short solid residence time (0.5-10 s) (Naik et al., 2010; Balat et al., 2009). Fast pyrolysis is mainly used to obtain high grade bio-oil, by decomposing biomass to generate vapours, aerosol, and some charcoal like char (Naik et al., 2010; Balat et al., 2009; Goyal et al., 2008). Depending upon the feedstock's, fast pyrolysis produces around 60-75 wt. % of bio-oil, 15-25 wt. % solid char and 10-20 wt. % non-condensed gases, which yields a dark brown liquid upon cooling and condensation (Naik et al., 2010; Demirbaş, 2001). A low temperature, high heating rate, and short gas residence time are conditions required, in order to boost liquid product yield from biomass pyrolysis (Balat et al., 2009). While, a high temperature, low heating rate, and a long gas residence time process would be preferential conditions, if the purpose were to maximize the yield of fuel gas (Demirbaş, 2001; Demirbas, 2007).

However, flash pyrolysis occurs when fine particle biomass (< 0.2 mm) is subjected to a temperature range of 1050-1300 K, under rapid heating rate (> 1000 K/s), and short residence time (< 0.5 s) (Brennan and Owende, 2010; Balat et al., 2009). It is thought to be a viable process option for the future production of biomass derived liquid fuels, a replacement for fossil-fuels mainly because of the achievable high biomass-to-liquid conversion ratio of (95.5%) (Brennan and Owende, 2010; Naik et al., 2010; Demirbas, 2006). Though, pyrolysis oils have the technical challenge of being acidic, unstable, viscous, and also containing solids and chemically dissolved water (Brennan and Owende, 2010; Balat et al., 2009). Hence, liquid bio-oils obtained from biomass by slow, fast or flash pyrolysis cannot be directly used as transportation fuels and need to be upgraded due to high oxygen and water content (Goyal et al., 2008).

Microalgal biomass pyrolysis has received extensive research efforts with reliable and potential outcome that could lead to its application at commercial

level (Brennan and Owende, 2010). Miao and Wu in (Miao and Wu, 2004) demonstrated an approach for enhancing the yield of bio-oil production from fast pyrolysis of *Chlorella prothothecoides* by manipulating its metabolic pathway towards heterotrophic growth. They reported bio-oil yield (57.9% dry wt. basis) from heterotrophic *Chlorella prothothecoides* biomass cells, which is 3.4 times higher than that from autotrophic biomass cell by fast pyrolysis (Brennan and Owende, 2010; Miao and Wu, 2004). This suggests that microalgal biomass is a potential feedstock for pyrolysis into liquid fuel. Miao et al., (2004) also reported bio-oil yields of 18% (HHV of 30 MJkg<sup>-1</sup>) and 24% (HHV of 30MJkg<sup>-1</sup>) from the fast pyrolysis of phototrophically grown *C. prothothecoides* and *Microcystis aeruginosa* respectively. Similarly, Demirbas, A. (2006) demonstrated the effect of pyrolysis temperature on the bio-oil yield (fuel properties) of mosses and algae biomass, with reported increase in yield from 5.7% to 55.3% for corresponding increase in temperature from 254 to 502 °C, and an ensuing drop in yield to 51.8% at 602 °C.

Circulating fluidized bed reactor, fixed beds, vortex reactor, entrained flow reactor, vacuum furnace reactor, wire mesh reactor, inclined rotating kilns, etc., are some of the most common reactor systems designed for performing pyrolysis (Goyal et al., 2008; Demirbas and Arin, 2002). Fast pyrolysis in an entrained- or fluidized-bed reactor is recommended for fine particle or powdery biomass feedstocks, as the choice of reactor type and heating systems affects the final product distribution (Demirbas and Arin, 2002).

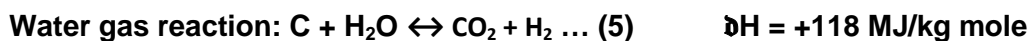
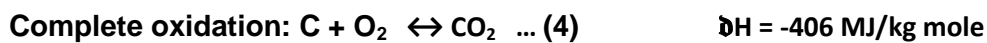
### **2.14.3 Gasification**

Gasification is the conversion of essential chemical energy of the carbon in biomass into gaseous fuel derivatives suitable for use in gas engines by heating biomass in a gasification chamber such as air, oxygen or steam at temperature, in the range of 800-1000 °C (Brennan and Owende, 2010; Cantrell et al., 2008; Demirbaş, 2001; Goyal et al., 2008; McKendry, 2002b). The by-products of gasification include char (a minor by-product) and primarily non-condensable, stable gases, CO, CO<sub>2</sub>, H<sub>2</sub>, and low molecular weight hydrocarbon gases (Naik

et al., 2010; Goyal et al., 2008). Biomass gasification can be achieved by two routes namely catalytic and non-catalytic process (Naik et al., 2010).

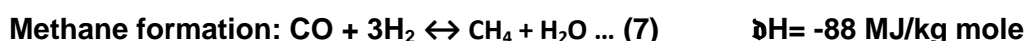
Technically, gasification includes both biochemical (AD) and thermochemical processes (use of air, oxygen or steam) at temperatures > 800 °C (McKendry, 2002b). However, for this study, the term gasification will refer only to the thermochemical conversion of biomass.

Gasification involves three process-chain heat reaction stages (McKendry, 2002b) as shown below:



The heat of reactions ( $\Delta H$ ) from the above three process equations indicates that the greatest energy is derived from the complete oxidation of C to  $CO_2$  i.e. combustion, whereas the partial oxidation of C and CO accounts for only 65% of the energy released during complete oxidation (McKendry, 2002b).

Unlike combustion that produces only a hot gas product, during gasification reaction, CO,  $H_2$  and steam can undergo further reactions (Demirbaş, 2001; McKendry, 2002b), yielding a product (syngas) consisting of a mixture of CO,  $CO_2$ ,  $CH_4$ ,  $H_2$ , and water vapour, as follows:



(Note that the arrows indicate that the reactions are in equilibrium and can proceed in either direction, depending on the reaction conditions of temperature, pressure and concentration of the reacting species).

The exploitation of microalgal biomass by gasification has been studied by a number of researchers (Brennan and Owende, 2010). Hirano et al., (1998) reported that microalgal biomass gasification at 1000 °C produced the maximum hypothetical yield of 0.64 g methanol from 1 g of biomass, with an estimated energy balance (described as the ratio of the energy of methanol produced to the total requisite energy) of 1:1. This is a marginally positive energy value (Brennan and Owende, 2010), which may be ascribed to the use of centrifuge process option during biomass harvesting. They achieved this outcome by determining the composition of produce gas from the partial oxidation of the microalga, *Spirulina* at temperatures of 850 °C, 950 °C and 1000 °C in order to evaluate the theoretical yield of methanol from the various gas compositions. Similarly, Sawayama et al., in (Sawayama et al., 1999) demonstrated a novel low temperature catalytic gasification process using high moisture content biomass of *C. vulgaris* with N cycling to obtain methane rich fuel. They indicated that the N component of the biomass which was converted into fertilizer quality ammonia during the gasification process could decrease the energy input for nutrient, if recycled as a source of nutrient.

Gasification has the key advantage of being applied on a wide variety of potential feedstocks as a biomass-to-energy conversion pathway (Brennan and Owende, 2010; Demirbaş, 2001). However, reliable literature data for microalgal gasification are sparse (Brennan and Owende, 2010). Consequently, there is need for more research particularly into the energy balance of drying microalgal biomass for gasification.

#### **2.14.4 Thermochemical liquefaction**

Liquefaction is used to describe the thermochemical conversion process of biomass in the liquid phase at moderate temperatures (300-350°C), and high pressure (5-20 MPa). This is supported by a catalyst to enhance the rate of reaction in the presence of high hydrogen partial pressure to yield bio-oil (Brennan and Owende, 2010; Demirbaş, 2001; Goyal et al., 2008). There is low interest in the conversion process due to the complex and expensive cost of

thermochemical liquefaction reactors (Demirbaş, 2001; Balat, 2008). Nevertheless, it has the advantage of converting wet biomass feedstocks into liquid fuels with HHV and lower oxygen content (Brennan and Owende, 2010; Huang et al., 2010; Balat, 2008). Liquefaction is the utilization of the high water activity in sub-critical conditions to decompose biomass feedstocks down to shorter and smaller molecular materials with higher energy density (Brennan and Owende, 2010; Huang et al., 2010).

There have been significant studies investigating the potential utilization of microalgal biomass feedstocks via thermochemical liquefaction to produce biofuel directly without the need of drying (Brennan and Owende, 2010; Huang et al., 2010). Huang et al., (2010) reported bio-oil yield of 37% of the total organic matter from the direct liquefaction of *Dunaliella tertiolecta* biomass with 78.4% water content. Dote et al., (1994) effectively achieved maximum liquid oil yield of 64% dry wt. basis with HHV of 45.9% MJkg<sup>-1</sup> by thermochemical liquefaction of *B. braunii* under the conditions of N<sub>2</sub> pressure of 10 MPa at 300°C using NaCO<sub>3</sub> as a catalyst with a positive energy balance (output/input ratio of 6.67 : 1). Similarly, Minowa et al., (1995) reported oil (comparable to fuel oil) yield of 42% dry wt. with HHV of 34.9 MJkg<sup>-1</sup> from the algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction and positive energy balance of 2.94:1. All these reports show that thermochemical liquefaction is a viable process option for the conversion of moist microalgal biomass into liquid fuel, as it does not require a drying process.

## **2.15 Comparative LCA studies of previous microalgae based biofuel production systems**

Ethanol and biodiesel (obtained from biomass feedstock's like rapeseed oil, palm, sugar beet, corn and vegetable oil) which have now attained commercial scale of production (Brennan and Owende, 2010), have been around for well over a century (Campbell et al., 2011). However, this practice has come under heavy criticism as it is seen by many as unsustainable and debatable (Mata et al., 2010) due to the priority use of food crop for human and animal sustenance, the potential impact of increasing food prices and the competition of biofuel with food production (food security). Although in some quarters it is considered as an additional spring of boosting income for poor farmers. Conversely, there have also been concerns over the twin issues of the effects (economics) of indirect land use change (biodiversity loss) and GHG balances from such production systems (Mata et al., 2010; Campbell et al., 2011; Rathmann et al., 2010).

Comparatively, the use of microalgae biomass as feedstock's for biofuel production has gained considerable momentum of interest; as they can be cultivated in areas unsuitable for terrestrial crops, can potentially grow at a much faster rate (Brennan and Owende, 2010; Mata et al., 2010). In addition, some species are exceptionally high in lipid accumulation, making them suitable candidates for biodiesel production (Khan et al., 2009; Li et al., 2008). However, most of the facilities for microalgal based biofuel production are pilot-scale facilities, with extensive research currently being performed on the feasibility, design up-scale and requirements for an industrial-scale facility in the near future (Campbell et al., 2011). Consequently, and most recently several researchers have studied microalgal biofuel production systems using LCA methods to quantify process energy consumption (in terms of inputs and outputs), determine the environmental burdens (primarily GHG emissions) and its economic viability (Yang et al., 2011; Clarens et al., 2010). LCA methodology can be used to account for all energy use and total emissions sustained during the production and use phases of a biofuel/product system (Edward et al., 2012).



For example, Kadam (2001), using LCA methodology demonstrated that GHG emission reductions benefit is potentially possible from power generation, by comparing electricity production via coal firing vs. cofiring of CO<sub>2</sub>-derived microalgae, respectively. The LCA results showed the associated benefits of recycling power plant flue gas CO<sub>2</sub> towards algae production, as it significantly reduced CO<sub>2</sub> emissions and used less coal. However, when monoethanolamine (MEA) solvent was employed to purify and concentrate the CO<sub>2</sub> in flue gas (Kadam, 2001), a lot of the benefits were lost due to high steam requirements for regenerating the MEA. Campbell et al., (2009) similarly examined various scenarios for microalgae biofuel production with regards to sequestering power plant flue gas during its growth phase on a life-cycle basis. Out of the nine power plants considered in this analysis, seven did not have provision for adequate land close by for algae ponds. Consequently, the authors investigated the possibility of flue gas transport and their results indicated that pressurized distribution required excessively high power demand, while low-pressure distribution network introduced limitations associated to capital, pipeline size and routing.

Lardon et al., (2009) reported a comparative LCA study of a virtual microalgae biodiesel production facility in Europe under two different culture conditions; (1) normal fertilizer use and (2) under N limitation. They compared the best scenario to that of first generation biodiesel and concluded that increasing algae biomass lipid fraction yield via N limitation was important and as well avoiding drying. Drying the harvested wet biomass to 10% moisture, similar to soybeans required more energy than is available in the harvested algae biomass. They also highlighted the potential of anaerobic digestion (AD) of the residue lipid-extracted algae (LEA) as a valuable option to reducing external energy demand. Clarens et al., (2010) reported a similar life cycle comparison from the United States comparing the impacts associated with algae biomass production to that of farming conventional crops (switch grass, canola, and corn) by using the heating value of their respective fuels. Apart from eutrophication and in total land use potential which algae perform favourably, their results showed that conventional crops have lower emissions, and water regardless of production

location. They however, failed to account for the subsequent transesterification of the algal biomass oil to produce biodiesel rather than combusting the biomass.

Also, (Jorquera et al., 2010) using LCA comparatively examined the energy life-cycle of producing biomass feedstock from the oil-rich microalgae *Nannochloropsis sp.* grown in both open ponds and PBRs. They demonstrated that the net energy ratio (NER) of PBRs was  $< 1$  and thus uneconomical whereas that of ponds (NER) was  $> 1$ , which agree with earlier life cycle energy analysis reports of Campbell et al., (2009).

Likewise, a group of researchers in the United Kingdom (Stephenson et al., 2010), compared the environmental sustainability of producing biodiesel from the freshwater alga *Chlorella vulgaris* cultivated in typical raceway pond and air-lift tubular PBR. Their results further confirmed that cultivation in raceway pond was significantly more environmentally sustainable than in tubular PBRs, as biodiesel produced from raceway pond cultivated microalgae had GWP  $\sim 80\%$  lower than fossil-derived diesel (on the basis of net energy content). However, the GWP of the biodiesel derived from PBR cultivated microalgae appeared to be significantly greater than the energetically equivalent amount of fossil-derived diesel. Their findings also show that GWP and fossil-energy requirement for such production facilities were predominantly sensitive to the following parameters: (1) algae oil yield during cultivation, (2) mixing velocity of cultivation facility, (3) possibility of recycling of culture media, and (4)  $\text{CO}_2$  concentration in the flue gas.

These analyses indicate that LCA can be a useful tool to evaluate new technologies for energy production, as it identifies the technological drawbacks and therefore supports the eco-indicators of an efficient and sustainable production system. However, there is need for a more complete LCA of microalgae based biofuel production. Indeed, previous studies have failed to consider the overall effect of process parameters, which has resulted in the inability to accurately, predict product yield/environmental burdens with variations in operating conditions. Consequently, this research work intends to

use LCA methodology to model and assess an integrated hypothetical microalgae biofuel production facility, in terms of input and output energy, resource use and the associated environmental burdens through wet and dry extraction process routes. Thus, a “cradle-to-gate” life cycle inventory of each process unit of the production process chain has been modelled and subjected to analysis to identify weak spots and possible energy saving parameters of each process unit. The LCA presented in this research, provides the prospect for evaluating alternative pathways and identifying greater integration opportunities with better economic advantage and lowering environmental burdens in relationship with existing models. The research work is distinctive in the sense that it considers several different technical options of key algae biomass production and conversion pathways. Numerous LCA results were cross-compared in order to identify the most significant opportunities for improvement aimed at understanding the burden of these parameters on production process. Thus, this study offers baseline information that will reduce the impact of the overall energy use and provide momentum for further technological advancement of microalgae biofuel production process.







# **CHAPTER THREE**

## **METHODOLOGY OF DATA COLLECTION**

### **3.1 LCA methodology overview**

The Life Cycle Assessment (LCA) methodology presented in this research is based on ISO 14040 LCA standards - Principles and Framework (ISO, 2006b). It describes LCA as “the collation and evaluation of the inputs, outputs and the environmental burdens of a product system throughout its entire life cycle”. However, the LCA presented in this report is a “*Cradle to gate*” (i.e. from raw material attainment, through production, excluding the use phase and waste management phases of the products life cycle). Likewise, potential environmental impact of all production scenarios was analysed using SimaPro 7.1: LCA software by Pre’ Consultants (Consultants, 2009).

As a result, the LCA presented in this study comprise of a number of steps or activities which are arranged into four phases to make up the LCA framework (ISO, 2006b; Henrikke, B and Anne-Marie, T., 2004). These are:

- Goal and scope definition,
- Life cycle Inventory analysis of all inputs/outputs,
- Life Cycle Impact Assessment (LCIA), which has to do with understanding the environmental consequences of all inputs/outputs) and
- The interpretation of the study results.

### **3.2 Goal and scope definition**

The goal and scope definition clearly states or explains the reason for carrying out the study, the requirements on the modelling to be carried out, and the intended application or audience of the results that the LCA study is meant for

(Finnveden et al., 2009) . In the case of this research, the goal is to develop lower material consuming, lower energy demanding and environmentally more sustainable microalgae biofuel production scenarios in order to support microalgae-based biofuel production process development.

The scope of the LCA study entails the comparison of the environmental profiles of four prospective production scenarios of (1) 100% recycling of production harvest water, (2) use of wastewater/sea water as culture media, (3) co-products economic allocation consideration, and (4) 70% induced increase in algae lipid yield, with that of the base-case scenario of each of the two selected microalgae biofuel production methods (transesterification and AD processes as shown in Figure 11 below). While the intended audience, of this study includes, but not limited to, biofuel developers and researchers.

Some other important features of the goal and scope definition process (often subjective) are; the functional unit, the choice of product/process alternative to be analysed, a description of the system boundary, an account of how allocation issues will be dealt with, the formulation of the reference flow for each alternative process route option and the assumptions/limitations (Henrikke, B and Anne-Marie, T., 2004; Finnveden et al., 2009; Rebitzer et al., 2004) . Overall, the goal and scope definition is the channel that helps to ensure consistency of the entire LCA (Henrikke, B and Anne-Marie, T., 2004).

### **3.2.1 Functional Unit,**

The functional unit for this research is 1kg of biofuel. It provides the reference to which the input and output data are related and harmonises the formation of the inventory.

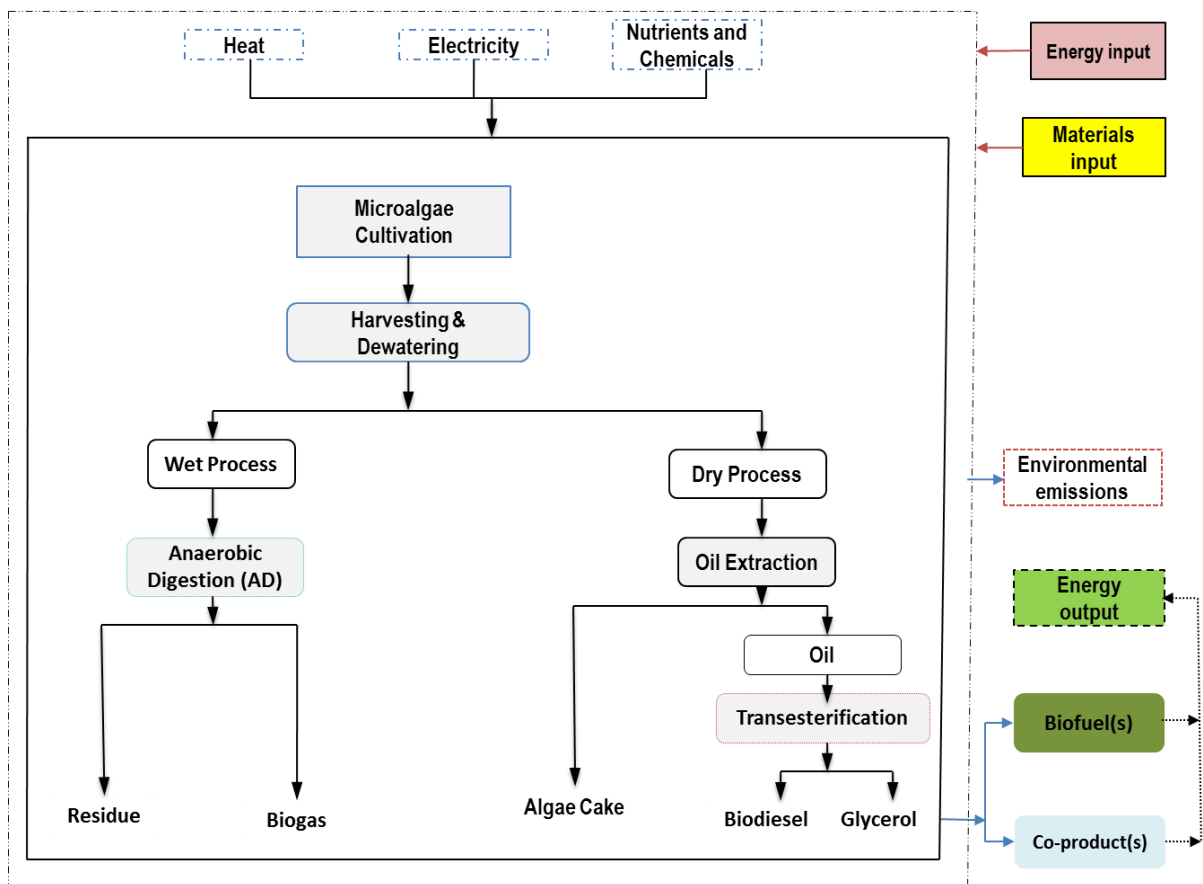
### **3.2.2 System boundary**

The system boundary in this study is set around the technical system of microalgal-based biofuel production chain of cultivation, harvesting and dewatering and the processing of the resultant microalgae biomass through specific biomass conversion technologies via dry and wet process routes into biofuels and co-products as shown below in Figure 10. Therefore, in line with the LCA goal and scope, the LCA study would estimate the total energy and



material inputs in form of heat, electricity, water, nutrients and chemicals required for each unit operations of cultivation, harvesting and dewatering, oil extraction and biomass conversion processes for both wet and dry process route. Also, the energy value in all the resultants biofuels and co-products produced for each process route would be computed and summed up as the total energy output from the system for either wet process or dry process route option.

Ultimately, all GHG air emissions familiar with biomass conversion processes from each unit process would be accounted for in terms of their global warming potential GWP. Although, it is assumed that CO<sub>2</sub> emissions which are the most significant GHG would be recycled into the production system as a source of carbon nutrient for microalgae growth.



**Figure 10 Microalgae biofuel production System Boundary** (Mathew et al., 2013b)

### **3.2.3 Allocation issues**

In accordance with ISO 14044 (ISO, 2006b) requirements and guidelines for carrying out LCA, and with specific regard to allocation issues related to processes with more than one function or product output. Allocation issues occur when a production process is shared amongst a number of products systems, making it problematic to determine which product/co-product to allocate the resultant environmental burden. The three types of allocation problems common in LCA practice (Finnveden et al., 2009) includes: (1) multi-input (a scenario where a process receives several waste products as input; e.g., incinerator), (2) open-loop recycling (a situation in which a waste product is reprocessed into another product; e.g. a used newspaper incinerated to recover heat and electricity), and (3) multi-output (a scenario in which a process leads to the production of several products; e.g., algae biodiesel transesterification). The problem of how to allocate emissions and material consumption between several products or processes is called allocation. Consequently, allocation issue is a very key methodological consideration in LCA (Heijungs and Guinée, 2007).

Predominantly, allocation issues arising from multi-functional production processes can be dealt with in two ways. The first is by apportioning (allocating) the resultant environmental loads (emissions and material consumption) between the products/co-products based on the physical outcome, such as mass or energy content of the output. Another procedure is using mass-economic allocation basis, by allocating the environmental burden on the basis of the mass/economic values of the resultant products. Otherwise, system expansion or dividing the production system into sub processes where possible is recommended to avoid allocation issues (Finnveden et al., 2009) . However, mass-economic allocation method is often used in most LCA's, as economic value is considered a worthy way of distinguishing waste from an output (Consultants, 2008) . Similarly, it expresses the comparative significance of a product output in a process relative to other co-products. Therefore, mass-

economic allocation procedure is adopted in this research, as the preferred method for dealing with allocation issues that may arise from the proposed algae biodiesel production process.

### **3.3 Life Cycle Inventory (LCI)**

The LCI analysis phase of this research involves the gathering and computation of quantitative input/output data associated with the production of the functional unit (1 kg biofuel) via two model process route options as depicted in Figure 12, a and b. It is important to note that the LCI analysis reported in this LCA study is based on a hypothetical production process layout extrapolated from lab-scale studies in current literature (Brennan and Owende, 2010; Campbell et al., 2011; Yang et al., 2011). Therefore, the analysis in this study may be significantly different if new technologies lead to completely different process layouts in future. As, the inventory data for each process unit are based on figures derived from a variety of academic resources, microalgae producers andecoinvent database. Also the information from the LCI compilation process is subsequently used as inputs data for the LCIA phase using SimaPro software.

### **3.4 Life Cycle Impact Assessment (LCIA)**

The Life Cycle Impact Assessment (LCIA) phase in this research aims at characterizing and establishing a relationship between the results of the LCI analysis of the production process and its potential impacts on resource use, human health and ecosystem quality (Henrikke, B and Anne-Marie, T., 2004). Thus, the LCIA phase essentially seeks to improve the understanding of the results outcomes in the LCI phase. For this study, all three impact categories (resource use, environmental impact, and human health impacts) according to the ISO 14042 (ISO, 1998) (now replaced by the new ISO 14044 (ISO, 2006a)) standards on impact assessment resulting from the production system, have been classified and characterised using Eco-indicator 99 methods in SimaPro.

Furthermore, each impact category has been characterised in terms of the relative contributions of emissions and resource consumptions of the production system to each impact category. The rationale is thus to make the results more environmentally significant, clear and easier to communicate. Consequently, all air emissions common with biomass conversion technologies, particularly greenhouse gases (GHG) such as CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> have been quantified and classified in terms of their global warming potential (GWP) expressed in kg CO<sub>2</sub>-equivalents. The CO<sub>2</sub>-equivalent factor denotes the quantitative value of the potential climate change impacts per kg unit emission of a given substance over a unit time frame (short term 20-50 years; long term 100-500 years) (Henrikke, B and Anne-Marie, T., 2004).

Similarly, for resource use, all energy and materials used have been accounted for in terms of how they impact on the ecology, while the human toxicological impact due to process emissions to air (acidification) and water (eutrophication) have been quantified using the Eco-indicator 99 method in SimaPro (Goedkoop and Spriensma, 2001). This method takes into account the complete environmental aspects (emissions, fate, exposure, effect and damage), although requiring lots of assumptions in the modelling process, which brings in some uncertainty (Consultants, 2008) . However, Eco-indicator 99 based-method LCIA results are easier to understand and evaluate, as they clearly indicate the best environmental performance for each production scenario.

### **3.5 Interpretation**

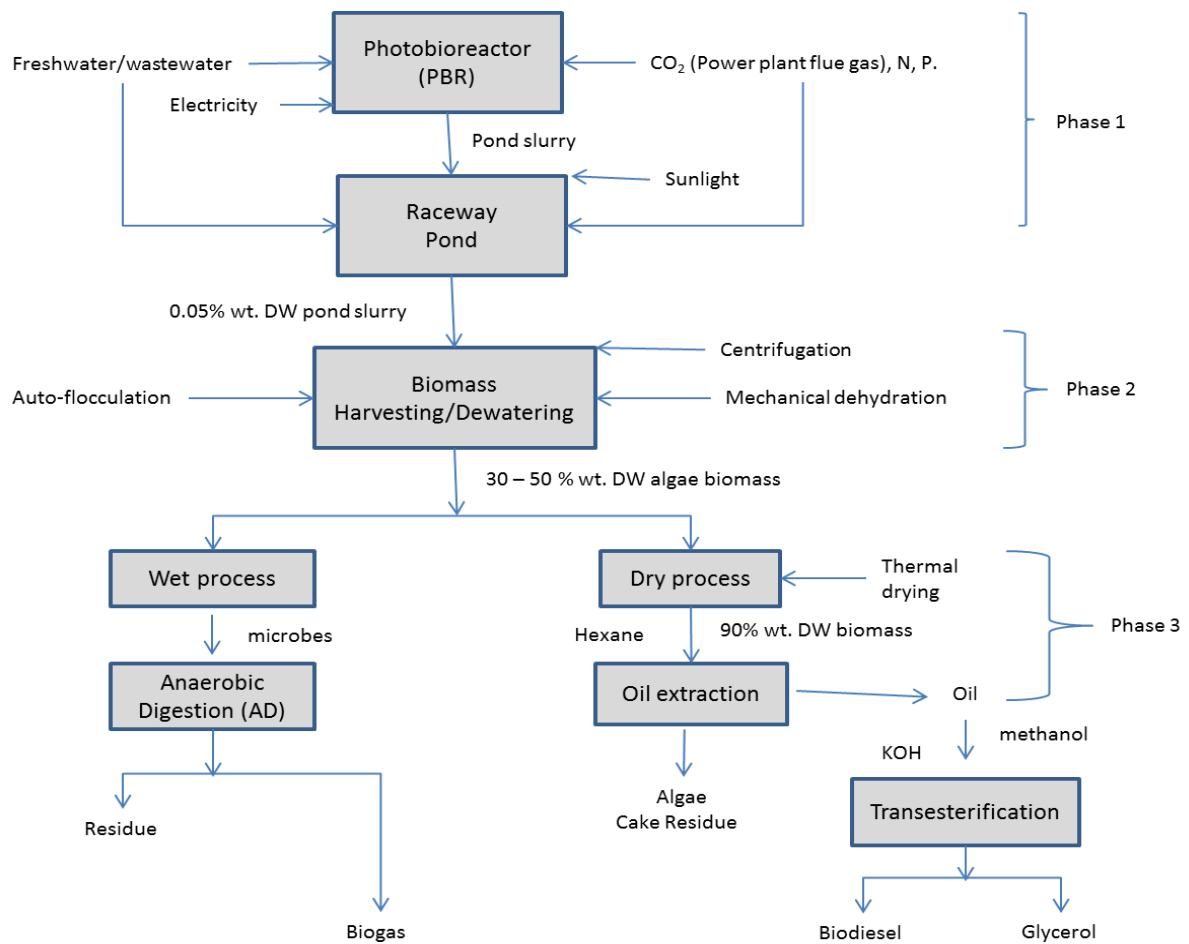
The interpretation stage of the LCA study highlights the significance and the strength of the evidence obtained and processed in previous stages, which helps in formulating conclusions and recommendations (ISO, 2006b). It accounts for general observation regarding contribution analysis, remarks regarding mismatch between inventory and impact assessment, appropriateness of impact assessment method(s), notes regarding the major uncertainties in the data and model, and conclusions and recommendations. There are different ways of interpreting the results depending on the kind of

study. It may include direct comparison with previously published LCA studies on similar product or process, uncertainty sensitivity checks of data, as a result of the many choices and assumptions that must have been made during the course of the LCA (Henrikke, B and Anne-Marie, T., 2004; Finnveden et al., 2009; Udo de Haes and Heijungs, 2007). One may also choose to stop after the inventory analysis and interpret the inventory results directly - such a study is called a life cycle inventory analysis instead of a life cycle assessment (Henrikke, B and Anne-Marie, T., 2004). Another process is to go through the characterisation factors, which reflects the degree of contribution of a LCI outcome and interpret the result from that level (Heijungs, 2002). It is the stage at which the final result and conclusion is determined.

In this research, Monte Carlo uncertainty analysis function in SimaPro was used to characterise the uncertainty and also promote the credibility of the results. Likewise, the interpretation phase entails drawing conclusions and formulating recommendations, based on the findings from both the LCI analysis and LCIA phases in line with the goal and scope of the research (as presented in the Results and Discussions chapter of this thesis).

### **3.6 Model description and case study**

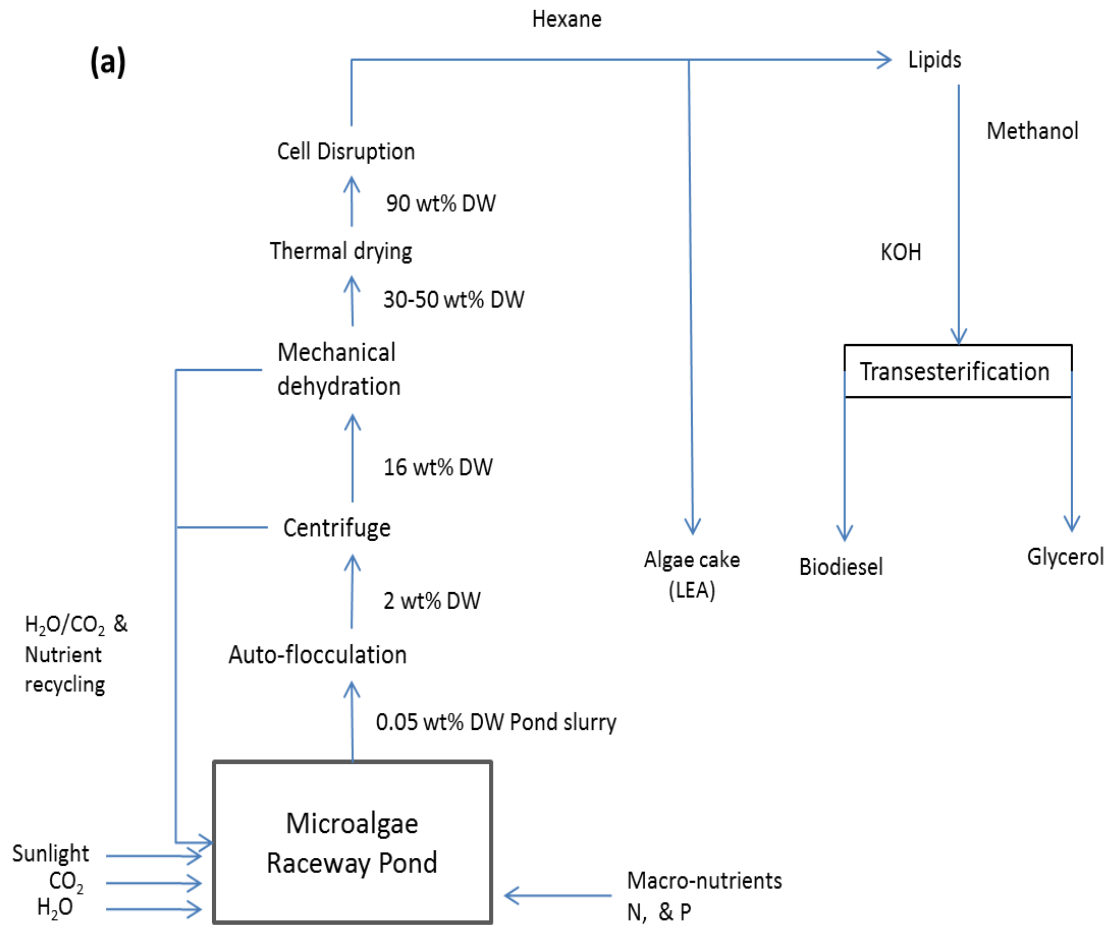
The LCA method adopted for this study is aimed at establishing reasonably best case process (with regards to lowered material and energy usage) scenario for microalgae-biofuel production, through the analysis of various process routes. Consequently, the study seeks to integrate existing models of microalgal-based biofuel production, in terms of microalgae cultivation with the option of utilising waste CO<sub>2</sub> from gas turbine power plant flue gas, biomass harvesting and processing option routes by developing a wide-ranging process model (see Figure 11). The model is aimed at providing an articulate description of an integrated possible route (wet & dry process routes) for producing 1 kg biofuel using microalgae biomass, utilizing all the algal biomass components as suggested by (Wijffels and Barbosa, 2010), in order to optimize the efficiency of the production system.

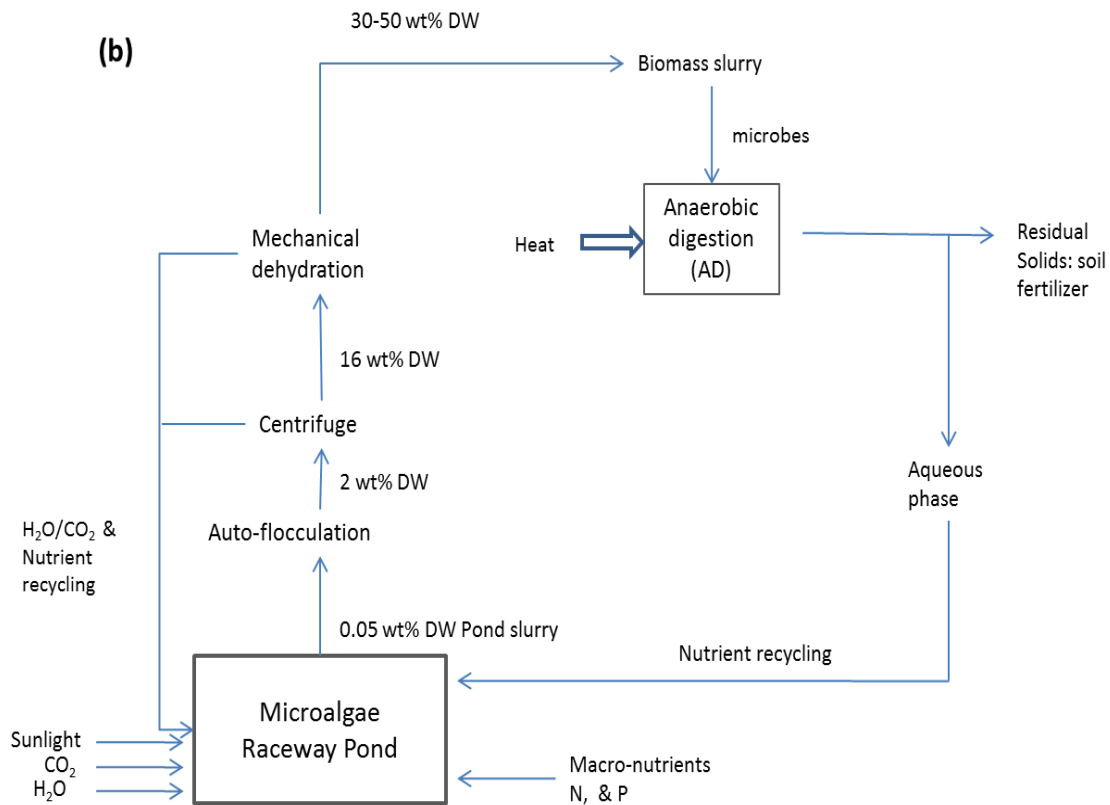


**Figure 11 Microalgal-based Biofuel Production Process Flow Diagram** (D.C. Mathew et al., 2013)

Furthermore, the “Cradle to gate” life cycle inventory of each process phase of microalgae cultivation (growth phase 1) using flue gas CO<sub>2</sub> from gas turbine power plant; biomass harvesting/concentration (phase 2); biomass thermal drying/oil extraction (Phase 3) for the biodiesel production route, and extracted oil/biomass-slurry processing options into liquid and gaseous biofuel derivatives. These processes have been modelled and analysed to identify weak phases/steps and possible energy/materials saving adjustments within the production system that could be made for each process chain. In addition, the biomass conversion process is assumed to occur via two microalgae-to-biofuel processes as shown in Figure 12, a & b through a combination of specific thermochemical and biochemical conversion routes:

- (a) Via a “dry process” (oil and energy extraction from dried algae) and  
 (b) Via a relatively “wet process” (energy extraction in the wet phase).





**Figure 12 Microalgae-to-biofuel process options, (a) the dry process route, and (b) the wet process route (adapted from Yang et al., 2011). The value of the DW denotes the input dry solid weight as a percentage of the designed post-process**

The two microalgae-to-biofuel conversion routes assumed for this study, as suggested by (Yang et al., 2011), are aimed at maximum extraction of bio-energy in the microalgal biomass with minimized fossil energy consumption. Consequently, the dry route combines several complimentary and low energy consuming drying techniques (Phases 2 & 3 in Figure 11) aimed at reducing the high energy consumption common with algae dewatering process (Sander, K. and Murthy, G. S., 2010). With the conversion of the dry algae biomass through transesterification of the lipids and subsequent combustion of the residue algae cake (LEA) in a combined heat and power (CHP) unit to offset heat and electricity demand. This is aimed at practically utilising all the carbon in the biomass. In the wet route option, instead, the entire microalgae to biofuel process chain takes place in a relatively wet phase (excluding Phase 3



operations of thermal drying and lipid extraction processes). The AD process ultimately yields biogas, a mixture of (CH<sub>4</sub> (60-70%) and CO<sub>2</sub> (30-40%)) with a residual solid that could be useful for soil fertilizer application. Both options have been analysed and assessed to evaluate which route can give better results. In terms of material usage, energy consumption and environmental loads associated with each consideration.

In addition, four prospective scenarios (earlier mentioned in the scope definition section of this report) which affect the microalgae biofuel production process were analysed. As understanding the influence of these factors on the production process would provide the insight that would lead to the formulation and development of a reasonably best case process model with regards to resource-use efficiency and sustainability of the process for microalgal-based-biofuel production. This information can provide momentum for further technological development of microalgal biofuel production process and reduce the impact of the overall energy use of future biofuel process.

### **3.7 Microalgae baseline production pathway overview**

The microalgae baseline biofuel production model adapted for this study is anticipated to offer an extensive set of options regarding different methodological choices. As a result the production phase is a two-stage hybrid system, as proposed by Huntley and Redalje (2006). The process begins with culturing *C. vulgaris* microalgae in PBR as it favours continuous cell division and high biomass yield (Brennan and Owende, 2010). Essential macro-nutrients (N & P) are supplied by urea (46% N content), and single superphosphate (P<sub>2</sub>O<sub>5</sub>), respectively. Also, secondary treated wastewater/sea water was used in place of freshwater as alternative source of nutrient/culture medium, so as to evaluate environmental load variations from both applications. CO<sub>2</sub> is assumed to come from gas turbine power plant flue gas coupled to the production process. The PBR is used to periodically provide a seed culture for the open raceway pond.

The preceding step of the growth stage (Phase 1 in Figure 11) is the transfer of the PBR slurry into the raceway pond, aimed at stimulating rapid biosynthesis of

desired lipid products due to environmental stress owing to the challenges common with maintaining optimal and stable growth conditions in the open (Yusuf, 2007). It is assumed that 30% of the production phase was set aside for biomass enhancement in N-sufficient setting in the PBR, whilst 70% of the production phase is assigned to lipid synthesis under N-limited environment. The whole routine is to induce the accumulation of high energy lipids in the biomass. Typical harvest-growth-harvest cycle is assumed to be 4-5 days (Richmond, 2004).

Biomass harvesting and thickening is the subsequent segment of the production process depicted as (Phase 2 in Figure 11). After the growth of microalgae to their harvest concentration, they are separated from the water by first aggregating the biomass from 0.05 wt. % DW to up to 2 wt. % DW using auto flocculation method. Auto flocculation is achieved by interrupting CO<sub>2</sub> supply to the algae system which causes algae to flocculate (Harun et al., 2010). This is followed by centrifugation and mechanically dehydrating the biomass to 30-50 wt.% DW and finally using thermal drying means to get the biomass to up to 90 wt.% DW, comparable to the solid content of soybeans (Lardon et al., 2009), for the dry extraction process route option. While for the relatively wet AD transformation route, thermal drying process is excluded.

The final stage of the LCA is the conversion of the bio-energy in the microalgae biomass into biofuels through selected biomass conversion processes. The dry process route involves using hexane extraction process and subsequent methanol transesterification of the resultant microalgal oil in the presence of an alkalis catalyst KOH (alkali-catalysed transesterification is about 4000 times faster than acid catalysed TAG transesterification reaction) into biodiesel and glycerol (Yusuf, 2007; Huang et al., 2010). Additionally, the residual algal cake (LEA) from the transesterification process is assumed to be utilised through a CHP plant to offset heat and electricity demand. This is carefully thought out to utilize all the algal biomass components in order to optimize the efficiency of the full chain. In the wet process case, AD is well-thought-out to be the choice conversion process as AD is considered to be particularly suitable for high

moisture content (80-90%) wet biomass material (Brennan and Owende, 2010; McKendry, 2002a).

Furthermore, the LCI of all energy and materials inflows and outflows in terms of total energy inputs, total energy outputs for both the resultant biofuels and co-products, and the environmental emissions of each unit process have been quantified and characterised as specified in the system boundary (Figure 10) for the whole production system, for both wet and dry process options. This would provide a basis for analysis and the development of a potential best case model in terms of the overall life cycle energy efficiency, resource use and the environmental sustainability of commercial microalgae biofuel production for each process route.

### **3.8 Life Cycle Inventory (LCI) data**

The LCI analysis covers the entire production process chain from microalgae cultivation to downstream biofuel conversion with the following major unit operations: cultivation, harvesting and dewatering, lipid extraction, transesterification and the use of algae cake residue via a CHP unit for co-generation of supplementary heat and electricity for the dry process option; while AD conversion process, is considered as conversion technique for the wet process option after cultivation, harvesting and dewatering the microalgae biomass to 30-50 wt. % DW.

It involves data collection, data estimation from stoichiometry balances where available data are poor, data aggregation where individual data are not available or are confidential, data validation and relating inputs and outputs data of each unit process according to the system boundary. Key inputs like electricity and chemicals have been obtained from the ecoinvent database.

#### **3.8.1 Algae cultivation data**

*C. vulgaris* has been chosen from the many microalgae species known in nature for this research as it is very robust and has been extensively studied with available quantitative composition and production data (Mata et al., 2010; Richmond, 2004; Chinnasamy et al., 2010) for freshwater, wastewater and

marine settings. The LCA has been set to exclude the production facility and its construction, as it is out of the scope of this research. Thus the energy and environmental burdens resulting from these items are not included. Also, since microalgae cultivation depends on the temperature and the location of production. These parameters are assumed to be favourable in this instance.

Materials and energy input flows for the cultivation process have been computed as shown in Table 5.

**Table 5 Input flows for microalgae cultivation per kg algae. [Source: (a)Yang et al., 2011; (b)Lardon et al., 2009; (c)Posten & Schaub 2009; (d)Schenk et al., 2008; (e)Liam et al., 2010 ]**

Input flow description	Unit	Energy equivalent (MJ)	Reference(s)
<b>PBR</b>			
Microalgae			
Water	117.8kg		estimated
Nutrient (N, & P.)	0.33kgN; 0.71kgP	28.3	(a), (b)
Electricity		23.45	estimated
<b>Raceway pond</b>			
CO <sub>2</sub>	1.8kg	24	(b), (c), (d)
Nutrient (N, & P.)	0.33kgN; 0.71kgP	28.3	(a), (b),(e)
Water	3726kg		(a)
Electricity (mixing/pumping)		32.79	(e)

### 3.8.2 Harvesting and dewatering process

Microalgae harvesting has been done in two steps. Initially by an induced settling process or auto-flocculating the biomass and then concentrating the algae by centrifugation and mechanical dehydration (as shown in Figure 13). These processes are aimed at reducing the burden of using chemical flocculants. Beside, chemical flocculants are very expensive for use in large scale production and they are thought to limit the application of the biomass sludge for downstream processes like AD (Schenk et al., 2008).

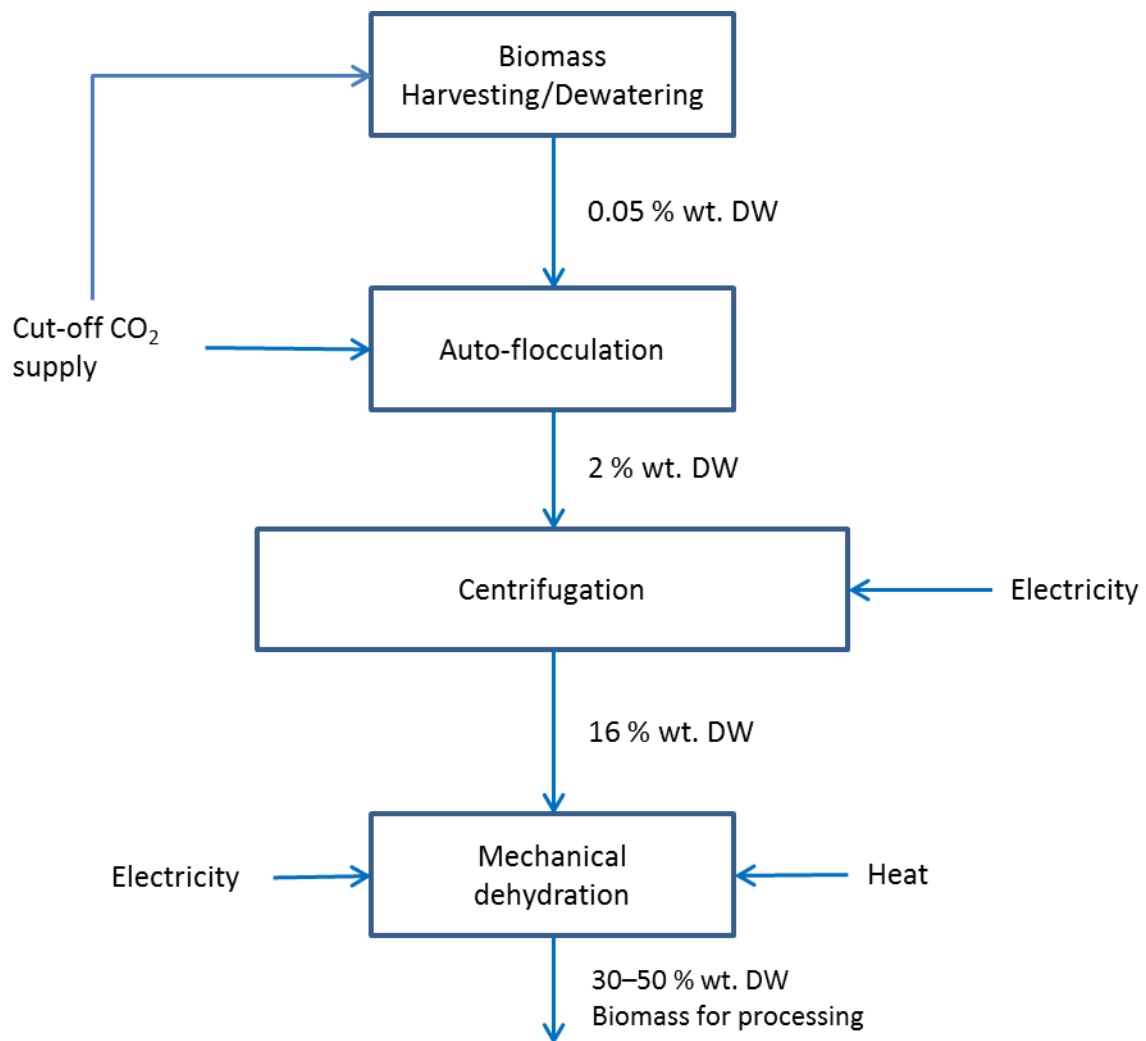


Figure 13 Biomass Harvesting/Dewatering process option

Also, for the algal dewatering, thermal drying although an energy consuming process has been chosen in the dry process option (See Phase 3 of Figure 11) since the water left over in the algae cake after mechanical dehydration of *C.vulgaris* to 30-50%wt DW is presumed to be mainly intracellular water (Xu et al., 2011), which can only be dried by thermal drying process. The input flows for this unit process have been computed as shown in Table 6.

**Table 6 Input flows for harvest and dewatering process per kg algae. Source: (1) Molina et al., 2003; (2) Liam et al., 2010; (3) Lardon et al., 2009**

Input flow description	unit	Energy equivalent (MJ)	Reference(s)
<b>Harvest</b>			
Electricity (slurry pumping)	9.08kWh	32.7	(2)
Auto-flocculation	Neglected		
Centrifugation (electricity)	8kWh	28.8	(1)
<b>Dewatering (dry process)</b>			
Electricity (mechanical dehydration)	2.36kWh	8.52	(3)
Heat (thermal drying)		81.8	(3)
<b>Dewatering (wet process)</b>			
Electricity (mechanical dehydration)	2.94kWh	10.6	(3)

### 3.8.3 Microalgae biomass conversion technology (Dry Process)

The biomass conversion process for the dry process route entails oil extraction from the 90% wt. DW biomass using hexane extraction process which is relatively inexpensive. Also, for this study, an alkalis catalyst KOH has been used for the methanol transesterification of the algal oil as it is 4000 times faster

than acid catalysed reactions (Yusuf, 2007). The choice of methanol is because it is relatively inexpensive.

The solid residue (LEA) is assumed to be utilised in a CHP unit for co-generation to offset electricity and heating demands in the production system. While the liquid component of the AD process with mineralised matter is re-injected into the production system and is considered as source of nutrient for the algae.

### 3.8.4 Microalgae oil extraction process

Since microalgae oil extraction is similar to that of soybean, the *C.vulgaris* biomass is pre-dried up to 90% wt. DW before being processed in the same fashion as soybeans oil. It is assumed that 70% of the microalgae oil is extracted using hexane, electricity and heat. The inflows for the oil extraction process have been computed as shown;

**Table 7 Input flow description for oil extraction per kg algae. Sources: (1) Lardon et al., 2009;**

Input flow description	Unit	Energy equivalent (MJ)	Reference(s)
<b>Oil Extraction</b>			
Hexane	15.2g		(1)
Electricity	0.42kWh	1.5	(1)
Heat	7.1MJ	7.1	(1)

### 3.8.5 Input flow description for the conversion technologies

Input flows for the conversion technologies per 1 kg delivered energy (biofuels) would be computed for each conversion process unit as follows.

**Table 8 Input flows for biomass conversion technologies per 1 kg delivered energy (biofuels). Sources: (1) Lardon et al., 2009**

Input flow description	Unit	Energy equivalent (MJ)	Reference(s)
<b>Transesterification</b>			
Algae Oil	0.89kg		Estimated
Algae cake (Allocation)	4.88kg	313.06	Estimated
Methanol	114g	0.40	(1)
KOH catalyst	273g		(1)
Heat		0.9	(1)
<b>AD</b>			
Biomass slurry	1kg		
Catalyst			
Enzyme			
Heat	0.9MJ		(1)

### 3.9 LCIA using Eco-indicator 99 method in SimaPro

The “cradle to gate” LCIA of each of the two production process chains has been analysed using Eco-indicator 99 method in SimaPro in comparison with four prospective alternative scenarios. Eco-indicator 99 method has been used to characterise all flows traversing the production system boundary into potential environmental damages in line with the goal and scope of this research. The categories of impacts are those due to: (1) fossil fuels use, (2) mineral use, (3) land use, (4) acidification/eutrophication, which is associated with the emission of acidifying substances/effects of discharging excessive high volumes of nutrients, (5) ecotoxicity, (6) ozone layer depletion, (7) radiation, (8) climate change, (9) respiratory inorganics, (10) respiratory organics, and



(11)carcinogens. This is focused on identifying the reasonably best environmental performance production scenario for each option.

Eco-indicator 99 method in SimaPro is an adaptable and flexible impact assessment tool that can be used for any LCA (Goedkoop and Spriensma, 2001). As it enables the user to quantify scores of environmental damages and also aggregate pre-calculated damage indicator up to single score level (Hajjaji et al., 2013) per unit of material or production process. Thus it is a useful tool for product/process development or environmental benchmarking of product systems. Eco-indicator 99 method in SimaPro is presented in three different versions (based on varied sets of model assumptions), for evaluating environmental damages and single score (Pt): Egalitarian (E), Hierarchist (H) and Individualist (I) versions. The key approaches related to the three prescribed versions in Eco-indicator 99 are summarised in Table 9 below. However, in this report the default Eco-indicator 99 Hierarchist (H) perspective has been adopted for evaluating potential damages associated with the production processes. As it aggregates average weighting set or damage category level taking into account, short term and long-term damages in line with international agreement (Hajjaji et al., 2013). Moreover, the hierarchical perspectives is widely more applied amongst policy makers and the scientific community, as it is based on facts that are supported by scientific and political groups (Goedkoop and Spriensma, 2001). The Intergovernmental Panel on Climate Change (IPCC) guideline for climate change is an example in this regard, with wide acceptance.

**Table 9 Typical value systems of the three different perspectives ((Goedkoop and Spruiensma, 2001)**

<b>Archetypes:</b>	<b>Egalitarian</b>	<b>Individualist</b>	<b>Hierarchist</b>
<b>Predictions:</b>			
Criteria	Argument	Experience	Evidence
Management style	Preventive	Adaptive	Control
Distribution	Parity	Priority	Proportionality
Perception of time	Long term dominates short term	Short term dominates long term	Balanced distinction between short and long term
Intergeneration responsibility	Present <future	Present >future	Present = future
Views of resources	Depleting	Abundant	Scarce
Perception of needs and resources	Can manage needs, but not resources	Can manage needs and resources	Can manage resources, but not needs
Energy future	Low growth (radical change now)	Business as usual	Middle of the road (technical fix)
Attitude to nature	Attentive	Laissez-faire	Regulatory
Attitude towards humans	Construct Egalitarian society	Channel rather than change	Restrict behaviour
Attitude towards resources	Need reducing strategy	Manage needs and resources	Increase resources
Perception (myth) of humans	Nature ephemeral	Nature benign	Nature perverse/tolerant
Perception of human nature	Born good, malleable	Self-seeking	Sinful
Attitude towards risk	Risk-aversive	Risk-seeking	Risk-accepting

### **3.10 Sensitivity/Data Quality (uncertainty) Analysis**

This is a procedure in LCA practice which helps identify the most dominant pollution causing activities in the LCA process (ISO, 2006a). It also indicates or flags up the most critical inventory data set in the LCA, for which slight variation in value would change the ranking between compared alternatives. Similarly, it highlights the effects of substitute methodological choices (e.g. different allocation methods) and the degree of uncertainty in the results due to assumptions/estimates/aggregates of input data (Consultants, 2008). It gives an indication of the robustness of conclusions drawn in an LCA study.

In this study, Monte Carlo analysis function tool in SimaPro is used to evaluate the effect of imprecise data on the results of the impact assessment. These include data uncertainties, uncertainties relating to the representativeness of the models, and uncertainties due to incomplete modelling (Consultants, 2008). The Monte Carlo technique is a statistical based method which assigns a numerical value which appears as lognormal distribution by sampling a Pedigree matrix originally developed by (Weidema and Wesnæs, 1996). The experimental dataset in ecoinvent utilises the Pedigree matrix to estimate a standard deviation (SD) (uncertainty distribution) for each input data (process used). Ecoinvent subsequently generates the representative numerical value based on specific uncertainty data you input during computation using SimaPro. The Monte Carlo function in SimaPro provides the opportunity to compare inherent difference between two alternative options or products and to know if such differences are significant or not. Such exploration of alternative options could help in identifying choices defining the baseline scenario.



# CHAPTER FOUR

## RESULTS AND DISCUSSIONS

### 4.1 Framework

The results in this study are based on investigations into the challenges limiting the microalgae (*C. vulgaris*) biofuel production process chain. The analysis herein is essentially with regards to material use, energy consumed and the environmental burdens associated with the production process. The goal is to determine the weak spots within the production system and identify changes in particular dataset that can lead to lower material use, energy consumption and lower environmental impacts than the traditional microalgae biofuel production system.

Consequently, detailed analysis were carried out, leading to the following: LCA overview of the microalgae biofuel pathway, transesterification process Life Cycle Inventory (LCI) results, Life Cycle Impact Assessment (LCIA) at characterisation level, single score impact assessment (by grouping and ranking), and LCIA results comparison of the baseline scenario of each of the two selected microalgae biofuel production models (transesterification and AD transformation process) with that of four proposed prospective alternative scenarios of; (1) 100% recycling of production harvest water, (2) utilization of wastewater/sea water as culture medium, (3) co-products economic allocation consideration and (4) 70% induced increase in algae oil yield strategy. These analytical procedures are aimed at identifying the most polluting step in the life cycle as well as, determining the most problematic environmental impact, and checking the effect of changes in scenarios or particular critical data to the overall results.

This chapter describes the results from the analysis of the effects and impact of changes in particular data sets and how these affect the overall algae biofuel baseline production process. The result analyses show how systematic change of variable input parameter could help understand the most critical variable, by indentifying the source of impacts and developing realistic alternative actions at

design/production stages. Indeed, these findings provide a starting point for future studies and actions, as previous studies have ignored using and comparing different algae biofuel production methods in practice. Furthermore, it could offer potential cost savings when used as a driver of innovation.

## **4.2 Microalgae biofuel pathway baseline case**

The LCA of microalgae biofuel pathway examined in this study is focused on assessing the pathways for the production of (1) 1kg biodiesel via transesterification of extracted algal lipid, and (2) 1kg biogas via AD transformation of microalgal biomass, respectively. Alternative scenarios that are thought to be less material consuming, less energy intensive, and less environmentally harmful are explored in this report. Hence, one key objective of this LCA research is to compare the material resource used and the potential environmental footprint for each proposed alternative production scenarios, with that of a reference (baseline) scenario. Consequently, a baseline model for each of the chosen biofuel production pathway has been specified. Key technical issues that affect LCA studies such as system boundary (which describes the scope within which resources are used) and emission footprints of the product system, allocation issues and how they are handled have been considered as specified in the methodology chapter.

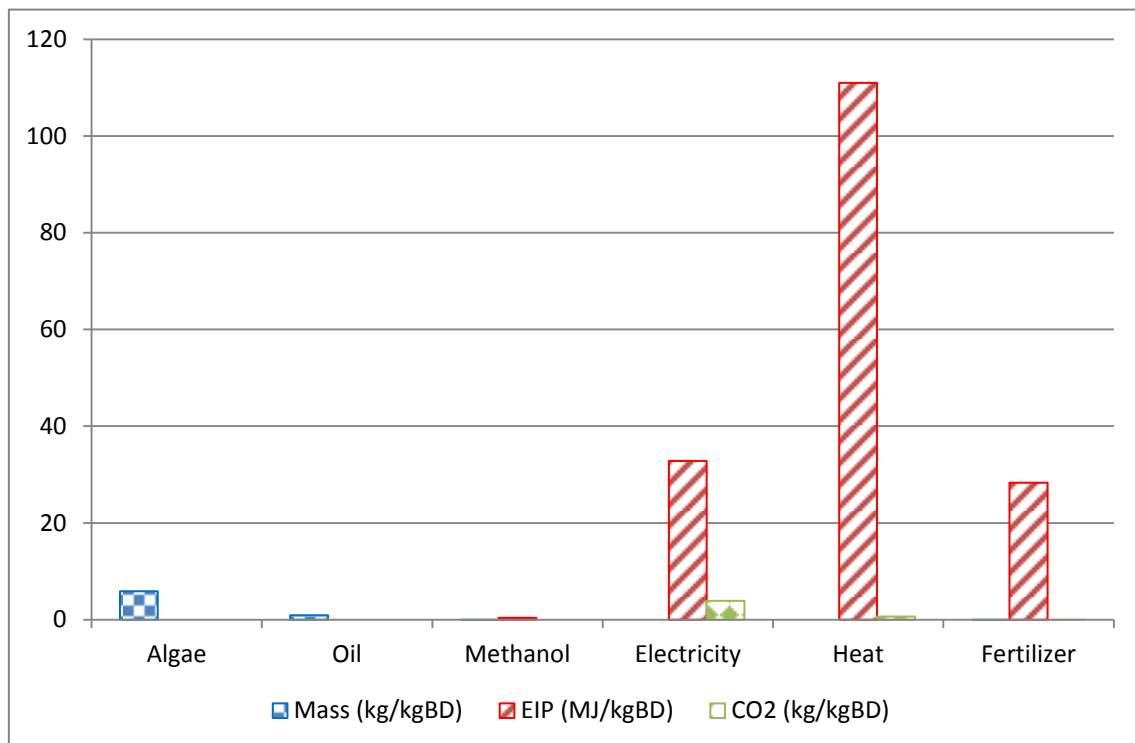
Furthermore, the microalgae Biodiesel (BD) transesterification production model used in this analysis is based on detailed process modelling of conventional BD production path way with an attempt to account for all mass and energy balances for each phase within the production system boundary. However, estimated values are assigned for key nutrients and all vital energy inputs when necessary. For example, algae CO<sub>2</sub> requirement, nitrogen (N) and phosphorous (P) concentration values were stoichiometrically evaluated and determined for microalgae cultivation.

Most of the data used were sourced from existing literatures (Brennan and Owende, 2010; Richmond, 2004), ecoinvent data base (Frischknecht and

Rebitzer, 2005), government agencies reports (Frank et al., 2011), other LCA inventories, and private communication with other researchers at conferences. However, process models data were used in some instances because no empirical or published data exists of a complete energy balance of microalgal to biofuel transformation model.

### 4.3 LCI results of biodiesel transesterification process

The result in Figure 14 below shows the process contribution analysis chart generated from the input/output data required to produce 1kg biodiesel (kgBD). It incorporates the quantitative mass requirement of algae biomass ( $5.88 \text{ kg}\cdot\text{kgBD}^{-1}$ ), algae oil ( $0.89 \text{ kg}\cdot\text{kgBD}^{-1}$ ), methanol ( $0.1 \text{ kg}\cdot\text{kgBD}^{-1}$ ), electrical input energy ( $32.79 \text{ MJ}\cdot\text{kgBD}^{-1}$ ), fertilizer requirement ( $28.3 \text{ MJ}\cdot\text{kgBD}^{-1}$ ) and  $\text{CO}_2$  emissions ( $4.63 \text{ kg}\cdot\text{kgBD}^{-1}$ ) resulting from the cultivation process, harvesting/concentration and processing of extracted algae lipids via transesterification, to produce 1kg biodiesel (as depicted in Tables 10 and 11 below).



**Figure 14 Process Contribution Analysis for the production of 1kg biodiesel (BD) via Transesterification (Domoyi et al., 2013)**

The LCI result indicates the material mass flows, energy flows and CO<sub>2</sub> emission drivers within the algae biodiesel production process which includes: energy consumed by the flat-plate PBR, hydraulic pumps for the open raceway pond, centrifuge, mechanical dryers, heating requirement to concentrate the biomass to 90% DWB (similar to that of soybean oil), fertilizer requirement, hexane oil extraction process and conversion techniques, respectively.

**Table 10 Transesterification process Input parameters to produce 1kg Biodiesel (BD), Note: EIP is energy input; while, EOP is energy output.**

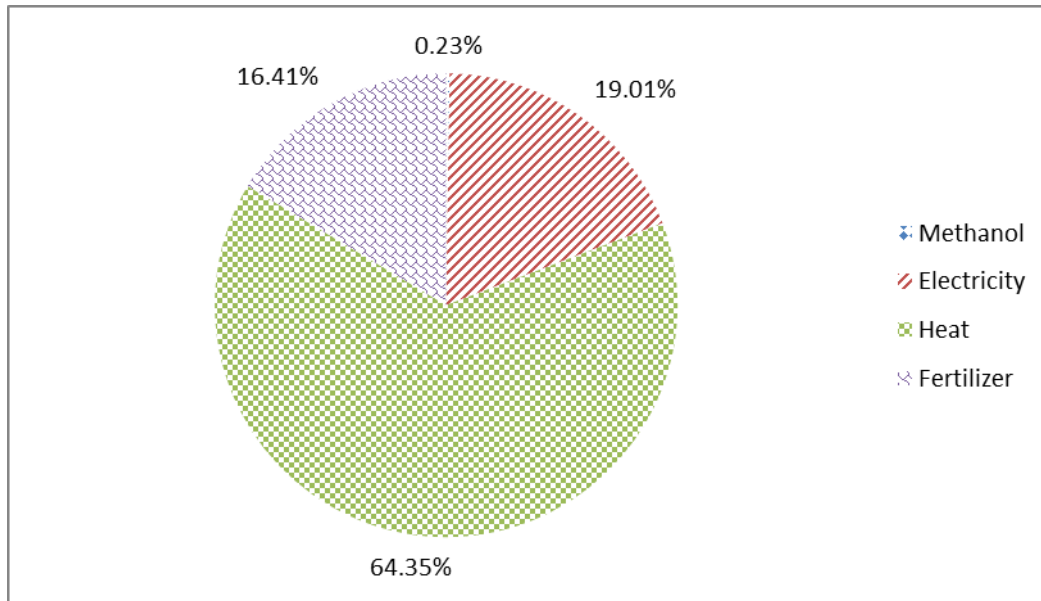
<b>INPUT</b>			
<b>Materials</b>	<b>Mass (kg·kgBD<sup>-1</sup>)</b>	<b>EIP (MJ·kgBD<sup>-1</sup>)</b>	<b>CO2 (kg·kgBD<sup>-1</sup>)</b>
Algae	5.882	0	0
Oil	0.896	0	0
Methanol	0.104	0.403	0
Electricity		32.796	3.917
Heat		111	0.638
Fertilizer	0.044	28.3	0.078
<b>Total</b>		<b>172.499</b>	<b>4.633</b>



**Table 11 Transesterification process output parameters**

<b>OUTPUT</b>			
<b>Materials</b>	<b>Mass (kg·kgBD<sup>-1</sup>)</b>	<b>EOP (MJ·kgBD<sup>-1</sup>)</b>	<b>CO2 (kg·kgBD<sup>-1</sup>)</b>
Biodiesel	1	37.8	2.86
Glycerol	0.018	0	0
Cake	4.882	313.057	0
<b>Total</b>		<b>350.857</b>	<b>2.86</b>

Similarly, Figure 15 below (pie chart) characterises the considered LCI input energy profile for the biodiesel (transesterification) production system. It depicts the quantitative percentage energy input value of each material, for the production of 1 kg BD. The results indicates that heating to dry the algae biomass to 90% DWB accounts for of 64% of the total input energy for the transesterification input energy profile. With electrical energy need at various instances within the production model and fertilizer requirement, contributing 19% and 16% of total energy input respectively as shown (Mathew et al., 2013b). These results are similar to recent LCA analyses reports (Clarens et al., 2010; Lardon et al., 2009; Collet et al., 2011), where electricity requirement were reported to represent 20% to 30% of the total production cost with heating obligation exceeding 50% correspondingly. Such contribution analysis evidence indicates where to focus attention on within the production system, and consider if the evaluation are sufficiently representative and significant. If significant, then more intensive data gathering can take place.



**Figure 15 Pie chart showing Energy Input (EIP) profile for producing 1 kg biodiesel via transesterification**

Thus, the LCI energy profile analysis is a valuable diagnostic procedure, as it provides information requirement which can help in identifying process improvement options in the production chain based on the relative contribution per process, within the production system. It also accounts for the total contribution of a process (e.g. electricity) that is used more than once within the production chain. Which although, may have a small contribution value in each occasion, but however with the total cumulative contribution of all instances being significant. Consequently, the process contribution analysis is a very useful and obligatory component of every LCA (ISO, 2006a). Besides, the LCI results are detailed, and it is not affected by the degree of uncertainties introduced in the LCIA phase.

#### 4.4 LCIA results of baseline biodiesel production model

In LCIA using SimaPro, the inputted inventory is analysed and characterised into comparable units (Goedkoop and Spriensma, 2001) based on specific substances that are emitted or used in the production process. For example, 1 kg Methane (CH<sub>4</sub>) emitted, is considered to be equal to 25 kg CO<sub>2</sub> equivalent (Henrikke, B and Anne-Marie, T., 2004) with regards to their GWP for a 100 years' time horizon. The data inputted in SimaPro data sheet (see Table 12) for the LCIA analysis of the baseline BD production model are similar to those in Tables 10 and 11.

The results for the characterization model of the baseline scenario for the production of 1 kg microalgae BD via transesterification is shown in Figure 16. Eco-indicator 99 method in SimaPro 7 has been used to characterise and evaluate potential environmental impacts. Again, the categorised impacts are: (1) fossil fuels use, (2) minerals use, (3) land use, (4) acidification/eutrophication, (5) ecotoxicity, (6) ozone layer depletion, (7) radiation, (8) climate change, (9) respiratory inorganics, (10) respiratory organics, and (11) carcinogens. However, most of the discussions about the results are focused on the contributions of energy (heat & electricity) usage, material consumption and environmental emissions within the production system towards the different impacts categories in line with the scope of this study.

**Table 12 SimaPro Input data sheet for the production of 1 kg biodiesel (baseline scenario), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation**

Product produced	Amount	Unit	Quantity	allocation
Algae Biodiesel (BD)	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	3726	kg	Lognormal	1.06
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.06

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.33	kg	Lognormal	1.06
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.71	kg	Lognormal	1.06
Soya oil, at plant/RER U	0.89	kg	Lognormal	1.05

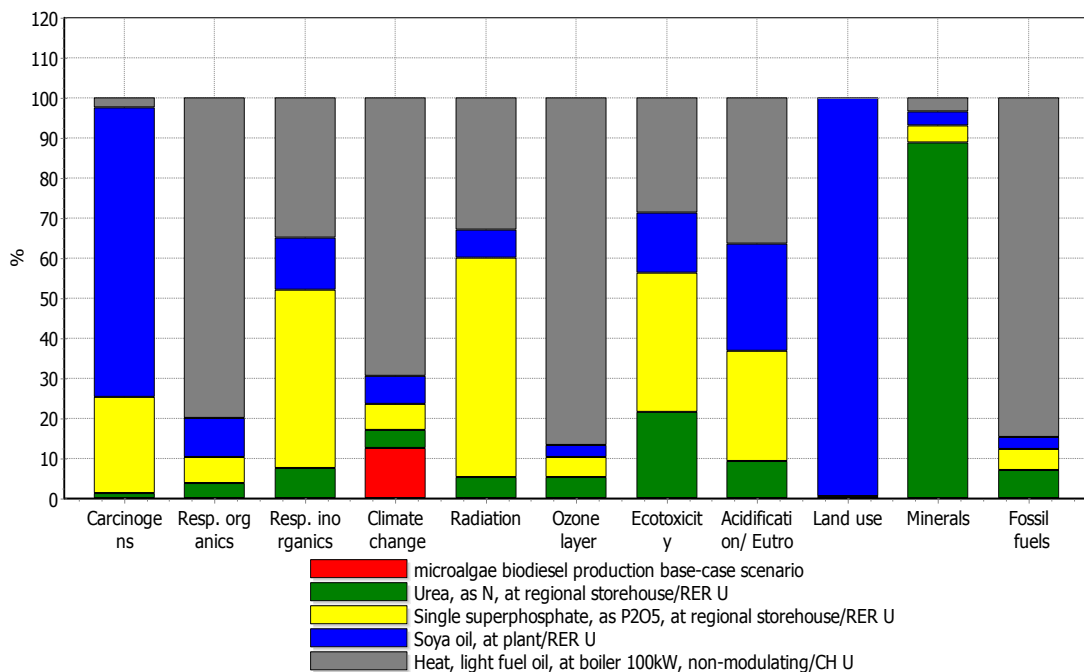
Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	172.49	MJ	Lognormal	1.21

Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.21

Thus, electrical and heat energy obligation represents the most contributing factor for most of the impact categories in Figure 16. It accounts for about 84.7% (30.7 MJ surplus) of the proportion of fossil fuel use, climate change (69.4%), ozone layer depletion (86.8%), and acidification/eutrophication

categories (36.6%), respectively. The high proportional contribution of energy use indicated for most of the impact categories relative to other resources is typically due to the concentration/drying requirement to get the algae biomass up to the requisite 90% DWB (similar to that of soybean). This is in order to ease downstream processes of lipid extraction and subsequent transesterification of extracted algae lipid into biodiesel (Yusuf, 2007; Lardon et al., 2009).



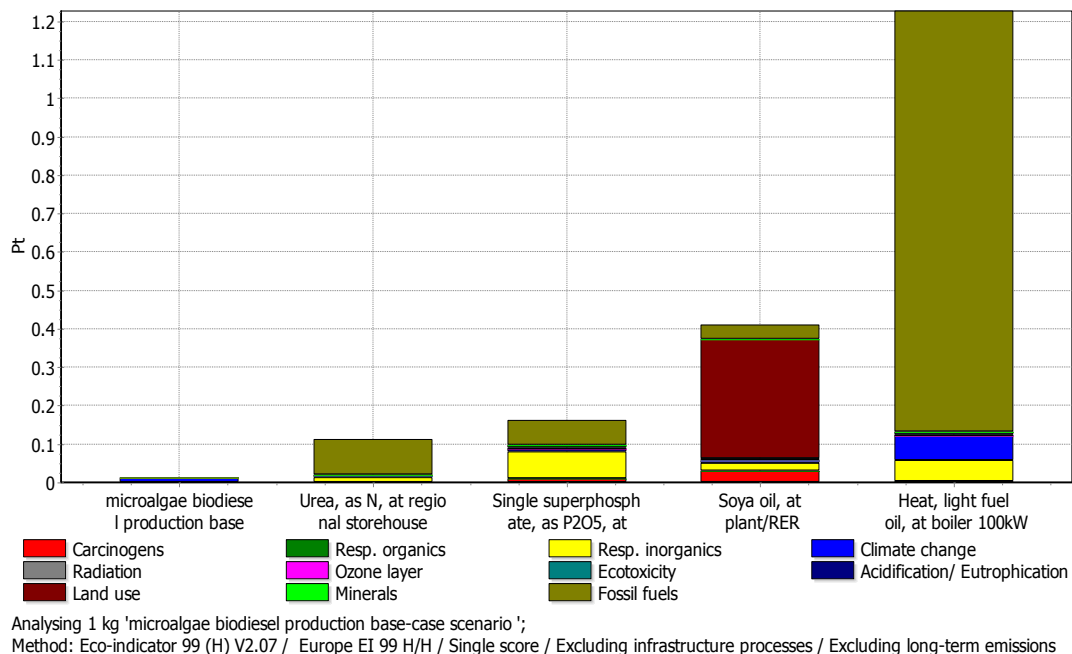
Analysing 1 kg 'microalgae biodiesel production base-case scenario';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Damage assessment / Excluding infrastructure processes / Excluding long-term emissions

**Figure 16 Analysing 1 kg microalgae biodiesel production (baseline scenario) at impact characterisation level**

Similarly, impact characterisation results of soya oil use signify 99.5% contribution for land use impact. This is typically because soya oil (similar to algae oil) process data was used as representative of microalgae oil (lipid), which is currently not included in SimaProecoinvent data base. This could possibly be the likely reason of the large value indicated for land use. Due to

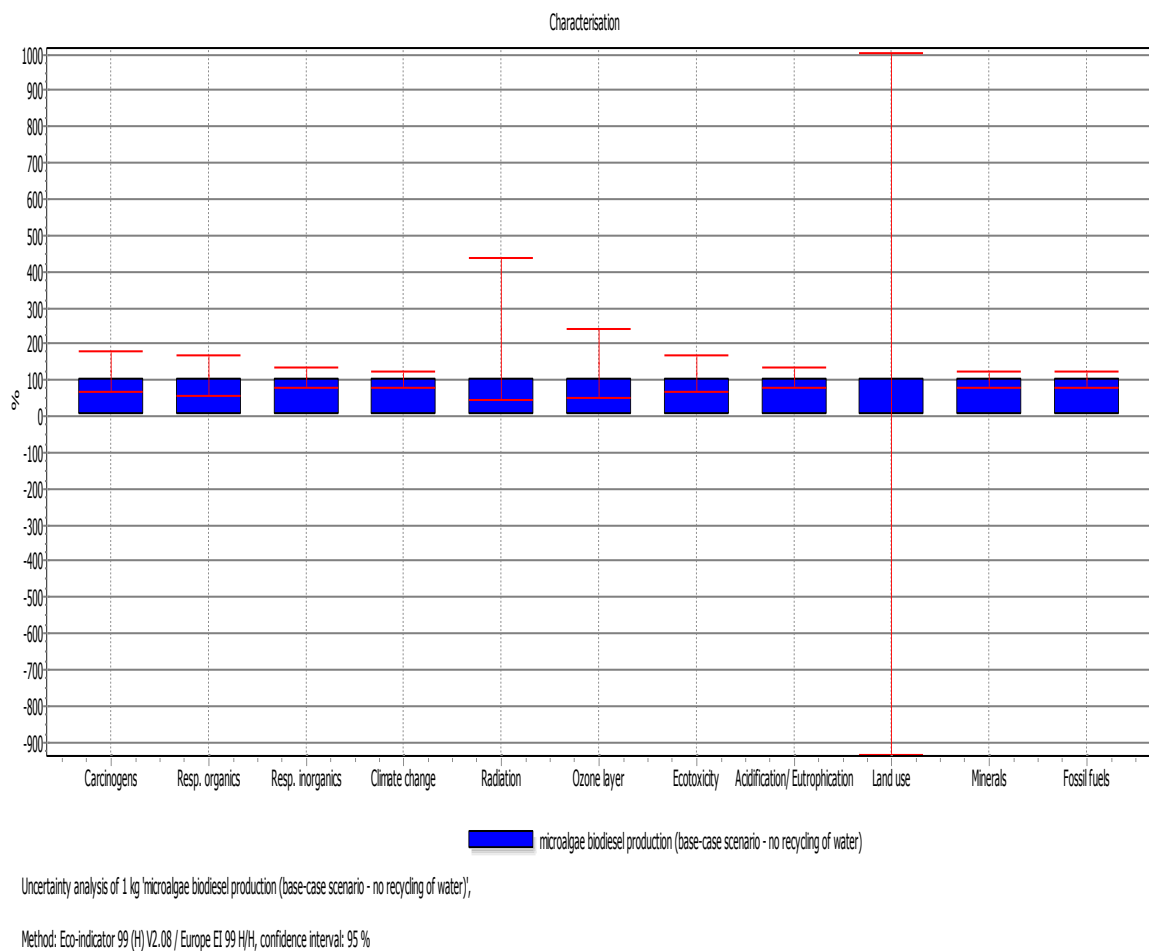
the huge land use requirement associated with soya bean plant cultivation and oil extraction.

Additionally, single score LCIA was carried out for the baseline BD production scenario as depicted in Figure 17. The single score impact ranking indicates that fossil fuel use (1.1 points), land use (0.31 points), respiratory inorganics (0.07 points), and climate change (0.06 points), respectively in a descending order of significance. Fossil fuel use apparently, accounts for the highest impact point of (1.1 points) amongst all impacts categories ranked for electrical/heat energy obligation and significantly, towards other unit process material requirement within the product system. The single score impact assessment scale applied here, is used to simplify the interpretation of results. As impact categories are grouped and ranked in order of significance. Though, it is regarded as an optional analysis technique (ISO, 2006b).



**Figure 17 Analysing 1 kg microalgae biodiesel production (baseline scenario) at single score impact level**

Furthermore, as a check of correctness (uncertainty) or otherwise of the dataset used, representativeness of the model, and incompleteness of the model. The baseline model was characterised using the Monte Carlo uncertainty analysis function in SimaPro with the distribution and standard deviation set for each input as shown in Table 12. Figure 18 shows the uncertainty analysis results for the baseline BD production model per impact category. The range of each bar chart expresses the 95% confidence interval. Obviously the score for land-use has a relatively high uncertainty. This may be due to the use of soya oil dataset in ecoinvent database in place of algae oil, which is not at the moment characterised in SimaPro as mentioned earlier. Also, the uncertainty scores for radiation and ozone layer depletion impact categories are also high. Most of the other scores have an uncertainty of above 100%, which is also high.



**Figure 18 Graphical representation of uncertainty analysis of 1 kg biodiesel production (base-case scenario)**

However, absolute uncertainties at characterisation level for single process or product stage are often quite high (Consultants, 2008; Goedkoop and Spriensma, 2001). Consequently, uncertainty analysis is more useful in analysing uncertainty of the difference between two products.



#### **4.4.1 LCIA of microalgae biodiesel production with 100% recycling of production harvest water**

The Life Cycle Impact Assessment (LCIA) result for producing 1 kg biodiesel with 100% recycling of production harvest water is presented in Figure 19. The SimaPro input process data for this production scenario are as shown in Table 13. The input data are based on the assumption of a 55% reduction in algae nutrient (fertilizer) requirement, due to 100% production water recycling as proposed by Yang et al., (2011). This consequently, reduced water demand from 3726 kg freshwater·kgBD<sup>-1</sup> to 591 kg freshwater·kgBD<sup>-1</sup> (Yang et al., 2011) after accounting for 15.9% evaporative losses/biomass drying.

**Table 13 SimaPro Input data sheet for the production of 1 kg biodiesel with 100% recycling of production harvest water**

Product produced	Amount	Unit	Quantity	allocation
Algae Biodiesel (BD)	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\wedge$ 2 or 2*SD
Water, unspecified natural origin	591	kg	Lognormal	1.05
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.06

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\wedge$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.18	kg	Lognormal	1.56
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.39	kg	Lognormal	1.51
Soya oil, at plant/RER U	0.89	kg	Lognormal	1.21

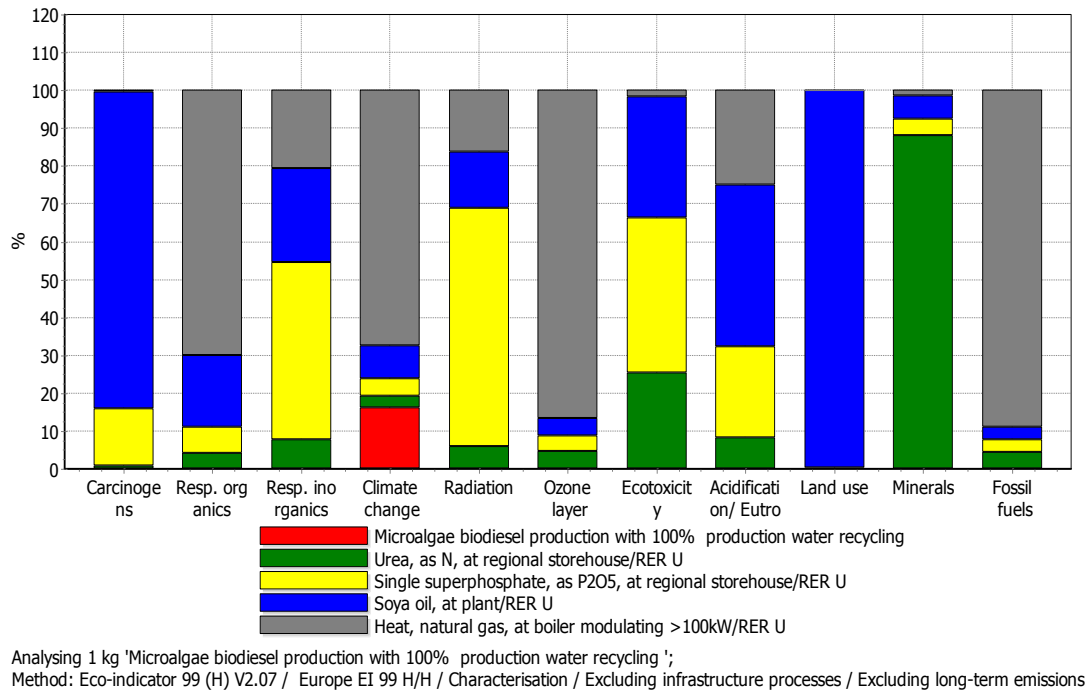
Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\wedge$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	172.49	MJ	Lognormal	1.62

Emissions to air	amount	Unit	Distribution	SD $\wedge$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.21

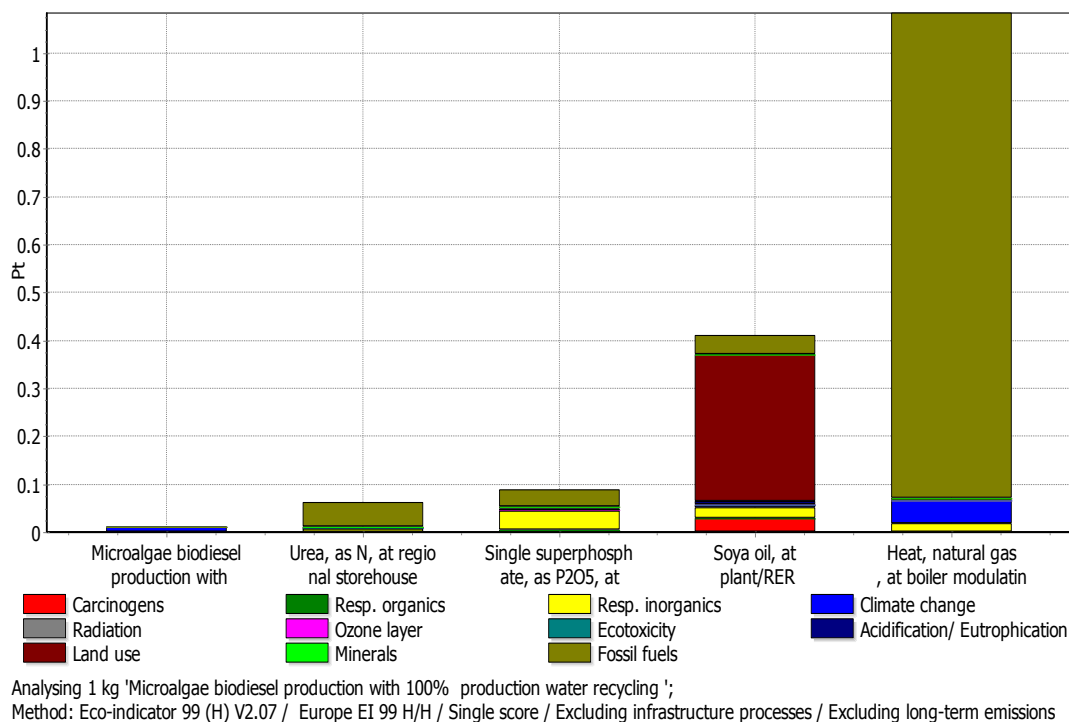
The LCIA characterisation results (Figure 19) for heat/electricity use indicated a 28.4 MJ surplus (88.9%) of total contribution towards fossil fuels use impact category. With soya oil, single superphosphate (as P<sub>2</sub>O<sub>5</sub>), and Urea (as 46% N

content) accounting for 1.11 MJ (3.48%), 1.02 MJ (3.21%), and 1.4 MJ (4.39%) respectively.



**Figure 19 Analysing 1kg microalgae biodiesel production with 100% production water recycling at impact characterisation level**

Likewise, for climate change impact category, heating/electricity contributed 67.4% of the total impact contributions, while the remaining proportion of impact is accounted for by soya oil (8.9%), P<sub>2</sub>O<sub>5</sub> (4.58%), and Urea as N (3.07%) separately. Similarly, heating/electricity obligation contributed 86.6% of the total impact extent for ozone layer depletion category, with soya oil (4.69%), P<sub>2</sub>O<sub>5</sub> (4.18%), and Urea as N (4.53%), accordingly. These results confirm the relatively high energy aggregate associated with algae cultivation, harvesting, concentration and drying to the required 90% DWB for lipid extraction and subsequent processing into biodiesel (Grierson et al., 2013).



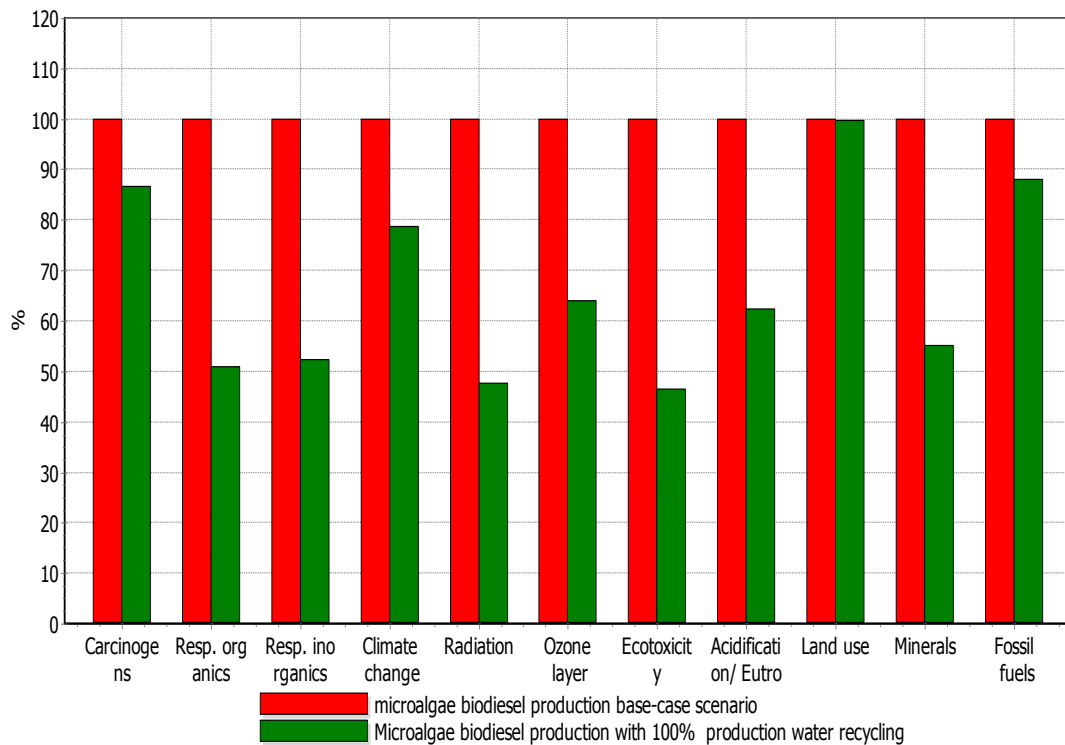
**Figure 20 Single score analysis of microalgae biodiesel production with 100% recycling of production harvest water**

In addition, Figure 20 above depicts the single score LCIA description of a microalgae biodiesel production system with 100% production harvest water recycling. In descending order of impact assessment ranking significance, the results indicate that; fossil fuel used showed the highest impact (1.01 points), followed by land use impact category (0.308 points), climate change (0.049 points), and respiratory inorganics (0.038 points) accordingly.

Equally, in order to evaluate the significance of the sensitivity prospect of recycling of production harvest water on the proposed microalgae biodiesel production system. LCIA results for the baseline microalgae (*C. vulgaris*) biodiesel production model were compared with that of a biodiesel production scenario incorporating 100% recycling of production harvest water using Eco-indicator 99 method in SimaPro. Figure 21 shows the comparison results of

both scenarios. Fossil fuel use impact category indicates 4.3 MJ savings (18.9%) in heat/electricity obligation due to 100% recycling of production water, compared to the baseline scenario of no recycling. Similarly, 100% recycling indicated a 78.6% contribution on climate change impact category relative to the baseline scenario. This translates to 21.4% reduction on climate change impact contribution when compared to the baseline production scenario of non-recycling of production harvest water.

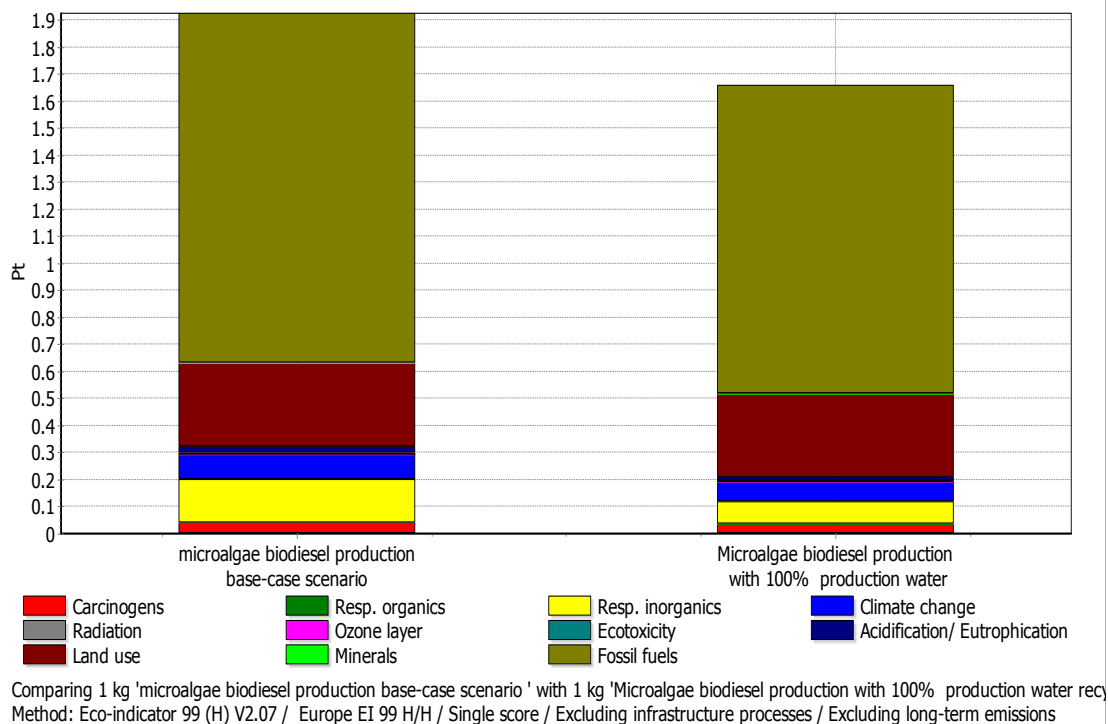
Also, LCIA contribution analysis for ozone layer depletion impact category, 100% recycling of production water indicated 63.9% in relation to the baseline scenario of no recycling. Which is 36.1% impact reduction for this category of impact. However, the most significant impact of the sensitivity prospect of 100% recycling of production water is on mineral use category 0.00265 MJ surplus (55%), compared to the baseline scenario of non-recycling of production harvest water. This translates to a 45% reduction and savings in mineral use obligation for the proposed algae biodiesel production model. This results validates earlier findings of the overall impact of recycling, as inorganic nutrient obligation increases with decrease or non-recycling of production harvest water (Yang et al., 2011). However, water recirculation may lead to excess nutrient concentration.



Comparing 1 kg 'microalgae biodiesel production base-case scenario ' with 1 kg 'Microalgae biodiesel production with 100% production water recycling'  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 21 Comparing 1 kg microalgae biodiesel production (baseline scenario) v. 1 kg biodiesel production with 100% recycling of production harvest water**

Additionally, single score LCIA was applied to show in clearer detail (Figure 22) the distribution for each individual substance, impact category and damage category. The comparison results of 1 kg biodiesel production (baseline scenario) with 1 kg biodiesel production with 100% production harvest water recycling, shows how inorganic mineral requirement need reduced individually: urea as N (from  $0.33\text{kg}\cdot\text{kgBD}^{-1}$  to  $0.18\text{kg}\cdot\text{kgBD}^{-1}$ ) and  $\text{P}_2\text{O}_5$  as P (from  $0.71\text{kg}\cdot\text{kgBD}^{-1}$  to  $0.39\text{kg}\cdot\text{kgBD}^{-1}$ ). Also, 100% recycling of production harvest water, brought about variable marginal reductions on all impact categories on the chart values compared to the baseline scenario.

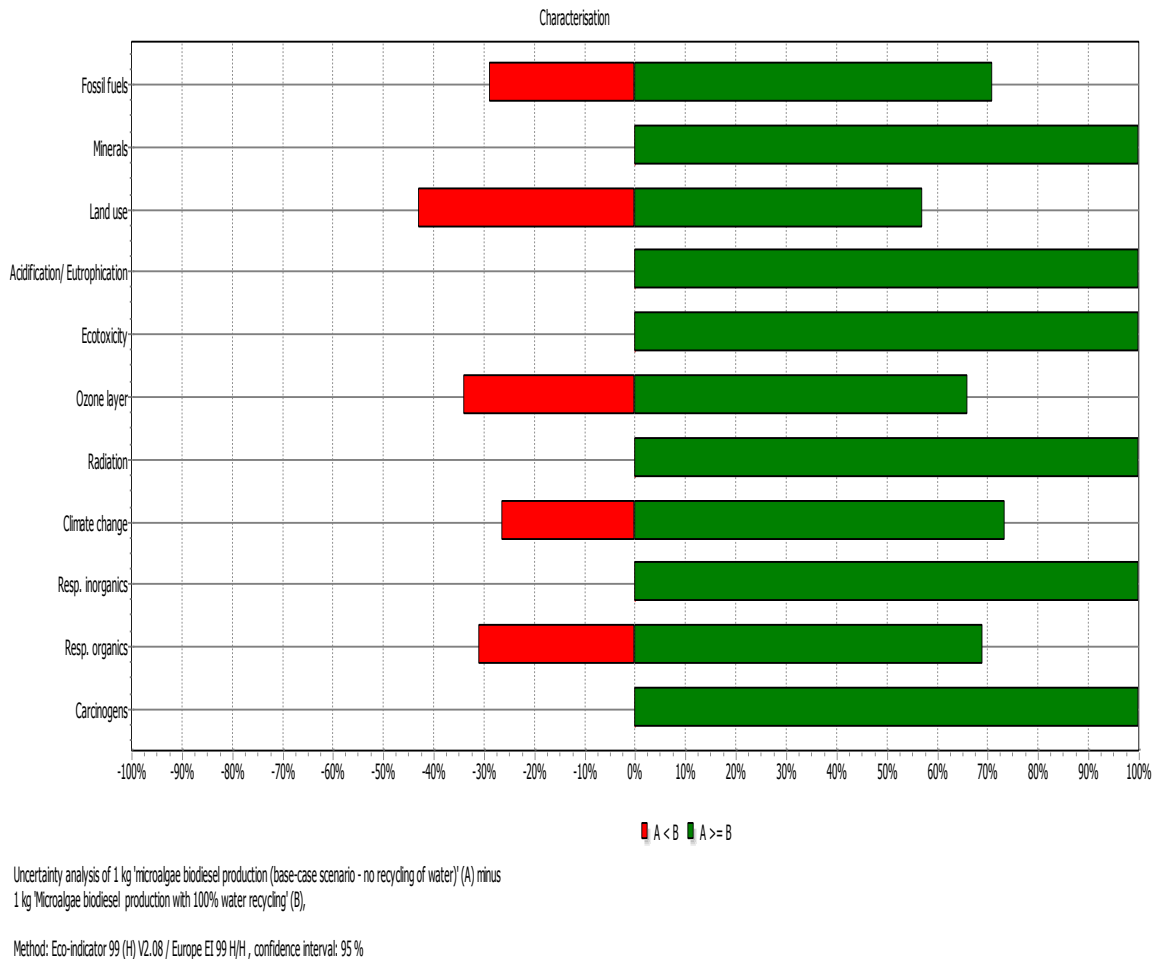


**Figure 22 Single score LCIA: comparing 1 kg biodiesel production (baseline scenario) with 1 kg biodiesel production with 100% production water recycling**

Furthermore, uncertainty analysis was carried out using the Monte Carlo uncertainty function in SimaPro with the distribution and standard deviation set of values for each input in Table 13. This is aimed at comparing the differences in results between the baseline models of producing 1 kg biodiesel from algae without recycling production harvest water with that of 100% recycling of production harvest water. Figure 23 below highlights the significance or otherwise.

The results indicates that 1 kg microalgae biodiesel production baseline scenario (process A) has higher score outcomes than the sensitivity prospect model of producing 1 kg biodiesel from microalgae with 100% recycling of production harvest water (process B), as shown per impact category. Although the absolute uncertainties for each impact category are high (Figure 23), the

value however, show that the differences shown in Figure(s) 21 & 22 are certainly significant. This confirms that there is clearly a significant difference between process A and B.

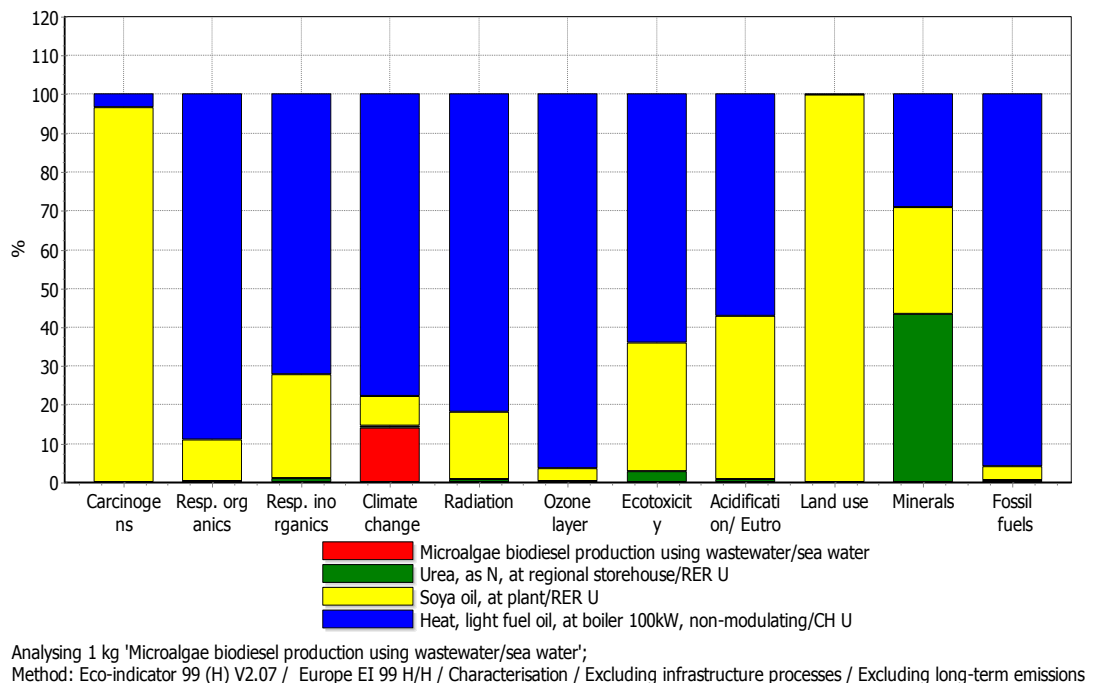


**Figure 23 Uncertainty analysis of 1 kg microalgae biodiesel production (baseline scenario) v 1 kg microalgae biodiesel production with 100% recycling of production harvest water**



#### 4.4.2 LCIA results of prospects of using wastewater/sea water

The utilization of wastewater/sea water application to grow algae is considered as a potential means of optimizing microalgae biofuel production system. As it brings about considerably enhanced economics (Brennan and Owende, 2010), with regards to minimizing the use of freshwater resources while also providing treated water for other application amongst other benefits. This prospect is thought to offer a potentially lower-cost and as such an environmentally sustainable means of treating wastewater compared to conventional wastewater treatment techniques (Pittman et al., 2011; Park et al., 2011). Consequently, in order to fully understand the trade-offs between using freshwater and the use of wastewater/sea water, a hypothetical scenario was analysed using SimaPro with results as shown in Figure 24 below. The SimaPro input data used for this analysis is as shown in Table 14.



**Figure 24 LCIA analysis of 1 kg microalgae biodiesel production using wastewater/sea water**

The results show electricity/heating requirement accounting for the highest percentage contribution in most of the impact categories except for land use impact and carcinogens impact categories. As indicated, electricity/heating obligations accounts for 96% (30.7 MJ surplus) contribution towards fossil fuels use impact category. Likewise, electricity/heating represents 77.8% of the contributions towards climate change impact, 96.3% towards ozone layer depletion, and 57.3% of impacts contribution due to acidification/eutrophication, respectively. The results apparently show that the percentage contribution of electricity/heating requirement increased for most of the considered impact categories with regards to the baseline scenario. However, this is based on the assumption of a reduction of 90% in water obligation, owing to the use of wastewater/sea water (Yang et al., 2011). The use of wastewater/sea water also, potentially eliminates  $P_2O_5$  obligation as reported by Yang et al., (2011) and relatively lowered urea requirement to 0.000259 MJ surplus (43.4%). This becomes clearer comparing it with the base-case scenario values for urea 0.00428 MJ surpluses (88.8%) and  $P_2O_5$  0.0002 MJ surpluses (4.15%), for mineral use respectively.

**Table 14 SimaPro Input data sheet for the production of 1 kg biodiesel using wastewater/sea water as culture media**

Product produced	Amount	Unit	Quantity	allocation
Algae Biodiesel (BD)	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	372.6	kg	Lognormal	1.05
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.21

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.02	kg	Lognormal	1.05
Soya oil, at plant/RER U	0.89	kg	Lognormal	1.06

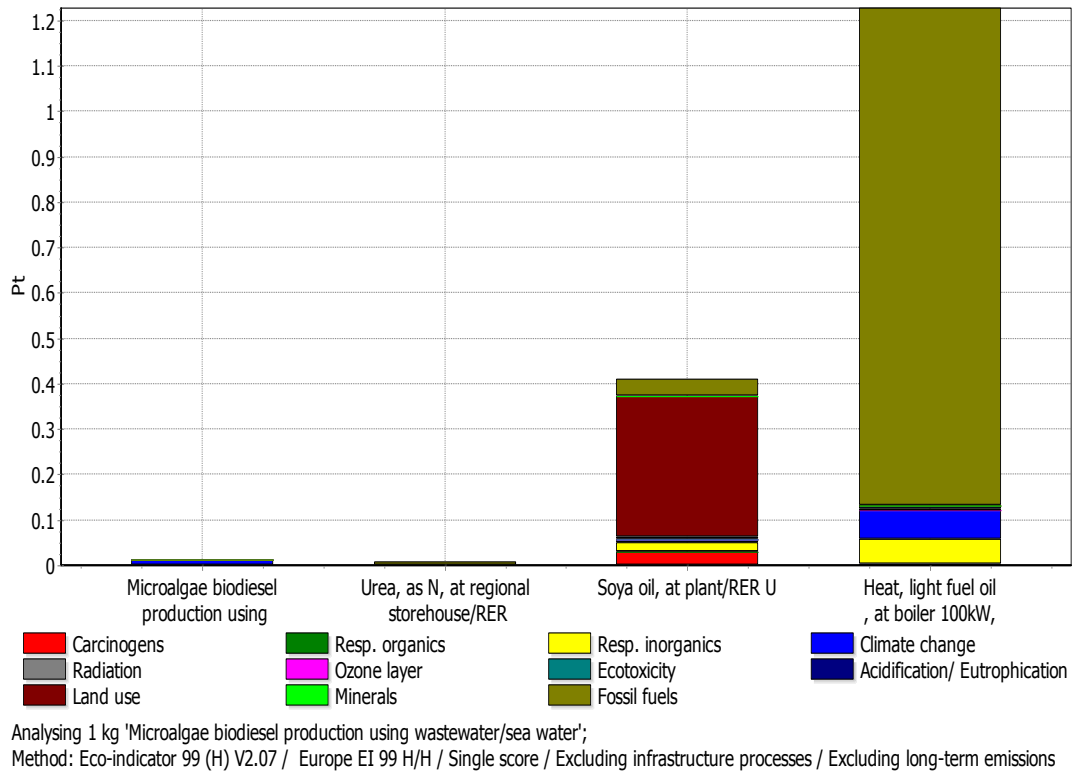
  

Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	172.49	MJ	Lognormal	1.21

Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.21

Furthermore, Figure 25 considers the LCIA characterised single score impact results of a biodiesel production scenario using wastewater/ sea water as culture media. The results highlight the quantitative contribution for each specific substance, impact category and damage category. The single score impact results in ascending order of impact indicates that, climate change impact category (0.064 point), land use impact (0.308 point), and fossil fuels use impacts (1.1 points) respectively.

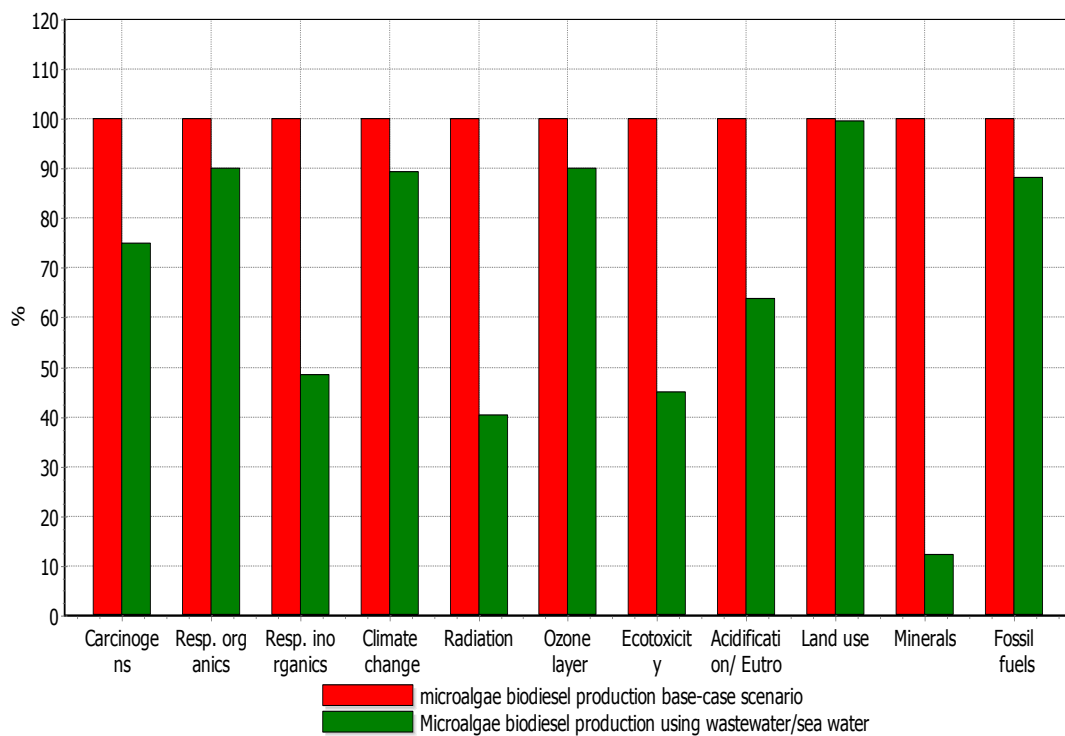


**Figure 25 single score impact analysis of 1 kg microalgae biodiesel production process using wastewater/sea water**

Additionally, in order to further assess the significance of the prospect of using wastewater/sea water as the production culture media for the proposed microalgae biodiesel production system. Comparisons were made for both production scenarios using eco-indicator 99 methods in SimaPro. Figure 26 below shows the graphical chart distribution of the difference between 1 kg microalgae biodiesel production baseline scenario (using freshwater) vs. 1 kg algae biodiesel productions using wastewater/sea water.

The results show that, using wastewater/sea water in place of freshwater, impacted positive difference potentially between the two process options. For instance, fossil fuels use impact category indicated a 4.2 MJ savings (11.8%) in

electricity/heat requirement due to the use of wastewater/sea water as against using freshwater. Similarly, the prospect of using wastewater/sea water as culture medium for producing 1kg microalgae biodiesel indicated the greatest impact margin for mineral use impact category. As the results show 87% offset (i.e. 0.0042 MJ surplus) in mineral use requirement, as a consequence of the application of wastewater/sea water, as an alternative to using freshwater as the culture media. Likewise, climate change impact category indicated a 10.7% reduction, ecotoxicity impact category 54.9% reduction, and other impact categories showed similar positive reductions as a result of using wastewater/sea water application.



Comparing 1 kg 'microalgae biodiesel production base-case scenario ' with 1 kg 'Microalgae biodiesel production using wastewater/sea water'; Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 26 Comparing 1 kg microalgae biodiesel production (base-line scenario) v 1 kg microalgae biodiesel production using wastewater/sea water**

#### **4.4.3 LCIA of biodiesel production scenario with co-products economic allocation consideration**

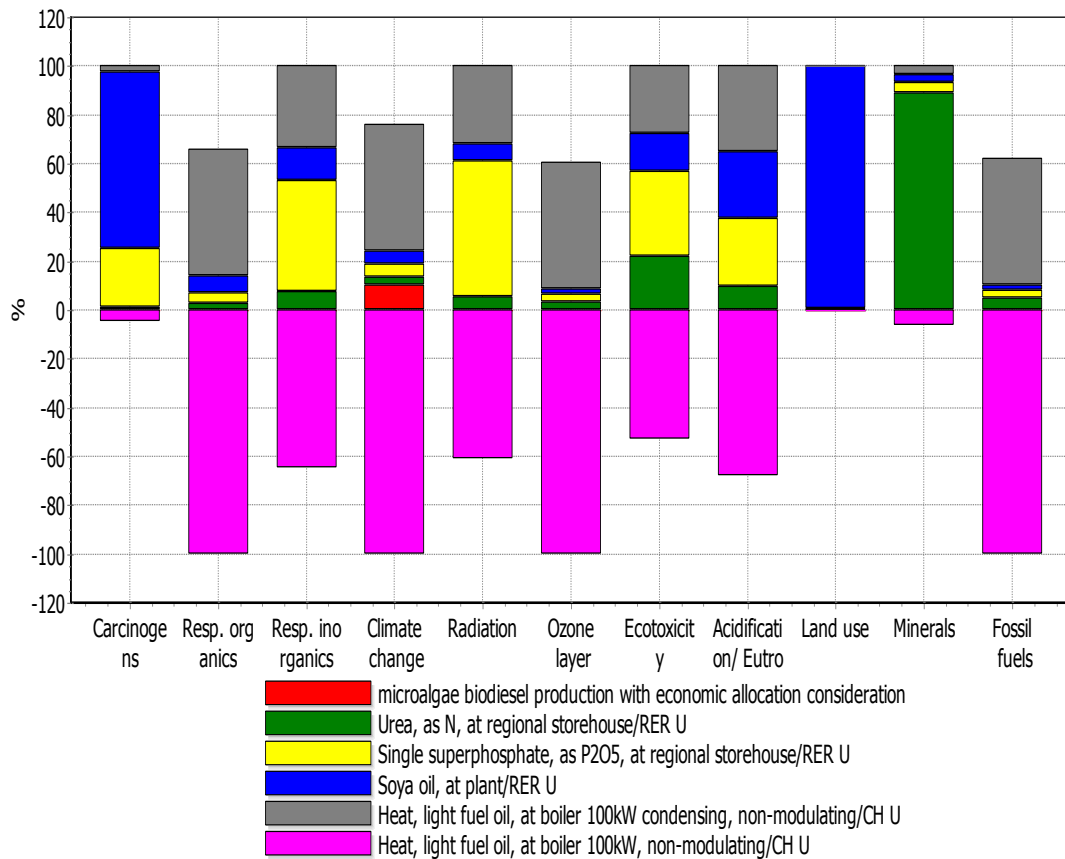
The LCIA analysis of the biodiesel production scenario integrating co-products allocation consideration aims at evaluating the significance of allocation consideration on the production system. It also, intends to assess how allocation can sways the material consumption and the environmental burden for a multi-output scenario, like the present microalgae biodiesel transesterification process. Consequently, glycerol is inventoried as co-product to the main output product (biodiesel) using mass-economic basis as shown in the data sheet (Table 15) inputted in SimaPro. Similarly, the residual 4.88kg algae cake is considered to be avoided products (i.e. materials/processes that are avoided through the use of this material/process). This is based on the assumption that the algae cake is combusted in a CHP plant to offset process heat and electrical energy (313.06MJ) demand via a hybrid approach combining allocation and displacement method. The result in Figure 27 below depicts the LCIA analysis characterisation of this scenario using the data in Table 15 as input data in SimaPro.

**Table 15 SimaPro Input data sheet for the production of 1 kg biodiesel with co-product allocation**

Product and Co-products	Amount	Unit	Allocation	Comments
Algae Biodiesel (BD)	1	kg	56.5%	1kg BD=37.8MJ/kg
Glycerol	0.02	kg	43.5%	1kg glycerol=26MJ/kg
Avoided Products	Amount	Unit	Distribution	SD $\Delta$ 2 or 2*SD
Heat/Electricity	313.06	MJ	Lognormal	1.05
Inputs from nature	Amount	Unit	Distribution	SD $\Delta$ 2 or 2*SD
Water, unspecified natural origin	3726	kg	Lognormal	1.06
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.21
Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\Delta$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.33	kg	Lognormal	1.06
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.71	kg	Lognormal	1.06
Soya oil, at plant/RER U	0.89	kg	Lognormal	1.05
Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\Delta$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	172.49	MJ	Lognormal	1.05
Emissions to air	amount	Unit	Distribution	SD $\Delta$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.21

As a consequence of how this impact on the results, electrical and heat energy requirements represent the most contributing feature for most of the impact

categories in Figure 27. The results indicates significant reductions as an outcome of the account of co-product allocation consideration, with fossil fuels use impact category accounting for 52% (16.4MJ surplus) of electrical and heat energy obligation. Similar reductions are depicted for climate change impact category (52%), ozone layer depletion (52%), and acidification/eutrophication (35.2%) respectively. Nevertheless, there was no change in the percentage of land use impact category (99.5%). This may be due to huge land requirement for soya bean cultivation.

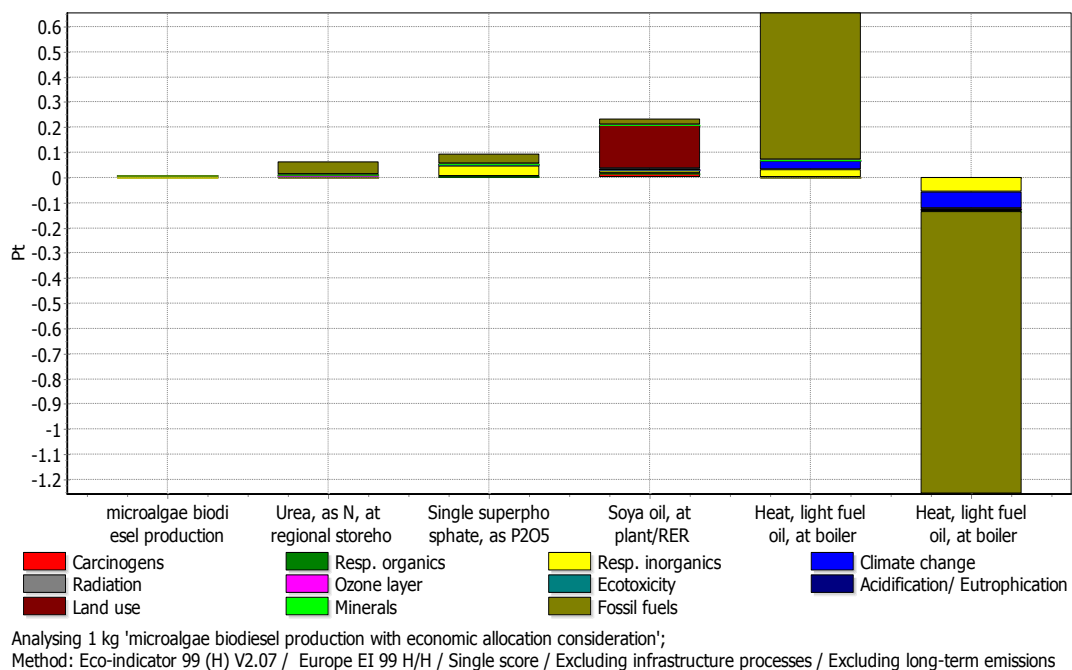


Analysing 1 kg 'microalgae biodiesel production with economic allocation consideration';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 27 Analysing 1 kg microalgae biodiesel production baseline scenario (with co-products economic allocation consideration)**



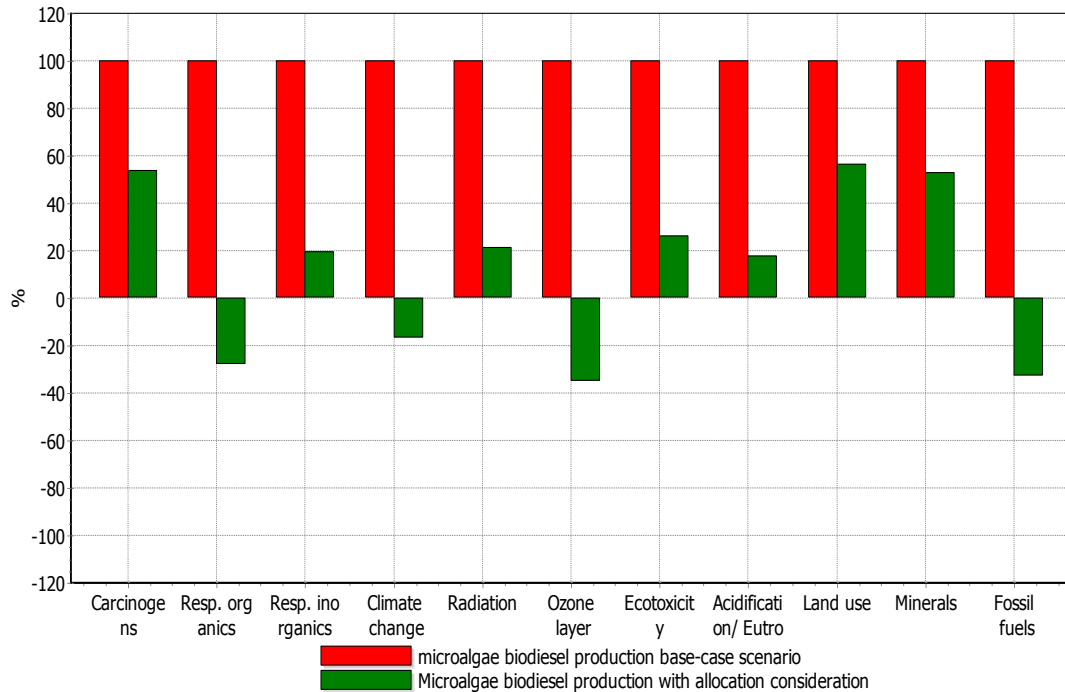
Also, Figure 28 below shows the single score impact analysis for producing 1 kg algae biodiesel baseline scenario (with co-products economic allocation consideration). The single score impact ranking analysis shows that electrical and heat energy obligation significantly reduced as indicated for fossil fuel use (from 0.58pt to -1.12pt). Climate change impact category changed (from 0.035pt to -0.066pt) and respiratory inorganics impact category changed (from 0.029pt to -0.057pt), respectively. Fossil fuel use apparently, seems to be the most significantly sensitive to the effect of co-product allocation consideration amongst all impact categories ranked for electrical/heat energy constraint. This result highlights the importance of co-products allocation consideration and avoided product included. Specifically with regards to electrical and heat energy commitment and also significantly with regards to other unit process material requirement within the algae biodiesel production process. However, it is dependent on the applicable data assumptions made.



**Figure 28 Analysing 1 kg microalgae biodiesel production bas-case scenario with co-products economic allocation consideration at single score impact level**

Furthermore, in order to fully understand and determine the extent of the effect of the sensitivity factor of co-products and avoided products allocation consideration inclusion in the LCA for the proposed algae biodiesel production model. LCIA results for the baseline model were compared with that of a scenario aggregating mass-economic allocation consideration of the resultant co-products (glycerine or propane-1, 2, 3-triol) and the residual algae cake using eco-indicator 99 method in SimaPro.

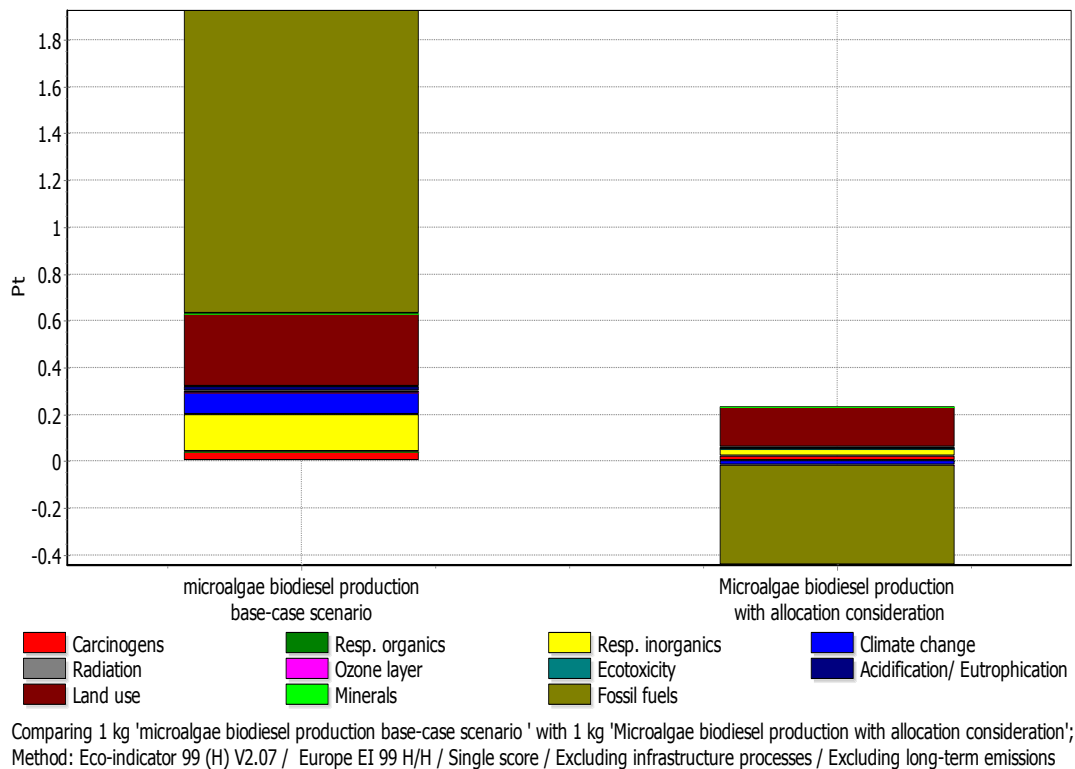
Figure 29 depicts the comparison characterisation results of both set-ups. Fossil fuels resource use impact category indicates -12 MJ surplus (-33%), with regards to heat/electrical energy requirement compared to the baseline scenario. Ozone layer impact category (-35%), climate change impact category (-16.9%), and respiratory organics impact category (-23.2%) also shown negative percentage reduction values respectively. The negative values indicated for fossil fuels use, climate change, ozone layer and respiratory organics impact categories represents a measure of the amount of additional energy (energy credit), required to compensate for future resource use. This is usually common with bio-based products and production processes, as it is reckoned that most bio-based processes take up more CO<sub>2</sub> and heavy metals during biomass growth stage (Lardon et al., 2009) than they emit in other segments of their entire production life cycle. Similarly, though positive reduction results, are also shown for mineral use impact 0.0025 MJ surpluses (52.7%), land use impact (56.6%) and across all other impact categories on account of co-product/avoided products allocation consideration. These results highlight the crucial effect of allocation outcomes consideration to LCA as a decision supporting tool.



Comparing 1 kg 'microalgae biodiesel production base-case scenario ' with 1 kg 'Microalgae biodiesel production with allocation consideration'; Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 29 Comparing 1kg algae biodiesel production (base-case scenario) with 1kg algae biodiesel production (with co-product economic allocation consideration)**

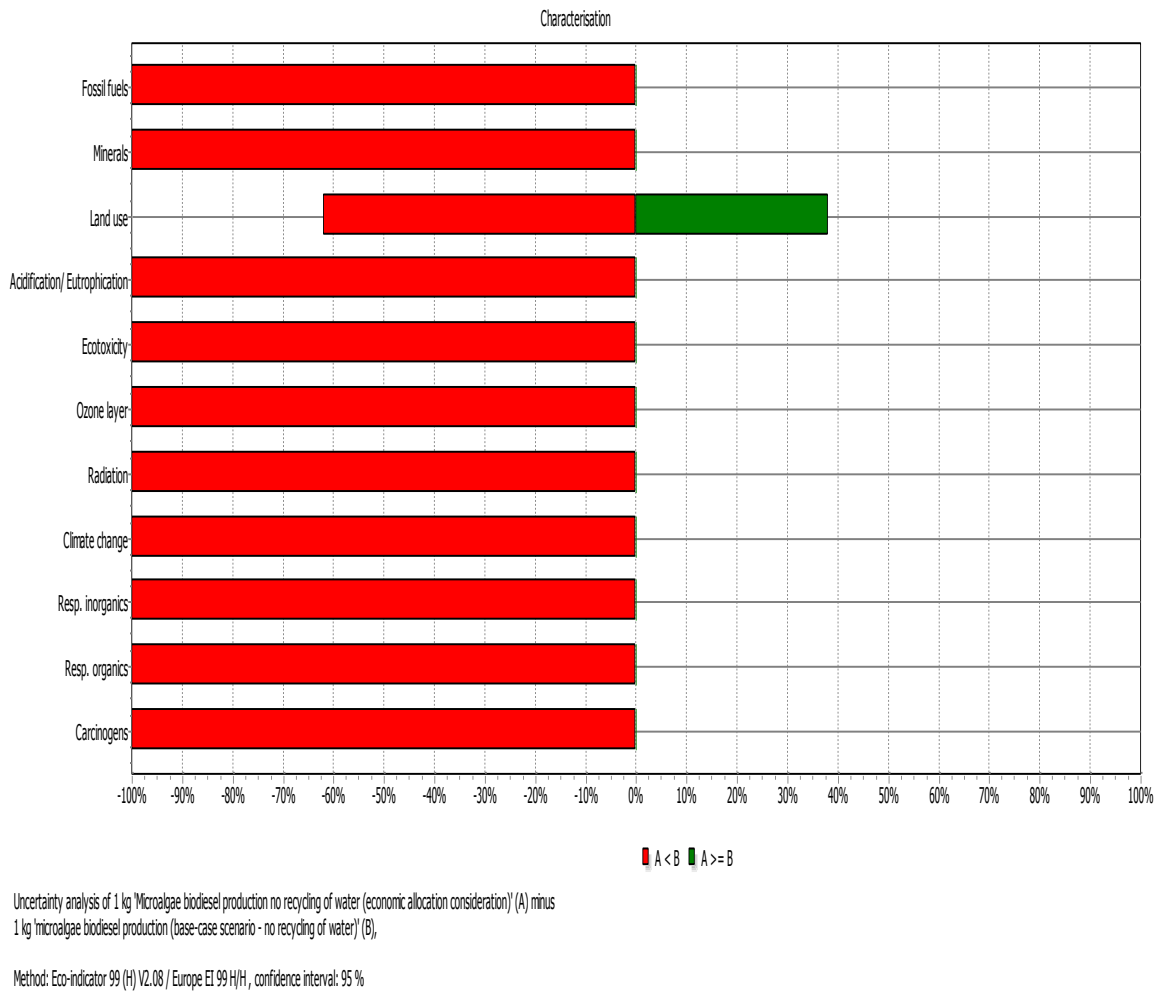
Additionally, single score LCIA characterisation comparison of 1kg microalgae biodiesel production (baseline scenario) with that of 1kg algae biodiesel production integrating allocation consideration set-up was carried out as shown in Figure 30. This is to show in clearer detail, the variation features of the distribution for each individual substance, impact and damage categories as an outcome of the effect of aggregating co-products allocation with the residual algae cake as avoided products. The single score impact ranking show that fossil fuel use requirement reduced from 1.29pt to -0.43pt, which equates to -33% reductions in fossil fuels use obligation. Land use impact showed a decrease (from 0.31pt to 0.18pt, i.e., 41.94%), while climate change impact decreased from 0.093pt to -0.0158pt (-16%). The result further supports earlier results and reflects how significant the effect of allocation is to LCIA results.



**Figure 30 Single score LCIA comparing 1kg microalgae biodiesel production (baseline scenario) with 1 kg biodiesel production integrating co-products economic allocation consideration.**

Furthermore, in order to compare the differences in result outcomes between the baseline's model of producing 1 kg microalgae biodiesel with that of 1kg algae biodiesel production using co-products economic allocation consideration. Uncertainty analysis was run using Monte Carlo uncertainty function in SimaPro to analyse the degree of uncertainty existent between the two scenarios using the distribution and standard deviation set of values for each input in Table 15. Figure 31 below shows the characterisation of the uncertainty for each impact category for both scenarios. Apart from the absolute uncertainty score for land-use impact which depicts relatively high uncertainty, the uncertainty values for all other impact categories, show that the differences depicted in Figure 29 and

Figure 30 are indeed significant. Which corroborate that there is clearly a difference between process A and process B.



**Figure 31 Uncertainty Analysis of 1kg algae biodiesel production with co-products economic allocation consideration (A) minus 1kg algae biodiesel production (baseline scenario) (B).**

#### **4.4.4 LCIA of biodiesel production assuming a 70% induced increase in algae lipid yield**

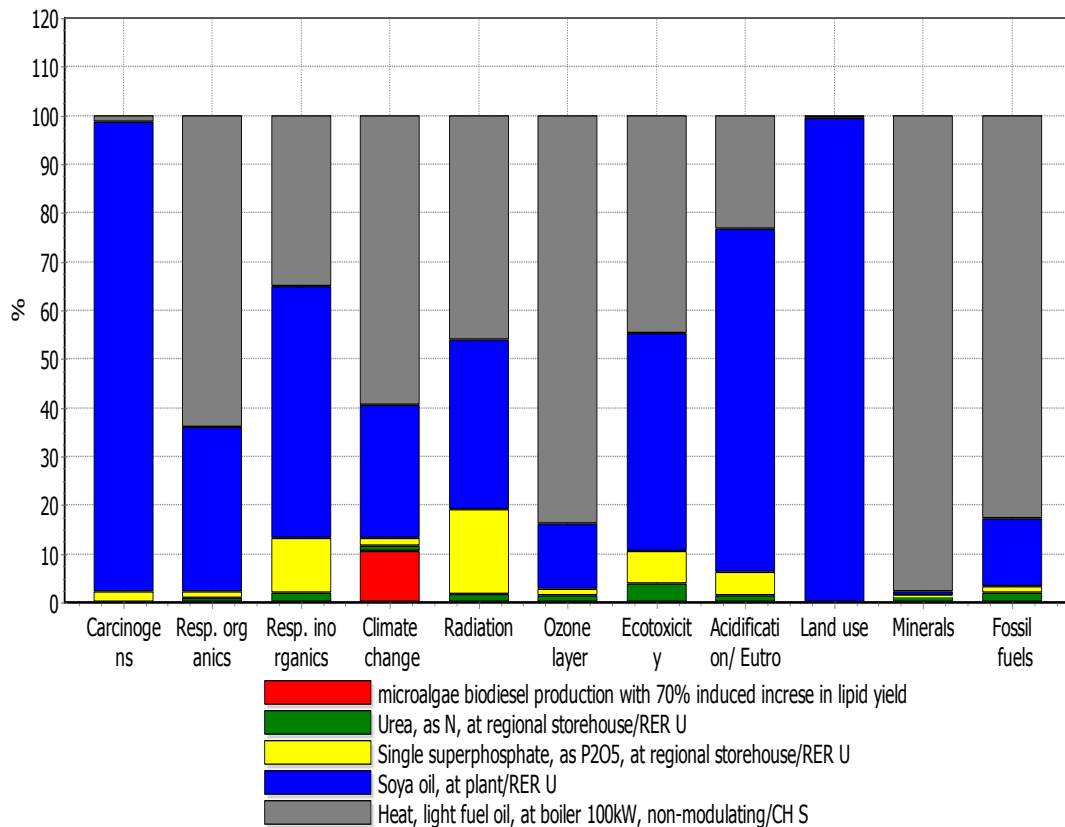
The prospects of potentially increasing microalgae lipid yield during cultivation, through biochemical engineering strategy, is considered to be a possible prospect (Rosenberg et al., 2008) for optimizing microalgae biodiesel production process. Given that the ability to regulate algal cell metabolic activities is a necessary step, in order to achieve full downstream processing capabilities of algae biodiesel production (Ehimen et al., 2010). Similarly, a number of algal species such as, *C. emersonii*, *C. minutissima*, *C. vulgaris*, and *C. pyrenoidosa* have been reported (Illman et al., 2000), to have amassed lipids of up to 63%, 57%, 40% and 23% respectively, on DWB in response to nutrient (N) limitation or low-N culture. Therefore, in order to fully explore and understand the consequence of such an approach and how increased lipid production enhances the economics and environmental sustainability of the algae BD production system. A hypothetical LCIA scenario for producing 1kg algae biodiesel, assuming 70% induced increase in biomass oil yield was analysed using Eco indicator 99 method in SimaPro with results as characterised in Figure 32 below. The results are based on the SimaPro inventory data sheet presented as Table 16.

**Table 16 SimaPro Input data sheet for the production of 1 kg biodiesel assuming a 70% induced increase in algae lipid yield**

Product and Co-products	Amount	Unit	Allocation	Comments
Algae Biodiesel (BD)	1	kg	100%	1kg BD=37.8MJ/kg
Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	3726	kg	Lognormal	1.21
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.21
Inputs from technosphere	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
(materials/fuels)				
Urea, as N, at regional Storehouse/RER U	0.09	kg	Lognormal	1.21
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.21	kg	Lognormal	1.21
Soya oil, at plant/RER U	4.12	kg	Lognormal	1.21
Inputs from technosphere	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
(electricity/heat)				
Heat, light fuel, at boiler 100kW non-modulating/CH U	172.49	MJ	Lognormal	1.21
Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.07

The results in Figure 32 indicate that electrical/heat obligation represents the major percentage contributor, across most impact categories except for land use impact category and carcinogens impact category. As indicated, fossil fuels use impact category 31.2 MJ surpluses (83%), climate change impact (59.6%), ozone layer depletion (84%), and acidification/eutrophication (23.3%) accordingly. However, mineral (particularly Urea, as N) use impact, shows the

least value in terms of percentage contribution per substance towards each impact categories as show. Mineral use impact (0.00117MJ surplus or 1.33%), fossil fuels use category (0.70MJ surplus or 1.87%), climate change impact (1.01%), ozone layer (1.38%), and ecotoxicity (3.81%) accordingly. It is therefore convenient to adduce that, stimulated increase in algal lipid yield through nutrient-limitation, implies corresponding reductions in growth mineral nutrient obligation (mainly Urea, as N) for the algae biodiesel production process.



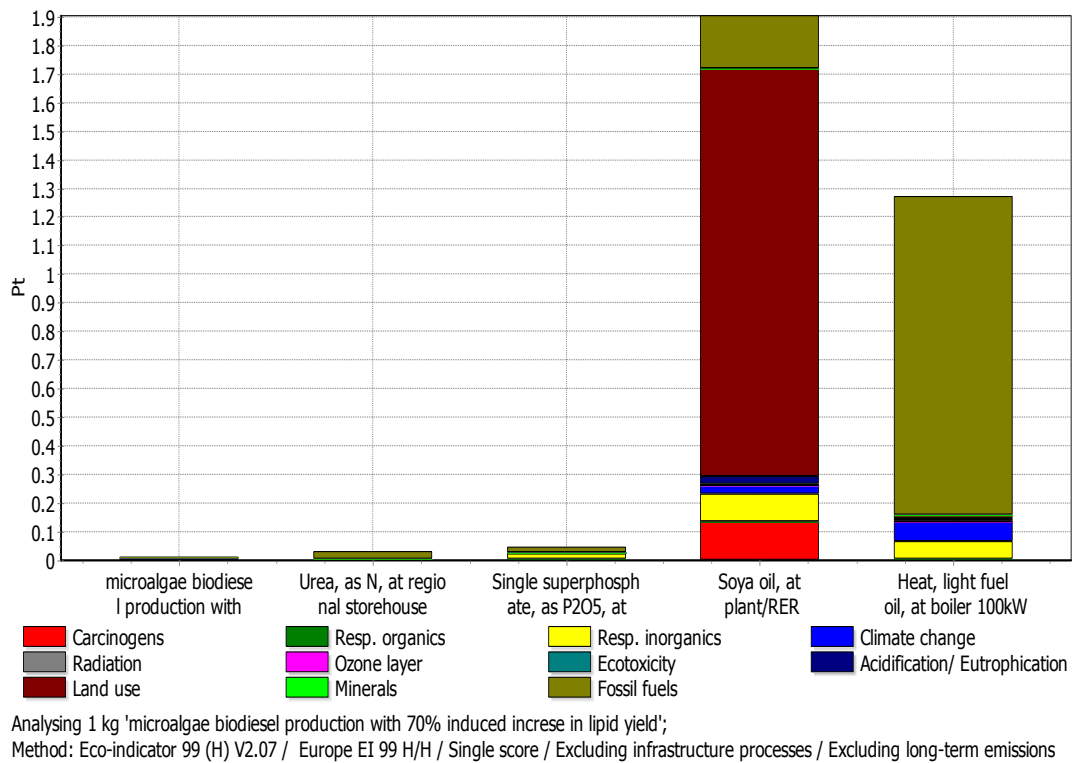
Analysing 1 kg 'microalgae biodiesel production with 70% induced increase in lipid yield';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 32 LCIA Analysis of 1 kg microalgae biodiesel production (assuming 70% biomass induced lipid yield)**



However, an inherent disadvantage of this technique is compromised (lowered) biomass productivity as a consequence of nutrient-starvation. Given that, the overall lipid productivity of microalgae, is a product of the algae cell lipid content multiplied by its biomass productivity (Courchesne et al., 2009). Consequently, it is recommended practice to use a two-stage (hybrid) cultivation strategy which incorporates the exceptional growth stages in PBR's and the open pond production system. With the first stage dedicated to biomass cell growth in nutrient-sufficient culture medium (PBR) and the later stage targeted for lipid synthesis under nutrient-limiting conditions in the open pond, as proposed by Huntley and Redalje (2006).

Also, Figure 33 below shows in more detail, the specification per substance, for each impact category and damage category as a result of a 70% induced increase in lipid stimulation. The single score LCIA, analysing 1kg microalgae biodiesel production with 70% induced lipid yield (excluding infrastructure processes/ excluding long-term emissions), show that: fossil fuels use in form of electricity/heat (1.11pts), soya oil (0.183pts), single phosphate as P (0.197pts), and urea as N (0.025pts) respectively in descending order of significance. Land use represents the biggest impact contribution of (1.43pts), followed by fossil fuels use (1.11pts) amongst all material substances required within the production system.

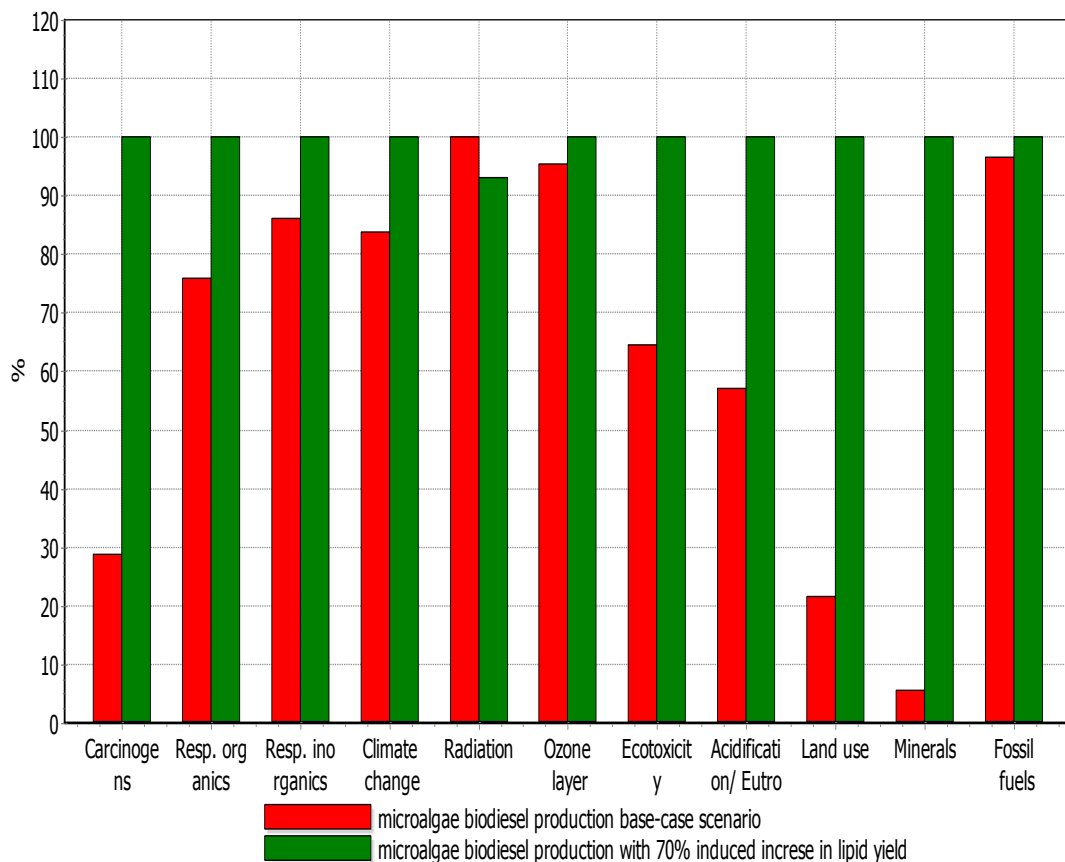


**Figure 33 Single score LCIA analysing 1 kg algae biodiesel production with 70% induced biomass lipid yield**

Additionally, in order to evaluate the significance of the sensitivity prospects of a 70% potentially induced increase in algae lipid yield during the growth phase, and how this impacts on the proposed microalgae biodiesel production system. Using eco-indicator 99 methods in SimaPro, LCIA results for the base-case scenario were compared with that of producing 1kg microalgae biodiesel, incorporating 70% induced increase in lipid yield as illustrated in Figure 34.

The LCIA characterisation results show negative impact values across all impact categories, due to 70% induced increase in algae lipid yield compared to the baseline scenario. With the most significant impacts being, due to mineral use obligation and land use impact categories accordingly. However, this result is in contrast and a surprising deviation from expected results. As mineral use obligation for the baseline scenario was higher (0.33kg-N and 0.71kg-P<sub>2</sub>O<sub>5</sub>), compared to that of the set-up with a 70% induced increase in algae lipid yield

of (0.09kg N, and 0.21kg- P<sub>2</sub>O<sub>5</sub>) accordingly, from the LCI data and analysed chart in Figure 32 above. Although, soya oil requirement increased relatively with 70% increase in algae lipid yield from 0.89kg soya oil for the baseline scenario to 4.12kg soya oil with 70% induced algae lipid yield strategy. This apparent deviation in impact results, particularly with regards to mineral use and land use impacts categories, may be due to the use of soya oil process data as representative data of microalgae oil, which at the moment is not inventoried in ecoinvent data base. Similarly, the seeming huge mineral use impact, and land use impact outcome as a result of the assumed 70% induced increase in algae lipid yield, may perhaps be typically due to the vast land and fertilizer use requirements associated with soya bean plant cultivation and oil extraction. Consequently, there is therefore need to improve and update the ecoinvent data base by including sector specific data for microalgae bio production processes, as it does not fulfil all sector needs for foreground data at the moment.

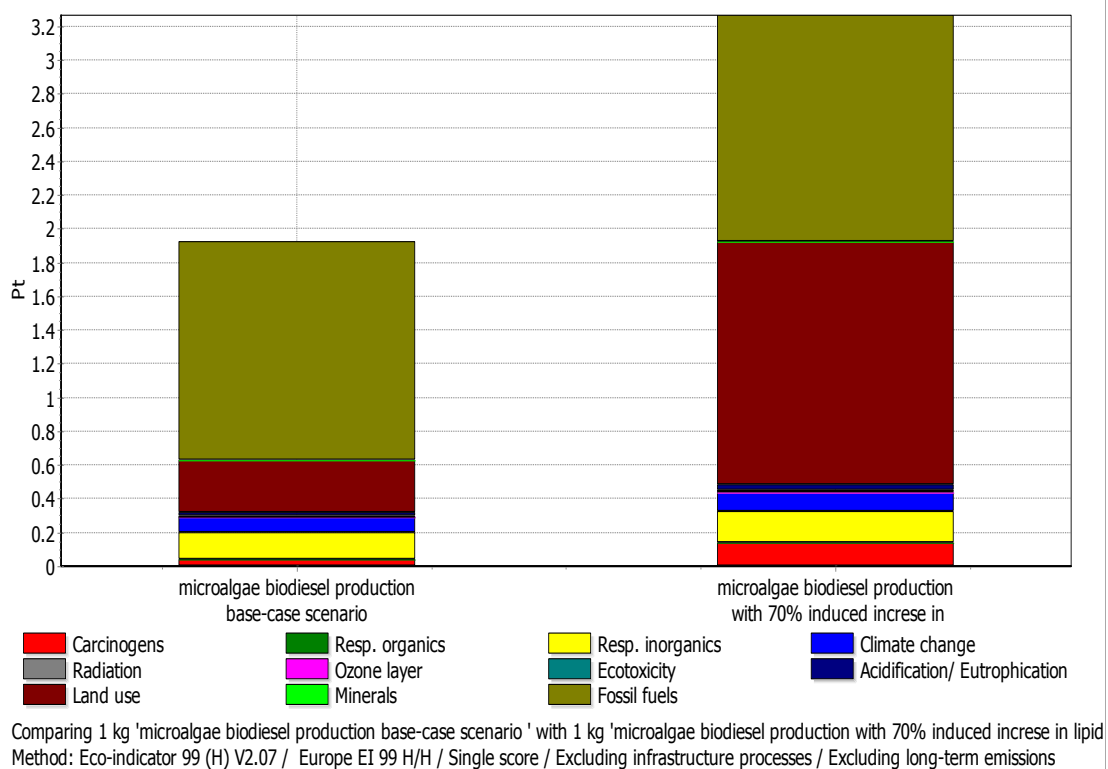


Comparing 1 kg 'microalgae biodiesel production base-case scenario' with 1 kg 'microalgae biodiesel production with 70% induced increase in lipid yield'  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 34 Comparing 1kg microalgae biodiesel production assuming a 70% induced lipid yield with that of 1kg algae biodiesel production (baseline scenario)**

Additionally, LCIA at single score impact level excluding infrastructural processes/excluding long-term emissions was applied with results as illustrated in Figure 35. The results indicates about 3.9% increase in fossil fuels use impact category, from 1.29pt for the baseline scenario to 1.34pt for the proposed 70% induced increase in algae lipid yield set-up. Similar increase is shown for the analysis chart value of land use impact, from 0.31pt for the baseline scenario to 1.44pt on account of an assumed 70% induced increase in algae lipid yield. This translates to over 365% increase in land use requirement as a consequent of induced increase in algae lipid yield. This is an unusually

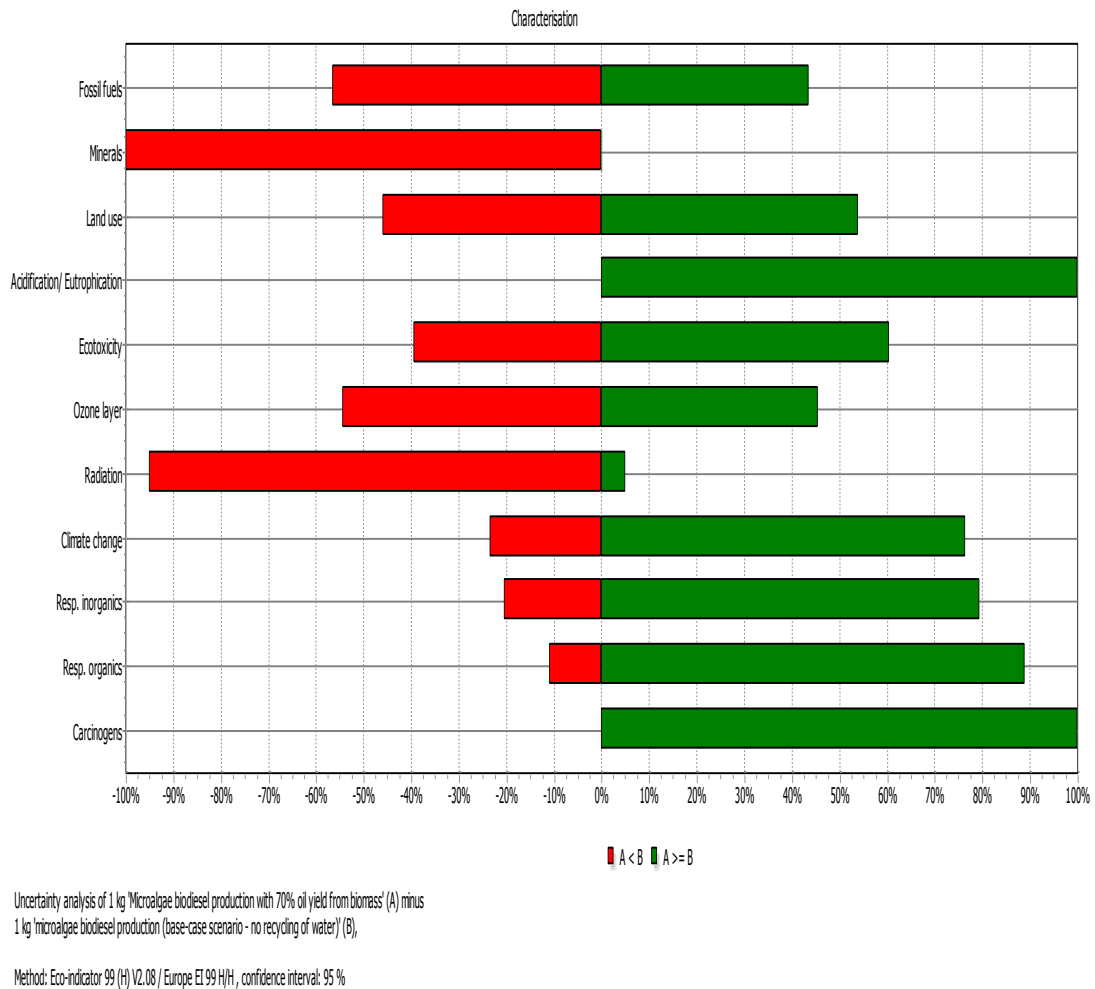
high range of value for such a variable change in lipid yield. This highlights the significance and implications of the difference shown in previous figures.



**Figure 35 Single score LCIA analysis comparing 1kg microalgae biodiesel production (baseline scenario) with 1kg algae biodiesel production assuming a 70% induced increase in lipid yield**

Furthermore, Monte Carlo uncertainty analysis was carried out to compare the differences in results outcome between the proposed set-ups with the integration of 70% induced increase in algae lipid yield and that of the baseline scenario. The uncertainty analysis results were generated using the distribution and standard deviation set of values for each input in Table 16. Figure 36 shows that process B (baseline scenario) has more numbers of comparison runs with higher score outcomes than the sensitivity prospects of the proposed model with 70% induced increase in lipid yield (process A). However, the

absolute uncertainty value for mineral use obligation for process A is relatively higher than the LCI data value. Therefore the difference may be considered significant.



**Figure 36 Uncertainty analysis of 1kg microalgae biodiesel production with 70% induced increase in lipid yield vs 1kg algae biodiesel production (baseline scenario)**

#### **4.4.5 LCIA comparison of baseline biodiesel model with all proposed alternative biodiesel production scenarios**

In order to examine the significance of changes in scenario on the microalgae biodiesel production system, essentially with regards to how changes in particular data could lead to lowered material use, energy consumption and lowered environmental burdens than the conventional (baseline) biodiesel production process. Eco indicator 99 methods in SimaPro was used to compare LCIA results of the baseline scenario with that of each of the proposed four sensitivity prospects of: (1) 100% recycling of production harvest water, (2) use of wastewater/sea water as alternative culture media, (3) co-products mass-economic allocation consideration, and (4) integrating a 70% induced increase in algae biomass lipid yield. As, it is anticipated that this diagnostic procedure would help in identifying the most polluting step or process within each life cycle, and establish the most challenging environmental burden for each set-up. The result for the LCIA characterisation comparison of all the processes for each impact category is shown in Figure 37. The results were generated incorporating inventory data for each production set-up and using the compare function in SimaPro.

Thus, for fossil fuels use impact category, 1kg microalgae BD production (with co-products mass-economic allocation consideration) has the least impact score of -12 MJ surpluses (-31.9%). While the set-up options of the use of wastewater/sea water and that of producing 1kg microalgae biodiesel with 100% recycling of production harvest water, indicated about similar impact scores of 32 MJ surplus (85%). Again, the negative impact value, which is common with bio-based processes, signifies the amount of energy credit required to compensate for future resource use. However, the proposed prospective model of incorporating a 70% induced increase in algae lipid yield, impacted the most on fossil fuels use impact category compared to the base-case scenario of producing 1kg microalgae BD as shown.

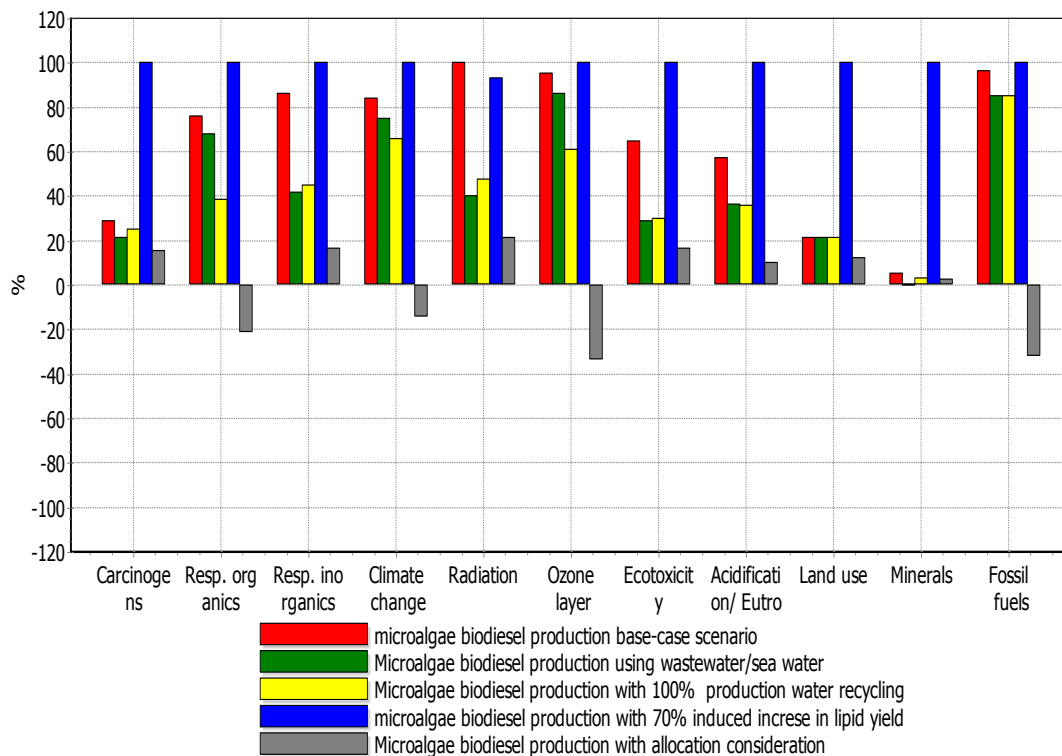
Also, for minerals use impact category, the prospect of the use of wastewater/sea water as culture medium clearly shows the least impact

obligation of 0.000598 MJ surplus (0.68%). This is closely followed by the set-up with co-products mass-economic allocation, with impact score of 0.00254 MJ surplus (2.89%). The sensitivity prospects of combining 100% recycling of production harvest water show mineral use impact scores of 0.00265 MJ surplus (3.02%), compared to the baseline scenario analysed chart value of 0.00482 MJ surplus (5.49%). The comparisons obviously indicate that, the use of wastewater/sea water application would bring about the largest reduction of 4.81% (best results) for mineral use obligation, relative to the baseline scenario for producing 1kg algae BD. However, not many algae species can survive in wastewater/sea water culture due to usually high nutrients/salt concentrations, presence of heavy toxic metals, and prevalence of invasive bacteria and zooplanktons in wastewater/sea water.

For climate change impact category, the set-up with co-products mass-economic allocation consideration indicated the best results impact score value of -14.2%. This is closely followed by the proposed scenario, incorporating 100% recycling of production harvest water, with analysed chart value of 65.9%. The set-up proposing the use of wastewater/sea water shows an analysed chart value of 74.5%, which is 9% lesser than the climate change impact value for the baseline scenario. However, the sensitivity prospect of a 70% potentially induced increase in algae lipid yields indicated the worst climate change impact outcome, amongst all the processes analysed as shown in Figure 37 below.

Likewise, the proposed set-up with a 70% induced increase in algae lipid yield indicated worst impact results across other impact and damage categories respectively, compared to the baseline scenario of producing 1kg microalgae BD as shown in the chart.





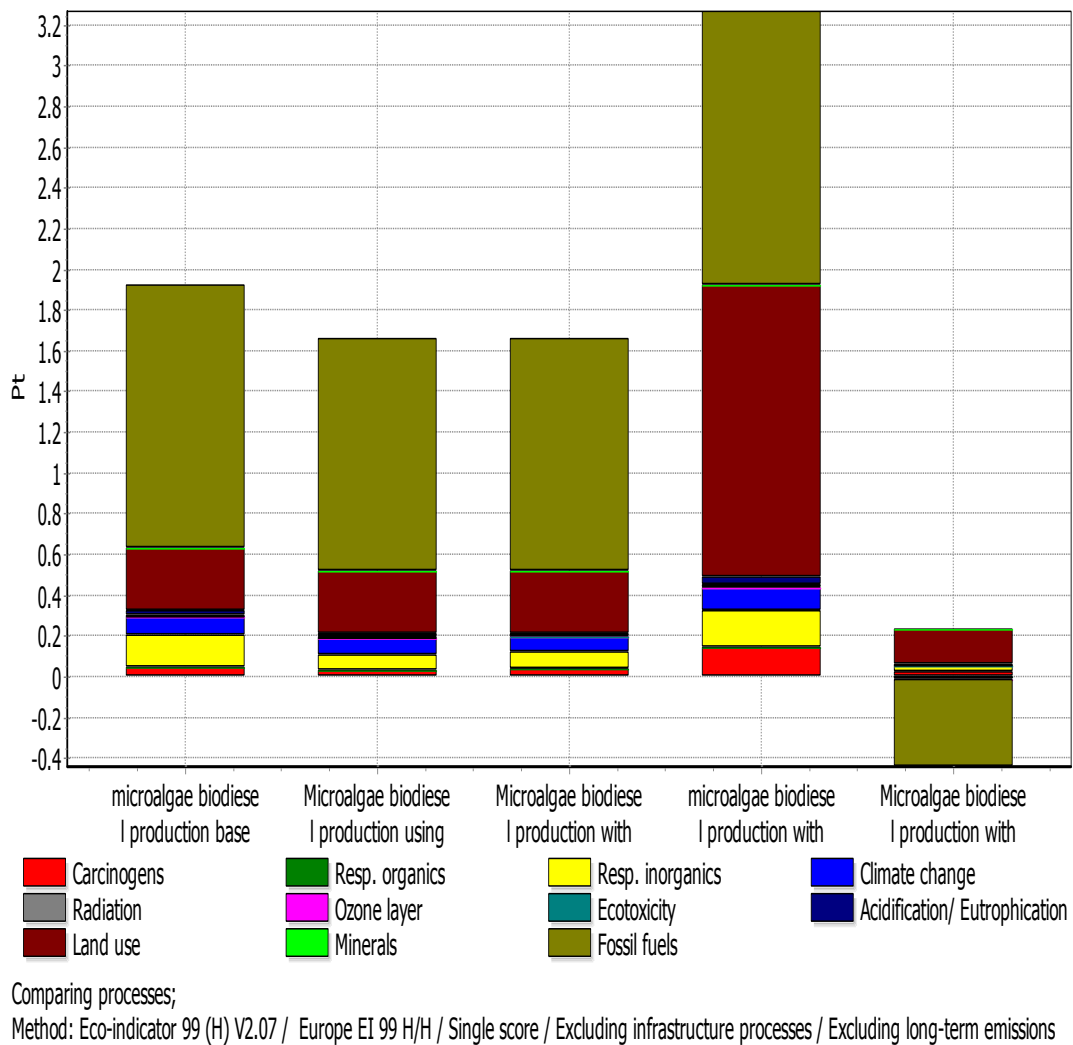
Comparing processes;  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 37 LCIA Comparing biodiesel production processes: (1) baseline scenario; (2) 100% recycling of production harvest water; (3) use of wastewater/sea water; (4) co-products economic allocation consideration; and (5) 70% induced increase in lipid yield**

Additionally, LCIA comparison of all the prospective processes of producing 1kg algae BD with that of the baseline scenario using single score eco-indicator chart was carried out with results as shown in Figure 38. Single score eco-indicator results are often clearer and devoid of technical environmental themes. Thus, the results highlights the impact of the proposed alternative set-ups with respect to lowering or otherwise, of the quantitative input for each specific substance, impact and damage categories respectively relative to the baseline scenario for producing 1kg algae BD.

Hence, the analysed single score chart results show that microalgae biodiesel production with co-products economic allocation consideration set-up indicated the best result outcomes as shown in Figure 38. Microalgae biodiesel production with co-products economic allocation consideration showed -0.21 pt. With the set-ups with 100% recycling of production harvest water and that with the option of utilising wastewater/sea water as culture medium, both showing 1.66 pt, compared to the baseline scenario with 1.92 pt. This is with regards to lowered material use, energy, and environmental burden associated with the product system.

However, the proposed scenario with 70% induced increase in algae lipid yield, had the highest eco-points score of 3.27pts. Its impact result is 0.35pts higher than that of the baseline scenario for producing 1kg microalgae BD. The higher impact values were more as a result of increase in land use obligation and fossil fuels use requirements. As land use impact indicates a score of 1.44pt compared to the baseline scenario value of 0.31pt for this category of impact. And fossil fuels use impact value score of 1.34pt in comparison with the base-case scenario value of 1.29pt.



**Figure 38 Single score LCIA comparing processes: (1) base-case scenario, (2) 100% recycling of production harvest water, (3) use of wastewater/sea water, (4) co-products economic allocation consideration, and (5) 70% induced lipid yield**

## 4.5 Energy Recovery via anaerobic digestion (AD) of algae biomass feedstock

In the AD energy recovery process model, the entire microalga to biofuel (biogas) transformation process takes place in a relatively wet phase (see Figure 12(b)). The whole idea is to examine an alternative approach devoid of thermal drying, in order to reduce the energy requirement and environmental cost of producing biofuel using microalgae as feedstocks. As thermal drying obligation and oil (lipids) extraction processes from the microalgae biomass which cumulatively accounts for over 50% of production energy cost are omitted. The energy recovery process involves the digestion of the organics in the algal feedstocks via anaerobic microbial metabolism to biogas, a mixture of CH<sub>4</sub> and CO<sub>2</sub>. Some influential process parameters conventional AD process used in Waste Water Treatment (WWT) is sensitive to, includes; solid and hydraulic retention time (HRT), feedstock nutrient and carbon contents, microbial biomass yield, biogas yield, biogas composition ratio, operating temperature, and digester configuration (Sialve et al., 2009).

However, even though, the biogas yield and gas composition ratios are key indicators of the performance and overall efficiency of the AD process. The energy recovery application of AD process as applied to microalgal biomass, in this study is different. As the focus of this research is on analysing the energy efficiency, material use, and the environmental burden associated with the AD energy recovery from microalgae biomass. Consequently, the results and discussions below focuses on the energy requirement, material use and environmental burdens associated with the AD of algal biomass to produce 1 kg biogas for a baseline scenario using Eco-indicator 99 methods in SimaPro.

In addition, the impact prospects of integrating 100% recycling of the microalgae production harvest water is also considered, because it affects energy and material use. Consideration is also made of the prospective use of wastewater/sea water as the medium for microalgae culture, as it is considered to have huge impact on growth mineral nutrient obligation and impacts on the energy and emissions profile of the production system. Also, the prospect of a

70% induced increase in algae biomass lipid yield through nutrient limitation, is analysed. Considering that lipids are considered high value substrates for AD process (Zamalloa et al., 2011), with reported high theoretical CH<sub>4</sub> yield, compared with that of carbohydrates and proteins (i.e. 1.0 LCH<sub>4</sub>g<sup>-1</sup>VS for lipids, 0.42 LCH<sub>4</sub>g<sup>-1</sup>VS carbohydrates, and 0.85 LCH<sub>4</sub>g<sup>-1</sup>VS proteins).

Furthermore, comparison is made between the baseline models of AD transformation of algae biomass to produce 1kg biogas with that of the sensitivity prospects of: (1) integrating 100% recycling of microalgae production harvest water, (2) use of wastewater/sea water, and (3) 70% induced increase in algae lipid yield production set-up. As it is anticipated that the comparison results would highlight which process route can give better results in terms of the inventory of resource consumption and environmental emissions associated with the production systems, in line with the project framework.

However, investigation shows that up till this moment, very little work has been reported in current literature concerning the anaerobic digestion of microalgae biomass. It is therefore anticipated that the results in this study would hence, provide process information for possible up-scale of the microalgae-biofuel production system.

#### **4.5.1 LCIA results of baseline model for the Anaerobic Digestion (AD) of algae biomass to produce 1kg biogas**

The technical considerations for the baseline production model of the energetic recovery of algae biomass via AD, to produce 1 kg biogas consists of three phases (See Figure 11). These are, microalgae growth stage (phase 1), the harvesting/dewatering stages (phase 2), and the AD of the 30-50% wt. DW biomass into biogas. It excludes the thermal drying and algal oil extraction stages (peculiar to the biodiesel production process), which brings about an energy savings of 111 MJ. Also, electricity demand for mixing, pumping pre-concentration of the algae biomass to the required 30-50% wt. DW algal biomass is 32.79 MJ. With fertilizer requirement amounting to 28.3 MJ, and AD

reactor thermal requirement equals 2.1 MJ. This brings the total energy demand, for the entire AD transformation process to 74.67 MJ. Table 17 presents the mass and energy flow as inputted in the SimaPro data sheet for the LCIA analysis.

**Table 17 SimaPro Input data sheet for the production of 1 kg biogas (baseline scenario) via Anaerobic Digestion (AD), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation**

Product produced	Amount	Unit	Quantity	allocation
Biogas	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	3726	kg	Lognormal	1.06
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.06
Biomass (biotic)	5.88	kg	Lognormal	1.07

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.33	kg	Lognormal	1.05
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.71	kg	Lognormal	1.05

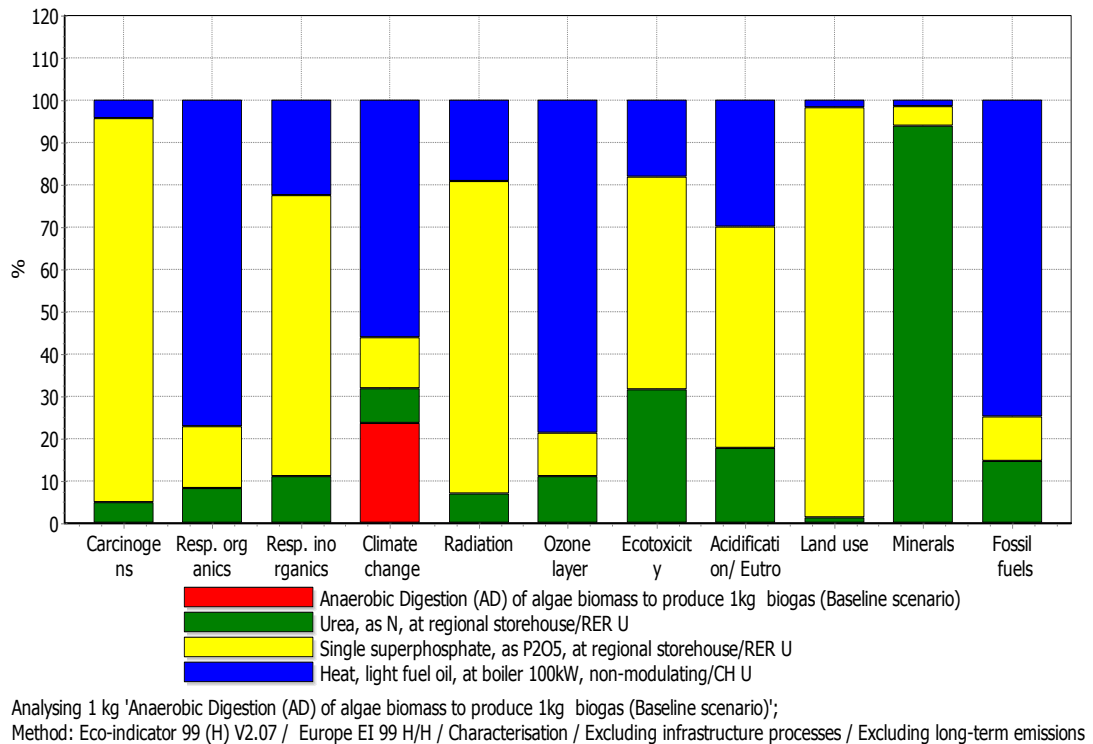
  

Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	74.67	MJ	Lognormal	1.21

Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.21

Figure 39 below shows the LCIA characterisation results of potential environmental impacts scenario of the baseline model for the AD of algal biomass to produce 1 kg biogas, using Eco-indicator 99 method in SimaPro. The results discussion is focused on energy usage, material requirements and the environmental burden associated with the production process in line with scope of this study. Thus, electricity/heat requirement represents the most contributing factors for most of the impact categories, indicating 75% (13.3 MJ) of the proportion of fossil fuels use for the entire AD process. Climate change impact category 56.1%, ozone layer 78.7% and acidification/eutrophication category 30.1% respectively.



**Figure 39 LCIA analysis of Anaerobic Digestion (AD) of algae biomass to produce 1 kg biogas (baseline model)**

These results indicate 43.1% relative reductions in electricity/heat process contributions compared to the baseline scenario of 1kg algae BD production via transesterification. The decrease is particularly, as a result of reductions in electricity/heat energy process contributions, as shown in Table 18. A consequence of the exclusion of thermal drying and algal hexane oil extraction processes, which when combined with the harvesting/pre-concentration phase's represents over 50% of the entire microalgae biofuel production cost (Moheimani, 2006). These results are similar to and within the range of values reported by Molina Grima et al., (2003).



**Table 18 Single score values of process contributions for the production of 1 kg microalgae biofuel. Excluding infrastructure processes/excluding long-term emissions, Note: N/A means not applicable.**

Resource use	1kg Algae biomass	Urea, as N	P <sub>2</sub> O <sub>5</sub>	Electricity/Heat	Soya Oil	Total value(point)
<b>Process</b>						
Anaerobic digestion (AD) to produce biogas	0.012	0.11	0.16	0.53	N/A	0.82
Biodiesel transesterification	0.012	0.11	0.16	1.23	0.411	1.92

#### **4.5.2 LCIA analysis of the impact of 100% recycling of production harvest water on the algae-to-biogas AD transformation system**

In order to examine the impact and the potential benefits recycling production harvest water would bring to the AD transformation of algal biomass to produce 1kg biogas. Especially, as it has to do with reductions in mineral nutrient requirement and water demand for the product system. LCIA characterisation of a prospective microalgal biomass AD set-up incorporating 100% recycling of production water was administered using Eco-indicator 99 method in SimaPro with input data as presented in Table 19. The results for the analysis are as shown in Figure 40.

**Table 19 SimaPro Input data sheet for the production of 1 kg biogas via Anaerobic Digestion (AD) of algae (with 100% recycling of production harvest water), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation**

Product produced	Amount	Unit	Quantity	allocation
Biogas	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	591	kg	Lognormal	1.05
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.06
Biomass (biotic)	5.88	kg	Lognormal	1.05

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.18	kg	Lognormal	1.07
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.39	kg	Lognormal	1.07

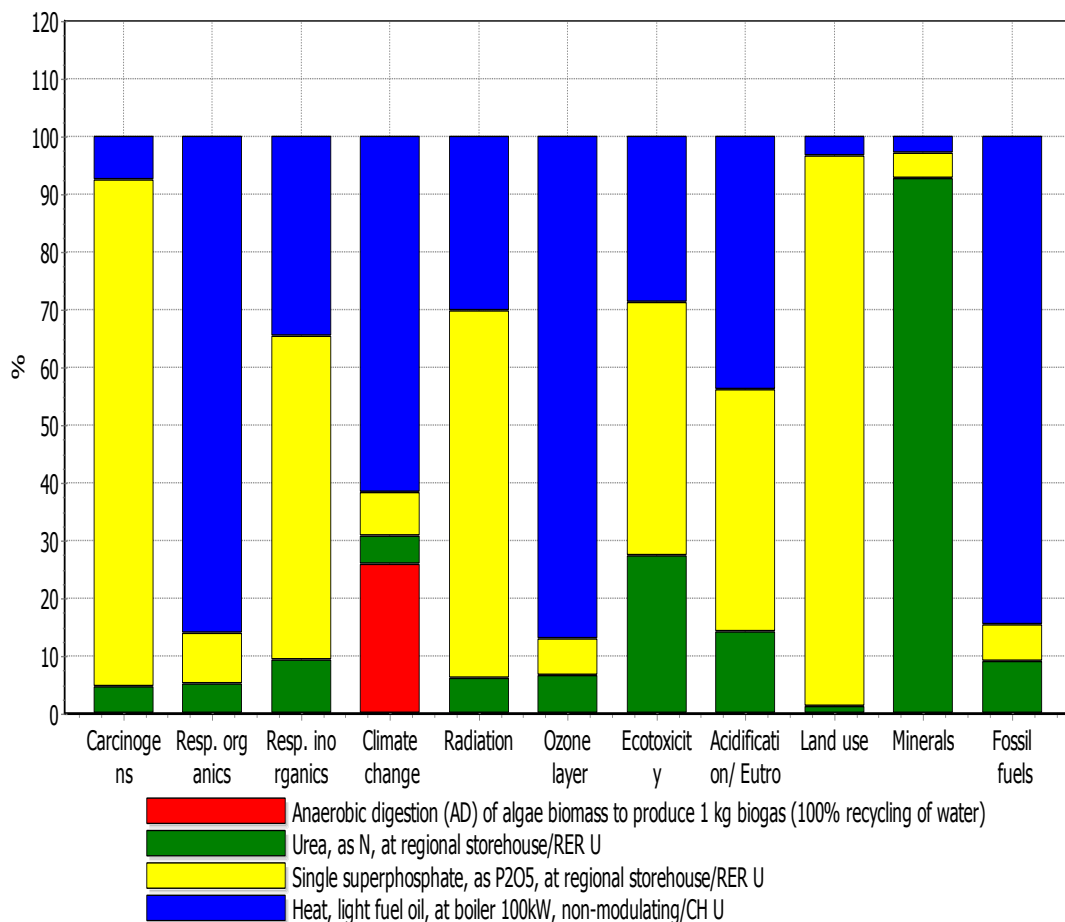
Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	74.67	MJ	Lognormal	1.05

Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.07

The analysed chart result (Figure 40) for fossil fuels use impact category shows 13.3 MJ surpluses (84.6%) as total contribution of electricity/heat requirements towards fossil fuels use. And urea as N, and single superphosphate as P<sub>2</sub>O<sub>5</sub>

inputs accounting for 1.4 MJ surplus (8.93%) and 1.02 MJ surplus (6.52%) total contribution towards fossil fuels use accordingly. For climate change impact category, electricity/heat requirements contributed 61.8% of the total impact contributions and the remaining proportion of impact accounted for by biomass production (25.9%), P<sub>2</sub>O<sub>5</sub> (7.39%) and urea as N (4.96%) respectively.



Analysing 1 kg 'Anaerobic digestion (AD) of algae biomass to produce 1 kg biogas (100% recycling of water)';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 40 LCIA analysis of AD of algae biomass to produce 1kg biogas (with 100% recycling of production harvest water)**

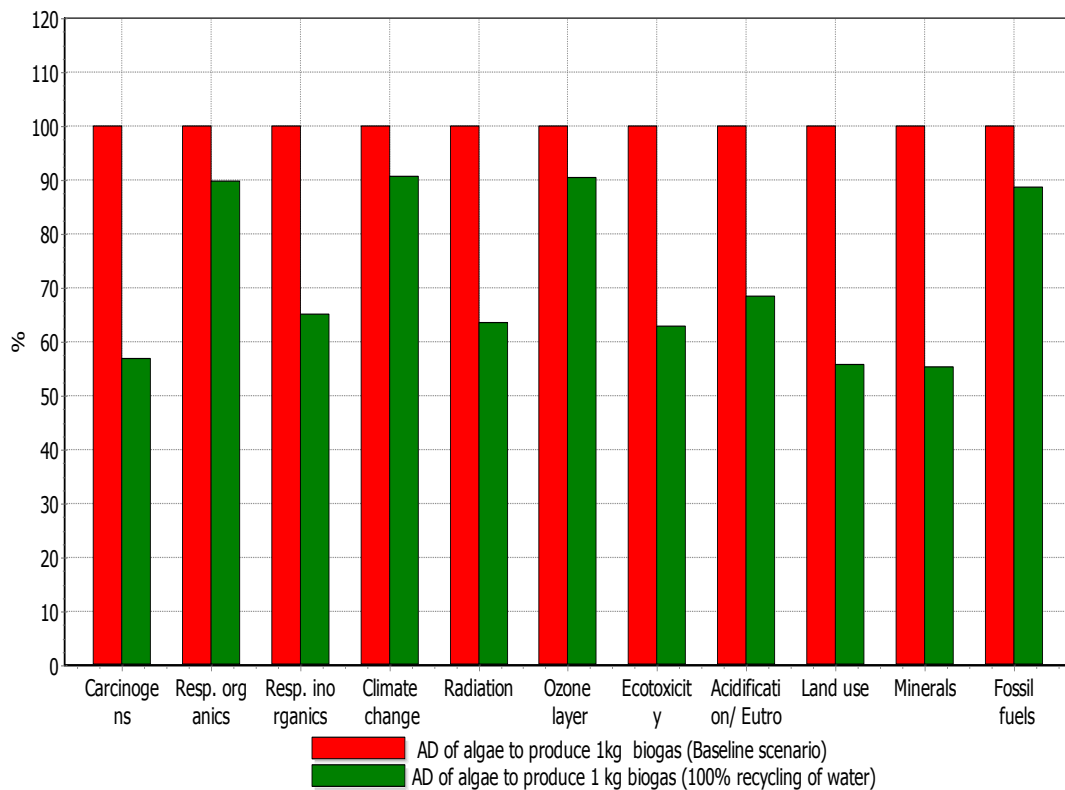
However, the impact of 100% recycling of the microalgae production harvest water is particularly obvious with regards to reductions in mineral use obligation as shown in Table 20. As recycling brought about 56.5% reductions in urea (as N) and 54.8% reductions in P<sub>2</sub>O<sub>5</sub> use, compared to the AD baseline model of producing biogas without recycling of production harvest water. These results are similar to those of Yang et al., (2011) and confirm earlier results of the impact recycling production harvest water have on nutrient usage (Richmond, 2004).

**Table 20 Single score values of process contributions for the production of 1 kg biogas via AD of microalgae. Excluding infrastructure processes/excluding long-term emissions**

Process/Resource use	1kg Algae biomass	Urea, as N	P <sub>2</sub> O <sub>5</sub>	Electricity/Heat	Total value(point)
Anaerobic digestion (AD) to produce 1kg biogas (baseline model)	0.01	0.11	0.16	0.53	0.82
Anaerobic digestion (AD) to produce 1kg biogas with 100% recycling of production water	0.01	0.06	0.09	0.53	0.69

In addition, comparison were made between the baseline model of AD transformation of algae biomass to produce 1kg biogas with that of the set-up incorporating 100% recycling of production harvest water with comparison LCIA results as shown in Figure 41. Sensitivity analysis was conducted to understand how critical this parameter is in quantifying the water footprint and the overall impact it has on the AD biogas production system. The comparison results show that AD of algae biomass to produce biogas incorporating 100% recycling of production harvest water showed significantly lowered impacts on the entire

range of impact categories relative to the baseline model. However, the greatest impact due to 100% recycling of production harvest water on the product system is in mineral use impact category. With a 55.3% reduction in mineral use obligation due to 100% recycling, compared to that of not recycling at all. Consequently, the mineral nutrient use obligation of the algae biogas production system increases with decrease in the rate of recycling production harvest water.

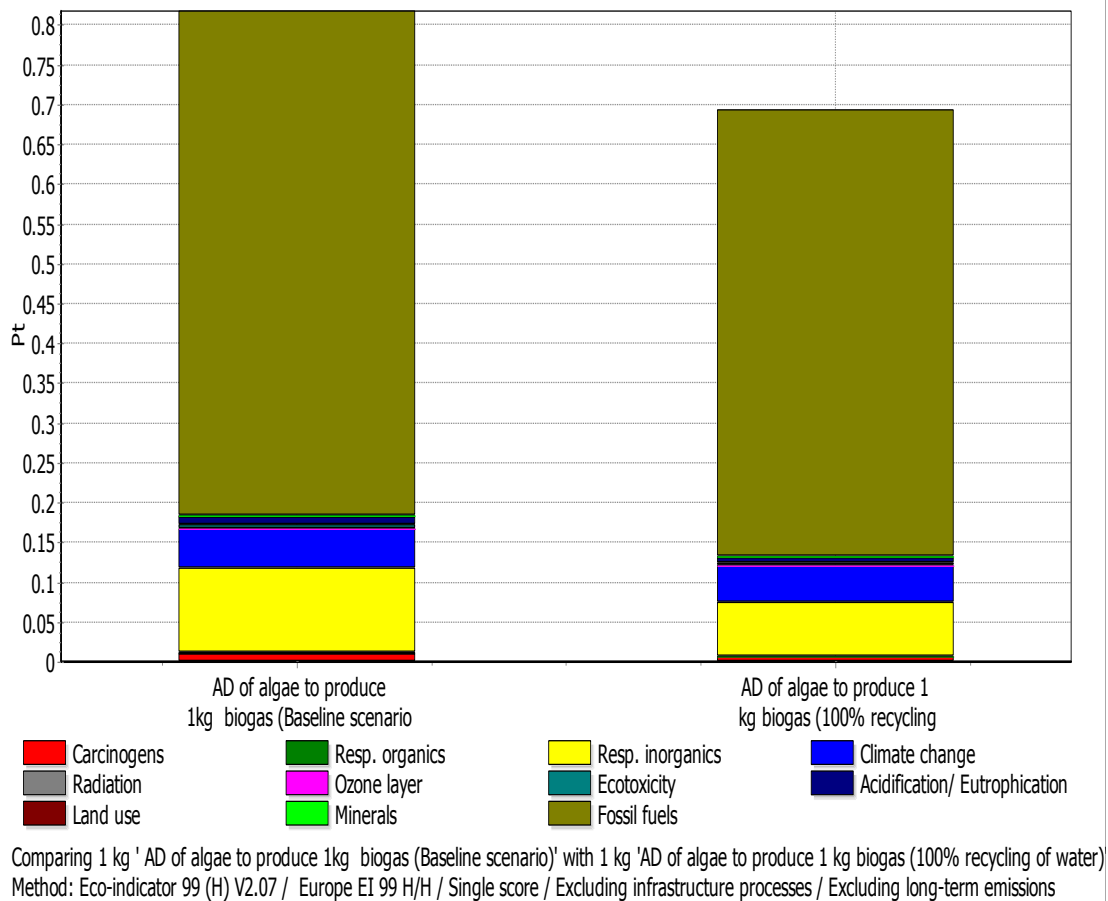


Comparing 1 kg 'AD of algae to produce 1kg biogas (Baseline scenario)' with 1 kg 'AD of algae to produce 1 kg biogas (100% recycling of water)  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 41 Comparing anaerobic digestion (AD) of algae biomass to produce 1kg biogas incorporating 100% recycling of production harvest water with AD of algae biomass to produce 1kg biogas (baseline model)**

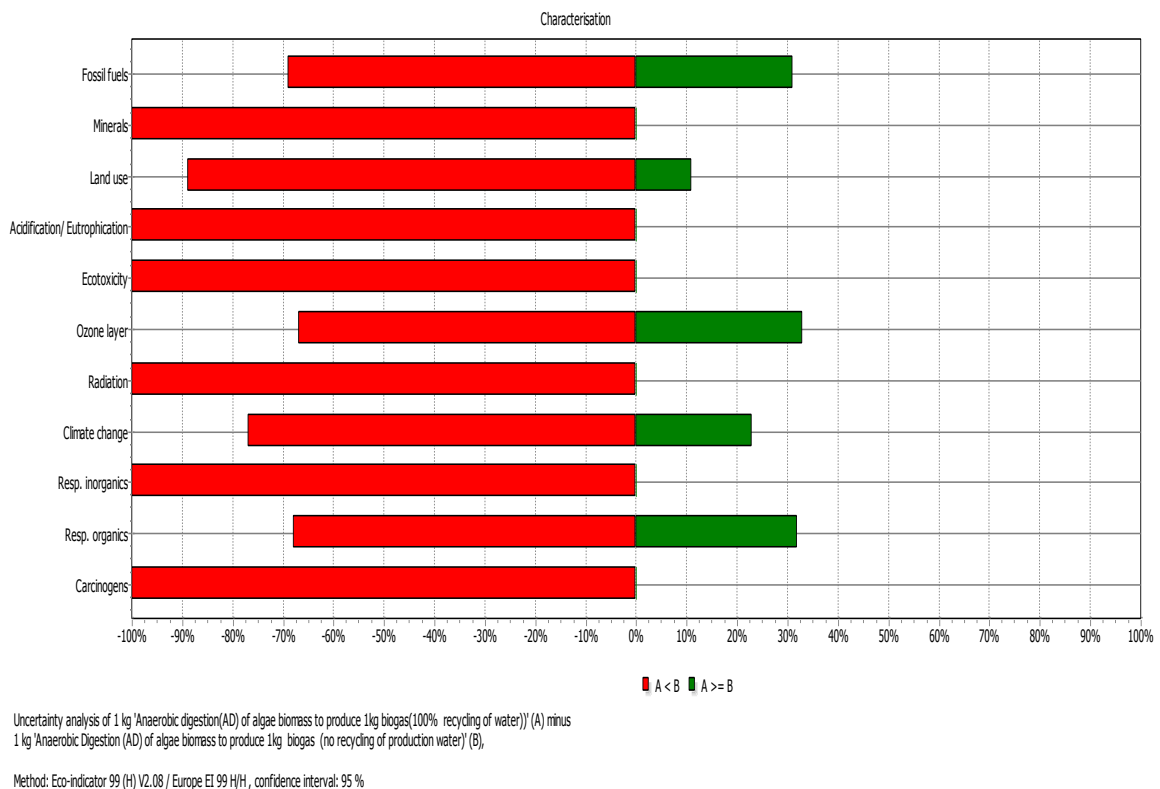
Similarly, single score LCIA comparison of the baseline model with the set-up integrating 100% recycling of production harvest water was carried out as

depicted in Figure 42. The single score LCIA comparison, show a 0.56 pt weight impact due to fossil fuels use impact category for the production set-up with 100% recycling. While the baseline production model indicates 0.63 pt weight impact due to fossil fuels uses impact. This translates to about 11.3% reductions in fossil fuels use obligation of the product system due to 100% recycling of production harvest water.



**Figure 42 Single score LCIA comparing 1kg biogas production from AD of algae biomass (baseline model) with that of set-up with 100% recycling of production harvest water**

Besides, uncertainty analysis were carried out using the SimaPro uncertainty function in order to compare how significant the results are, between the baseline model and the production scenario with 100% recycling of production harvest water. The uncertainty results (Figure 43) are based on the distribution and standard deviation sets of values for each input in Table 19. The results show that the baseline model (process B) has lower outcomes values per damage category than the sensitivity scenario with 100% production harvest water recycling (process A). The uncertainty analysis results in Figure 43 therefore indicate that there is significant difference between the two processes.



**Figure 43 Uncertainty analysis of algae-to-biogas AD transformation process (with 100% recycling of production harvest water) vs algae-to-biogas transformation (base-case scenario)**

### **4.5.3 LCIA analysis of the prospects of using wastewater/sea water as culture medium for the algae-to-biogas AD production process**

Eco-indicator 99 method in SimaPro has been used to analyse the consequences of using wastewater/sea water as alternative microalgae growth medium of the production system. The result presented in Figure 44 below, depicts the LCIA characterisation results of the AD of algae biomass to produce 1kg biogas using wastewater/sea water as the production culture medium. Table 21 presents the inventory data used for the analysis.



**Table 21 SimaPro Input data sheet for the production of 1 kg biogas via Anaerobic Digestion (AD) from algal biomass (using wastewater/sea water as culture media), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation**

Product produced	Amount	Unit	Quantity	allocation
Biogas	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	0	kg	Lognormal	1.05
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.05
Biomass (biotic)	1	kg	Lognormal	1.05

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.02	kg	Lognormal	1.07

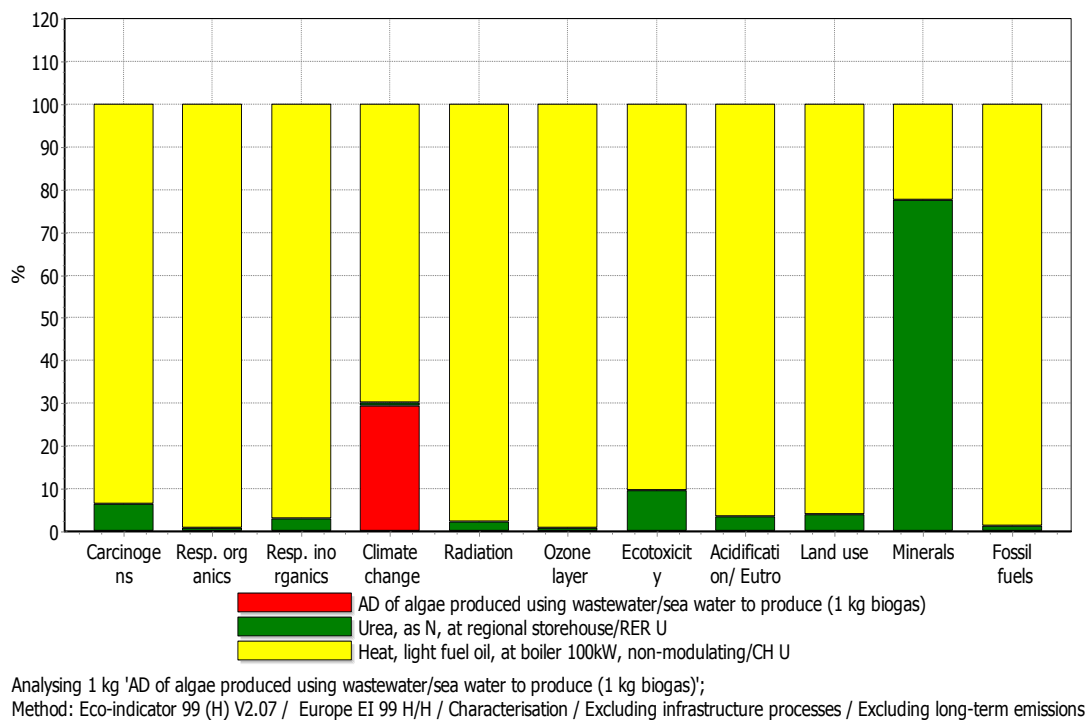
Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	74.67	MJ	Lognormal	1.06

Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.07

From the results in Figure 44 below, electricity/heat energy requirement visibly dominates the percentage contribution across most of the impact categories analysed in the chart. Except for mineral use impact category, where urea as N use obligation accounts for 77.5% of the total percentage contribution towards mineral use impact. It clearly shows that the use of wastewater/sea water

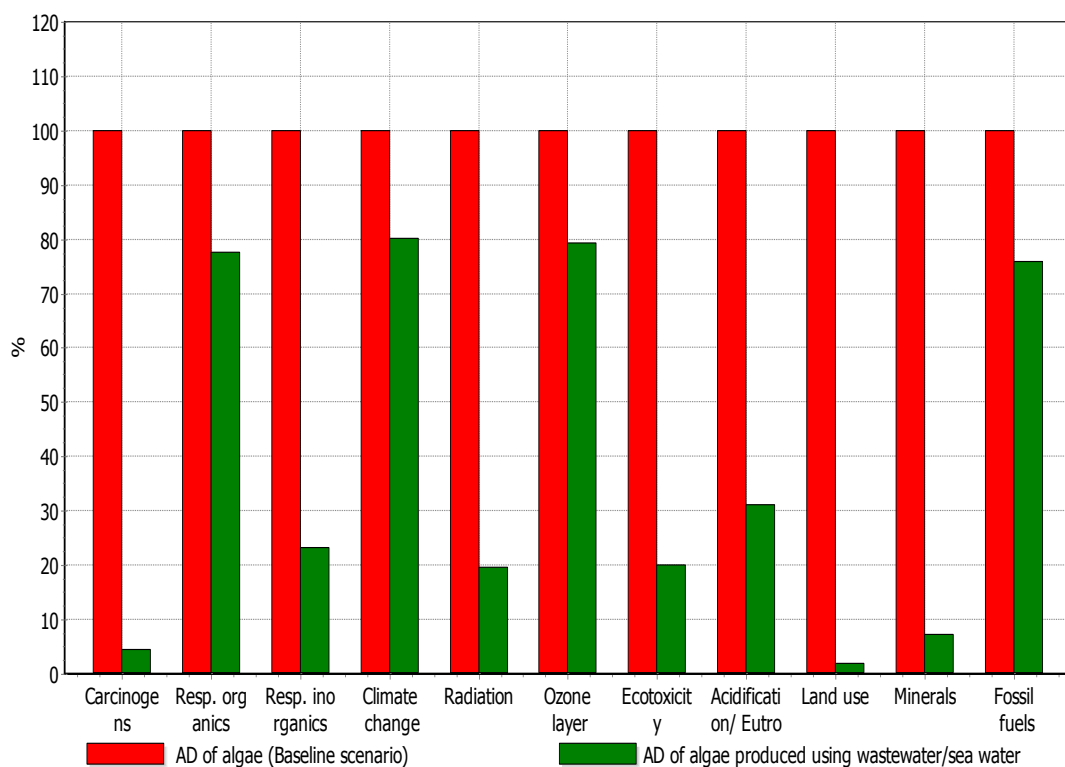
completely eliminates the requirement for single superphosphate as  $P_2O_5$ , while also reducing the demand for freshwater.



**Figure 44 LCIA results for AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium: wastewater here represents secondary effluents characterised by low biological oxygen demand (BOD) and chemical oxygen demand (COD) but high in inorganic N and P.**

Likewise, the extent of the impact of the prospective use of wastewater/sea water as the growth medium for culturing microalgae biomass was examined by comparing the baseline model with that of a potential set-up using wastewater/sea water as an alternative to freshwater. Results outcome for this scenario are as depicted in Figure 45. The results chart indicates that using wastewater/sea water brought about significant overall reductions across all of the impact categories with regards to the baseline model of producing 1kg biogas from AD of algae biomass. More so, the highest reductions impact was for land use, showing an impact reduction value of about 98.1% for land use

requirement. This is closely followed by minerals use impact, indicating a reduction of 92.65% as a result of using wastewater/sea water as alternative growth culture medium. With significant reductions across other impact categories: fossil fuels use impact categories 24.2%, ozone layer impact category 20.6%, and climate change impact category (20% reductions) respectively, as shown.

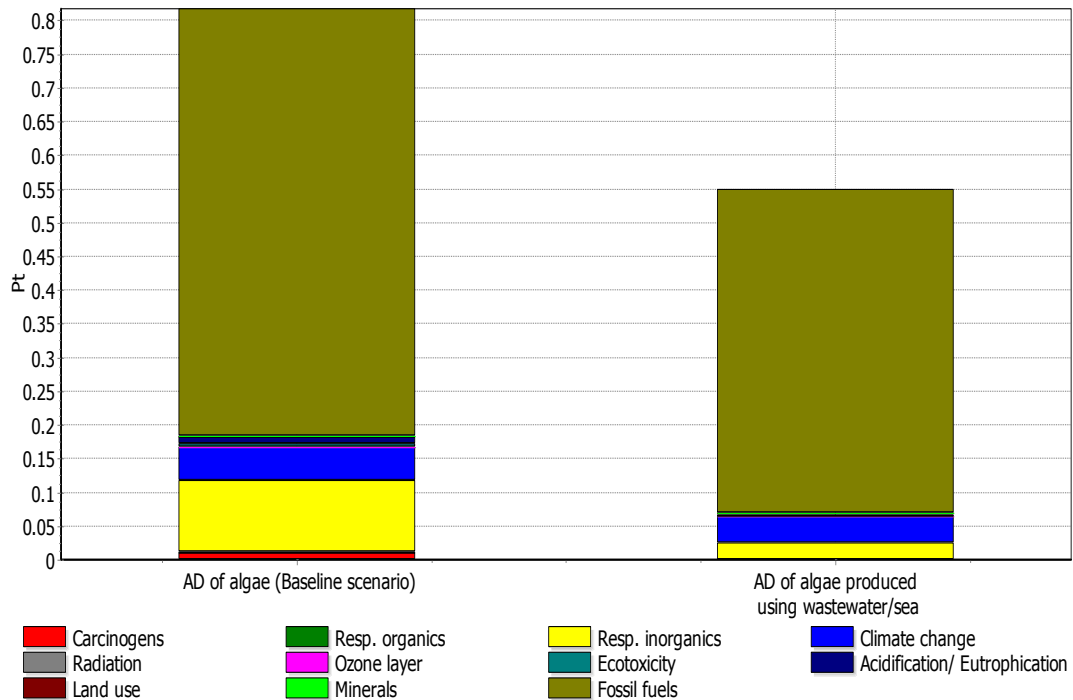


Comparing 1 kg 'AD of algae (Baseline scenario)' with 1 kg 'AD of algae produced using wastewater/sea water';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 45 LCIA comparison of AD of algae biomass to produce 1kg biogas (baseline model) vs AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium**

Additionally, Figure 46 depicts the single score impact characterisation comparison of the baseline AD production model with that of the production scenario using wastewater/sea water as culture media. The chart in Figure 46 highlights the share variation for each process material, impact category and

damage categories as shown. Eco-indicator 99 method in SimaPro has been used for this analysis, excluding infrastructure processes and long-term emissions. Using wastewater/sea water as algae growth/production medium would see fossil fuels use impact reduce by 0.12pts (i.e. from 0.64 pt for the baseline model to 0.52pts for the prospective model) in contrast with the baseline production model. Also, the prospects of using wastewater/sea water for the production system reduced climate change impact by 0.09pts (i.e. from 0.29pts for the baseline scenario to 0.20pts for the prospective set-up).

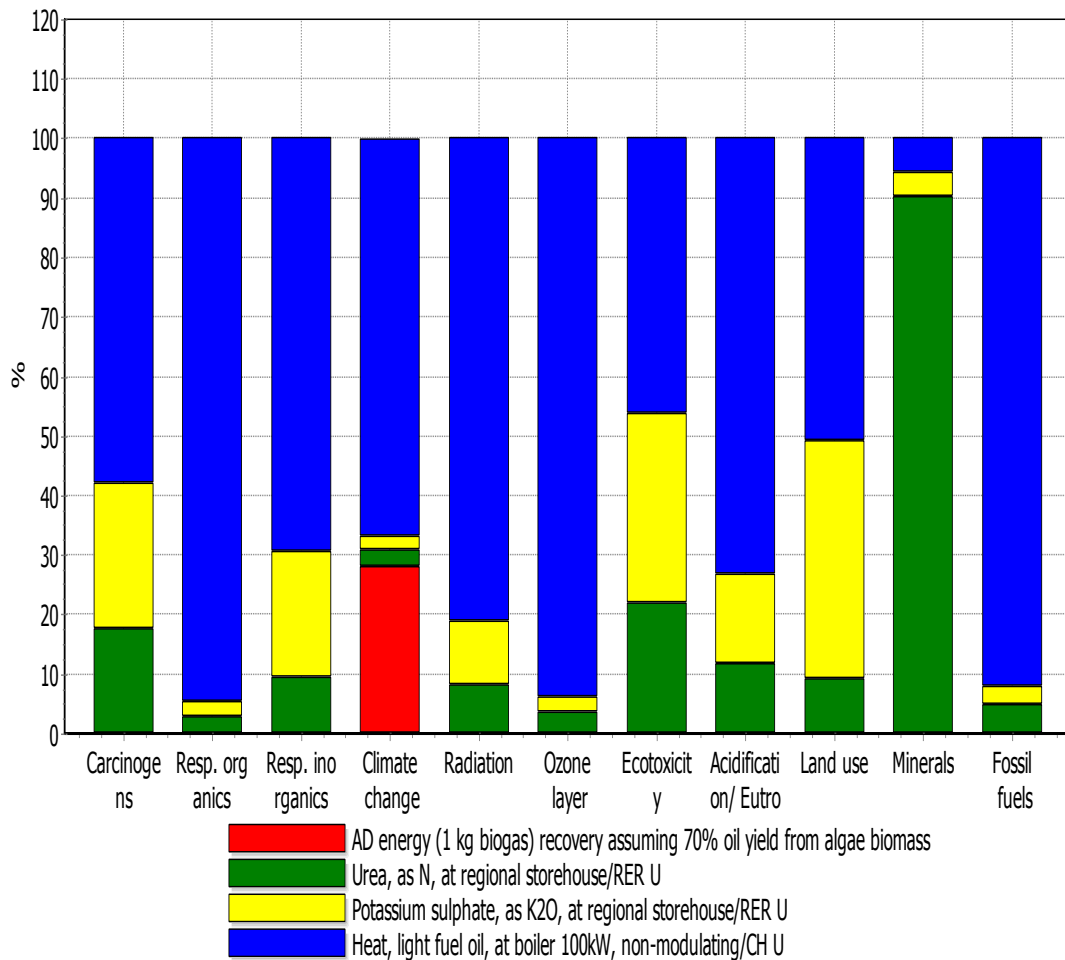


Comparing 1 kg 'AD of algae (Baseline scenario)' with 1 kg 'AD of algae produced using wastewater/sea water';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Single score / Excluding infrastructure processes / Excluding long-term emissions

**Figure 46 Single score LCIA analysis comparing AD of algal biomass to produce 1kg biogas (baseline model) with AD of algal biomass to produce 1kg biogas using wastewater/sea water as growth media**

#### **4.5.4 LCIA analysis of the prospective impact of induced increase in algae oil yield on the algae-to-biogas AD transformation process**

The LCIA result outcome in Figure 47 show the analysis of 1kg biogas production set-up via AD of algal biomass, assuming a 70% induced lipid yield through nutrient limitation. This result is based on the inventory data presented as Table 22. The analysed chart result shows that electrical/heat requirements signify the major contributor, across most impact categories. As clearly, electrical/heat accounts for 92% of total fossil fuels use impact. While also representing 67% of climate change impact, 93.8% contribution towards ozone layer depletion impact, and 73% of acidification/eutrophication impacts contributions, respectively. However, urea as N (nutrient requirement) accounts for the largest percentage contribution towards mineral use impact category. With its value representing over 90% of the total impact contributions due to minerals use obligation for the microalgal AD production system. This result value apparently seems anomalous, as it appears to be inconsistent with nutrient (particularly, N) reduction practice, which is a prerequisite for induced increase in algae lipid yield. The outcome also seems inconsistent with expected results, as input data for N were lowered, compared to the clearly huge outcome values.



Analysing 1 kg 'AD energy (1 kg biogas) recovery assuming 70% oil yield from algae biomass';  
 Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 47 LCIA analysis of 1kg biogas production via AD of algal biomass with 70% induced lipid yield**

**Table 22 SimaPro Input data sheet for the production of 1 kg biogas (with 70% induced lipid yield) via Anaerobic Digestion (AD), Note: RER= Europe, U=Unit process, CH=Swiss, SD=Standard deviation**

Product produced	Amount	Unit	Quantity	allocation
Biogas	1	kg	Mass	100%

Inputs from nature	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Water, unspecified natural origin	3726	kg	Lognormal	1.05
Carbon, in organic matter, in soil	15.39	kg	Lognormal	1.05
Biomass (biotic)	1	kg	Lognormal	1.05

Inputs from technosphere (materials/fuels)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Urea, as N, at regional Storehouse/RER U	0.09	kg	Lognormal	1.05
Single superphosphate, as P <sub>2</sub> O <sub>5</sub> at regional storehouse/RER U	0.21	kg	Lognormal	1.05

Inputs from technosphere (electricity/heat)	Amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
Heat, light fuel, at boiler 100kW non-modulating/CH U	74.67	MJ	Lognormal	1.05

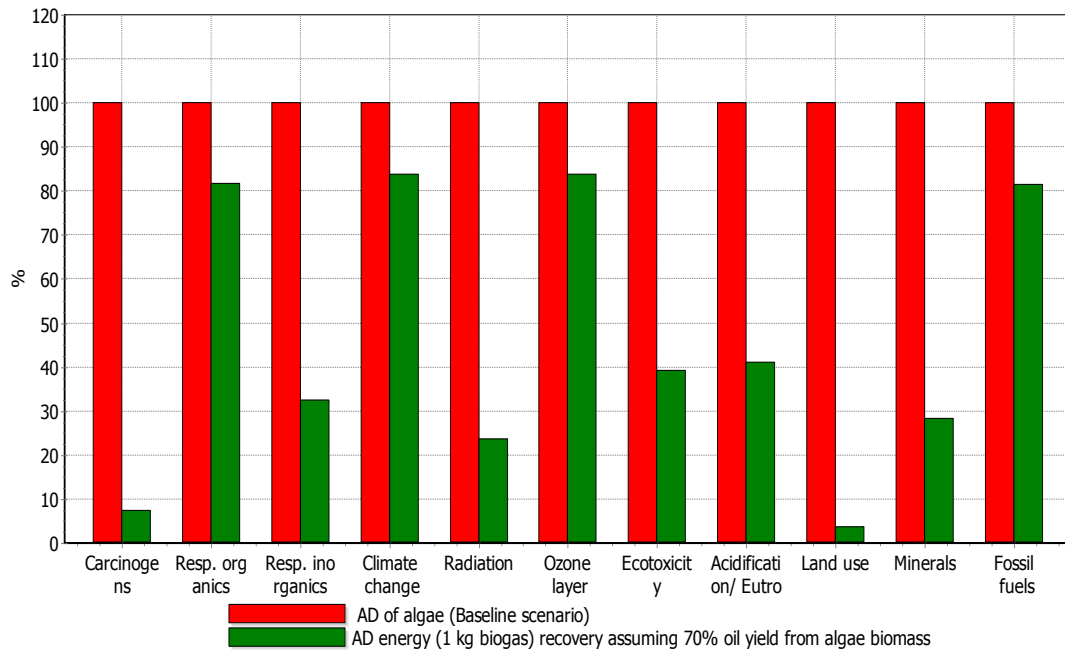
Emissions to air	amount	Unit	Distribution	SD $\lambda$ 2 or 2*SD
CO <sub>2</sub>	2.86	kg	Lognormal	1.05

Furthermore, the consequence of the prospects of incorporating a 70% potentially induced increase in algae biomass lipid yield, and how this strategy influence the algal biogas production system was examined with results as

shown in Figure 48. The study results are based on the comparison of LCIA outcomes of the baseline AD model with that of the production scenario integration a nutrient limiting strategy that induces increase in biomass lipid synthesis. The comparison results were characterised using eco-indicator 99 method in SimaPro.

The LCIA comparison results show that induced increase in algal biomass lipids brought about significant reductions across all impact categories, in comparison with the baseline production model. The characterisation chart results of the AD energy recovery process, assuming 70% induced increase in lipid yield show the following impacts reductions: fossil fuels use 18.6%, climate change 16.3%, ozone layer depletion 16.1%, and ecotoxicity 60.7% reductions accordingly. With the most considerable impacts reductions being indicated for land use impact category 96.39%. This is closely followed by carcinogens impact category 92.61%, and minerals use impact categories 71.6% in that order. The 71.6% reductions indicated for minerals use obligation is anticipated, as induced lipid increase in microalgae is associated with growth mineral (particularly N) reductions. However, it further strengthens the doubts expressed about the percentage contribution of Urea as N, indicated for mineral use impact in the results in Figure 47.

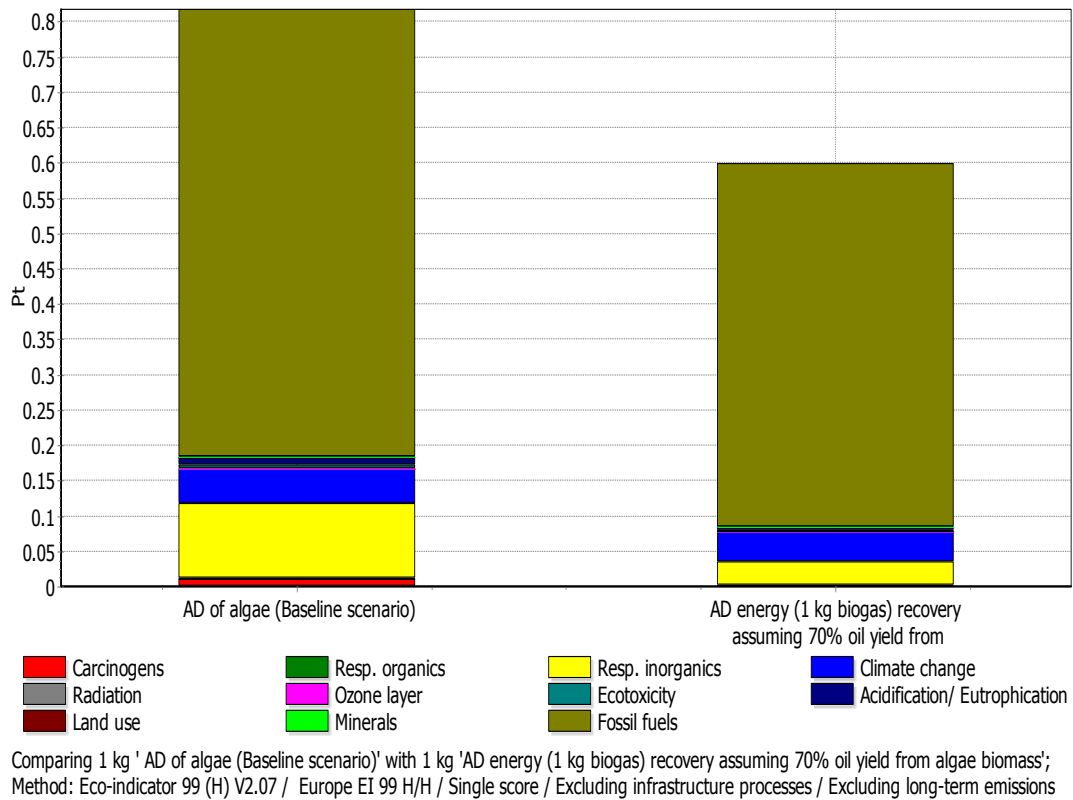




Comparing 1 kg 'AD of algae (Baseline scenario)' with 1 kg 'AD energy (1 kg biogas) recovery assuming 70% oil yield from algae biomass'; Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

**Figure 48 Comparing 1 kg biogas produced from AD of algal biomass (baseline model) with that of producing 1kg biogas via AD of algal biomass (assuming 70% induced increase in lipid yield)**

Additionally, in order to examine each individual production process in a bid to examine the share allocation for individual materials, impact category and damage categories respectively. Single score impact characterisation comparison of both production systems excluding infrastructural processes/excluding long-term emissions was administered using eco-indicator 99 method in SimaPro with results as depicted in Figure 49. In comparison with the baseline model, incorporating 70% induced increased in microalgae biomass lipid synthesis would bring about the following reductions/savings on the product system. 0.12pts savings for fossil fuels use impact category (i.e. baseline model; 0.63pt and prospective model; 0.52pt), and climate change impact category 0.01pt (i.e. baseline model; 0.05pt and prospective model; 0.04pt) accordingly.

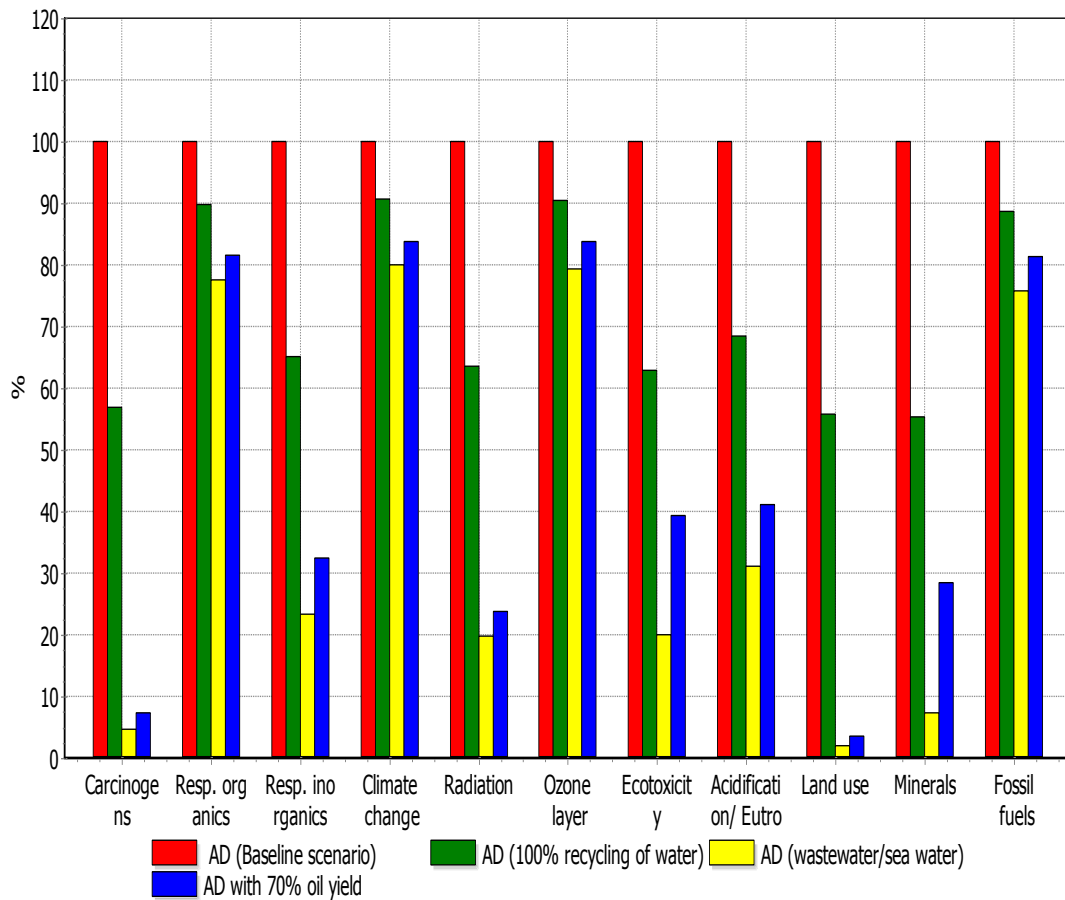


**Figure 49 Single score LCIA analysis comparing AD of algal biomass to produce 1kg biogas (baseline model) with AD of algal biomass to produce 1kg biogas assuming a 70% induced increase in lipid synthesis**

#### **4.5.5 Comparing the baseline model of producing 1kg biogas from AD of algae biomass with that of all proposed prospective alternative production scenarios**

Determining the effects and how variations in particular production data sway the microalgae AD transformation system, as in previous segments of this section, is not sufficient for performing a complete LCA. A comparison of the LCIA outcomes of these proposed scenarios with that of the baseline model is needed to determine the extent such variations in scenarios have on the production system. This assessment is essentially, in line with the project framework which is aimed at lowering material consumption, reducing energy use and significantly lowered environmental impacts production alternatives. As doing this would help identify the prospective scenario that offers the best solution, with regards to the least polluting process and also the most environmentally challenging burden common with each scenario. Figure 50 illustrates the LCIA characterisation comparing all processes with the baseline production model. The results were generated based on the inventory data for each production set-up and using the compare function in SimaPro.

Accordingly, AD of algae biomass to produce 1kg biogas utilising wastewater/sea water apparently offers the best result value for fossil fuels use impact, with a score of 13.4 MJ surpluses (75.8%), compared to the baseline model. This is closely followed by the production process integrating the strategy of a 70% induced increase in algae biomass lipid synthesis, indicating 14.4 MJ surplus (81.4%) score as a consequence of fossil fuels use. However, AD transformation of algae biomass to biogas with 100% recycling of production harvest water indicted the least impact reductions in terms of fossil fuels use relative to others. Showing 15.7 MJ surplus (88.7) impact scores, due to fossil fuels use in comparison with the baseline production model.



Comparing 1 kg 'AD (Baseline scenario)', 1 kg 'AD (100% recycling of water)', 1 kg 'AD (wastewater/sea water)' and 1 kg 'AD with 70% oil yield'; Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Characterisation / Excluding infrastructure processes / Excluding long-term emissions

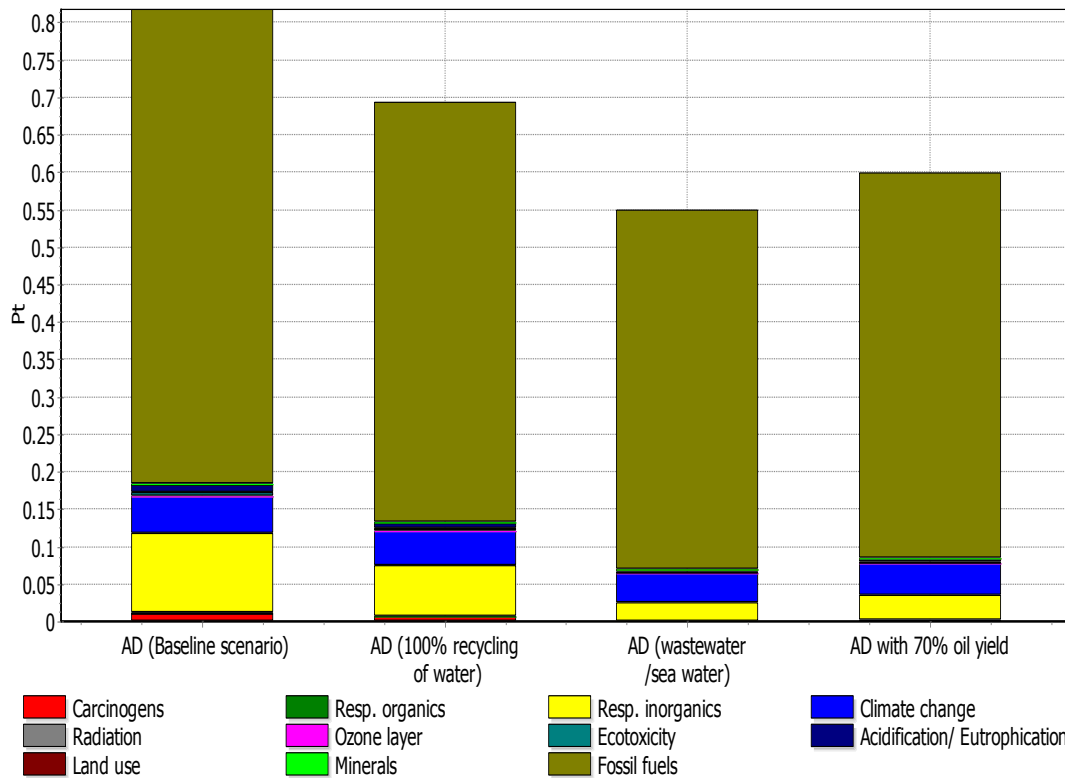
**Figure 50 LCIA Comparing processes: (1) AD of algae biomass to produce 1kg biogas assuming 70% induce increase in lipid yield, (2) AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium, (3) AD of algae biomass to produce 1kg biogas with 100% recycling of production harvest water, and (4) baseline AD production model**

As for mineral use impact category, it appears that all proposed alternative strategies brought about significant reductions in mineral use. With the potential use of wastewater/sea water as culture media for the production set-up, visibly indicating the smallest value of impact score of 0.000334 MJ surpluses (7.35%) compared to the baseline model. This translates to a whopping 92.65% reduction in mineral use obligation for the production system, due to the use of wastewater/sea water. This result outcome is very close to the 94% reductions

in mineral usage reported recently by Yang et al., (2011), as a consequence of using sea/wastewater for algae cultivation. Similarly, the prospective strategy of inducing 70% increase in microalgae biomass lipid yield, via nutrient limitation indicated significant reductions in mineral use with analysed chart value of 0.00129 MJ surpluses (28.4%), relative to the baseline model. Also, the prospects of 100% recycling of production harvest water also brought about considerable reductions in mineral use, with chart score value of 0.00252 MJ surplus (55.3%) comparative to the baseline model. This translates to a 44.7% reduction in mineral use requirement as a consequence of recycling, compared to the baseline model. Consequently, the potential of using wastewater/sea water to grow algae culture would offer the best reduction results, with regards to mineral use obligation compared to other proposed parallel production scenarios. However, wastewater/sea water tolerant algae species are very limited in algae-based biofuel application.

With regards to climate change impact, the production model adopting wastewater/sea water as its preferred culture media indicated the least impact score of 80% relative to the baseline production model. This is closely followed by the prospect of inducing 70% increase in algae biomass lipid yield, showing 83.7% (i.e. 16.3% reductions), in contrast to the standard production model. However, the production set-up incorporating 100% recycling of production harvest water impacted the least reduction on climate change impact, with an impact score value of 90.7% (i.e. 9.3% reductions) relative to the standard production model.

Furthermore, Figure 51 below depicts the single score LCIA comparison of all the proposed alternative scenarios with that of the standard (baseline) AD production model. It illustrates which components that contribute the most weights in terms of material use, environmental impact and damage categories specific to each production scenario based on eco-indicator 99 methods in SimaPro.



Comparing 1 kg 'AD (Baseline scenario)', 1 kg 'AD (100% recycling of water)', 1 kg 'AD (wastewater/sea water)' and 1 kg 'AD with 70% oil yield'; Method: Eco-indicator 99 (H) V2.07 / Europe EI 99 H/H / Single score / Excluding infrastructure processes / Excluding long-term emissions

**Figure 51 Single score LCIA comparing processes: (L to R) (1) AD of algae biomass to produce 1kg biogas assuming 70% induce increase in lipid yield, (2) AD of algae biomass to produce 1kg biogas using wastewater/sea water as culture medium, (3) AD of algae biomass to produce 1kg biogas with 100% recycling of production harvest water, and (4) baseline AD production model**

Thus, the production scenario proposing the exploitation of wastewater/sea water to culture algae biomass indicates the best results outcome. With regards to lowered material/energy use and impact to environment, based on the analysed chart results. As it indicates 0.48 pt for fossil fuels use category, relative to the baseline model chart result of 0.63 pt attributed to fossil fuels use. This translates to 24.17% reductions in fossil fuels use, as a consequence of the alternative application of wastewater/sea water in place of freshwater-use as standard culture media. Closely following these results, is the production scenario integrating the prospective of 70% induced increase in algae biomass

lipid production. As it indicates 0.515 pt, suggesting an 18.64% offset in fossil fuels use compared to the baseline production model. The scenario proposing the prospects of combining 100% recycling of production harvest water, impacted the least impact reduction on fossil fuels use category (11.37%), compared to other proposed parallel production scenarios.

Likewise, the production set-up proposing the use of wastewater/sea water for growing algae brought about significant reductions also, in its climate change footprint. Indicating analysed chart value of 0.4 pt due to climate change impact relative to the baseline production model value of 0.5 pt. This translates to 19.8% reductions in climate change burden, due to the alternative use of wastewater/sea water as culture media. The production set-up integrating 70% induced increase in biomass lipid yield strategy, also impacted significantly on climate change. With analysed chart impression of 0.041 pt relative to the baseline production model. This turns out to be 16.2% reductions in climate change footprint of the baseline production system, by incorporating this strategy. Similarly, the scenario with 100% recycling of production harvest water brought about the smallest impact reduction in its climate change footprint, in comparison with other proposed alternative production set-ups. With a 9.2% reductions in climate change burden of the baseline production model, as a consequence of combining 100% recycling of production harvest water.

Therefore, the prospects of the use of wastewater/sea water for cultivating algae biomass for biogas production via AD seems to offer the best results, amongst the entire prospective alternative improvement scenario analysed. However, the application of wastewater/sea water as culture media has its inherent limitations. Future research focus on deploying bioenergy should consider the large-scale implications of the use of wastewater/sea water in order to advance the feasibility of algae biofuel as carbon-neutral replacement for fossil fuels.

# **CHAPTER FIVE**

## **CONCLUSIONS AND RECOMMENDATION FOR FURTHER STUDIES**

### **5.1 Introduction**

LCA methodology using Eco-indicator 99 approach in SimaPro software has been successfully used to characterise the direct and indirect inputs and outputs of two baseline microalgae biofuel production processes: (1) BD production via transesterification of extracted algae lipids; and (2) AD of a relatively wet algae biomass to produce biogas. The goal was to determine the weak spots within the production systems and identify possible changes in scenarios that can lead to lower material use, lesser energy consumption and lower environmental burdens than the standard microalgae biofuel production system. As the baseline models are currently considered to be very energy intensive. Thus, four prospective alternative production scenarios were examined to evaluate the extent of their impact on the production system. The alternative scenarios are: 100% recycling of production harvest water; use of wastewater/sea water as alternative microalgae culture medium; co-product mass-economic allocation consideration; and integrating a 70% induced increase in algae lipid yield strategy. Using SimaPro package, these scenarios were simulated and analysed by comparing them with the baseline production models (set of generic algae biofuel production scenarios) with conclusions and the associated limitations and recommendation for future study presented herein.



## 5.2 Conclusions

The initial LCI analysis results in this study successfully identified the quantitative percentage energy input and output values of each material for the production of 1 kg BD via standard transesterification process. Heating requirement to get the algae biomass to 90% DWB represents 64% of the total input energy, with electrical energy at various instances within the production system and fertilizer nutrient obligation contributing 19% and 16% respectively. Thus, the main objective of the present study which is to analyse and quantify the impact of materials and energy inputs on the production process has been successfully achieved.

Also, to examine the potential environmental impacts of each production scenarios, LCIA characterisation of each process options have been successfully screened using Ecoindicator 99 approach in SimaPro at both characterisation levels and at single score impact levels respectively. LCIA characterisation results for the baseline scenario of producing 1 kg BD indicated that, heat and electrical energy requirements represent the most contributing factors towards most of the impact categories relative to other resources. As it showed 84.7% (34.7 MJ surplus) of the proportions of fossil fuels use impact, 69% for climate change, 86.8% of ozone layer depletion impact and 36.6% of impact due to acidification/eutrophication. Except for the impact characterisation results of soya oil use, this accounted for 99.5% of land use impact category. A result outcome attributed to the use of soya oil plant production data (associated with huge land requirement), as representative data for microalgal oil (lipid) which is currently not included in SimaPro ecoinvent data base.

Likewise, for the baseline AD of algae-to-biogas production model, electricity/heat requirements seem to be the most dominating contributing factor towards most of the impact categories followed by single superphosphate requirement as  $P_2O_5$ . Screened results indicated 75% (13.3 MJ) contribution towards the proportion of fossil fuels use, climate change impact category 56.1%, ozone layer impact category 78.7%, and acidification/eutrophication category 30.1% respectively. However, results for the AD transformation

process showed 43.1% relative reductions in heat/electricity process contribution (see Table 18) compared to the baseline BD production scenario. This is due to the exclusion of thermal drying and hexane oil extraction processes (which cumulatively represent over 50% of the algal biofuel production cost) in the AD transformation process.

In recognition of the huge impact contribution values for heat, electricity and fertilizer nutrient requirements (urea as N, and  $P_2O_5$  as P) towards the overall LCI energy profile and LCIA results of the algae BD and AD of algae-to-biogas production systems. This study has proposed, examined and identified process improvement options in form of alternative materials and process set-ups, which brought about considerably lowered material usage, lowered energy consumption, and lowered environmental burdens than the traditional algae biofuel production system. The process scenarios examined includes: impact of 100% recycling of production harvest water, use of wastewater/sea water as alternative algae culture media, impact of co-product allocation consideration, and a production scenario integrating a 70% induced increase in algae lipid yield. Again, the analysis and evaluations of these proposed alternative process models accomplished the second objective requirement stated in this research.

LCIA results for producing 1 kg BD integrating 100% recycling of production water, showed that heat/electricity use represents 28.4 MJ surpluses (88.9%) of total contributions towards fossil fuels use impact category. With soya oil, single superphosphate (as  $P_2O_5$ ), and Urea (as 46% N content) accounting for 1.11 MJ (3.48%), 1.02 MJ (3.21%), and 1.4 MJ (4.39%) respectively for the same impact category. The LCIA results values for the baseline BD production set-up and the production scenario integrating recycling showed a similar pattern, except for water demand which reduced from 3726 kg (freshwater)·kgBD<sup>-1</sup> to 591 kg (freshwater)·kgBD<sup>-1</sup> after accounting for evaporative losses/biomass drying in the later scenario. However, the consequences of the prospect of recycling production harvest water on the proposed algae BD production system can be evaluated by comparing the LCIA results of these two scenarios. The LCIA comparison results showed that recycling of production harvest water

brought about 4.3 MJ savings (18.9%) in heat/electricity obligation due to fossil fuels use, relative to the baseline BD production scenario with no recycling. Recycling also reduced climate change impact burden by 21.4% and 36.1% impact reductions for ozone layer depletion impact categories, relative to the baseline scenario of non-recycling of production harvest water. The biggest impact on the proposed BD production system due to recycling was a 45% reduction and savings in mineral use obligation in comparison with the baseline scenario of non-recycling. It is important to note that this results confirms earlier reports of the overall impacts of recycling (Brennan and Owende, 2010; Yang et al., 2011), as non-recycling of production harvest water increases inorganic nutrient use requirement.

Similarly, LCIA comparison results of the baseline AD transformation of algae biomass to biogas with that of a scenario incorporating 100% recycling of production harvest water, indicated significantly lowered impacts on the entire range of impacts categories due to recycling. The most significant impact reduction was in mineral use impact category, with a 55.3% reduction in mineral use obligation as a result of recycling. 100% recycling of production harvest water also brought about 11.3% reductions in fossil fuels use obligation relative to the baseline product system.

In the LCIA comparison analysis to fully understand the potentials of the trade-offs between using freshwater and the use of wastewater/sea water as algae growth media, results showed that the later application impacted positively on the production systems. As it indicated a 4.2 MJ savings (11.8%) in heat/electricity requirement, due to the use of wastewater/sea water as alternative growth media for the BD production system. Similar reductions were also indicated for climate change impact (10.7% reductions), ecotoxicity impact category 54.9% reductions and across most impact categories. The most significant reduction margin was 87% offset in mineral use requirement indicated as consequence of the use of wastewater/sea water as alternate culture medium.

Likewise, the use of wastewater/sea water for the AD transformation of algae-to-biogas product system, showed a 98.1% impact reduction for land use impact relative to the baseline production system of using freshwater as algae culture growth media. Other significant reductions due to wastewater application are: 92.65% reductions in mineral use impact category, 24.2% reductions in fossil fuels use impact obligation, and a 20.6% reduction in ozone layer depletion impact category accordingly.

The impact of co-product allocation consideration on the LCIA results of the production system was analysed for the BD transesterification process, using mass-economic basis. It requires integrating the 0.018 kg glycerol (43.5% allocation) from the product system as co-product to the main output product of 1 kg BD (56.6% allocation), and the residual algae cake (LEA) as avoided products. This is based on the assumption that the residual LEA is combusted in a CHP plant to compensate for heat and electrical energy (312.91 MJ) demands within the production system boundary. Results for the LCIA analysis incorporating co-products allocation consideration, showed fossil fuels use impact reduce to 52% (16.4 MJ surplus) from 84.7% (34.7 MJ) for the baseline scenario. Similar reductions are indicated for climate change impact category (52%), ozone layer depletion (52%) and acidification/eutrophication (35.2%) reductions accordingly. However, land use impact category remained unchanged at 99.5%, an outcome attributed to the huge land requirement obligation associated with soya bean oil plant cultivation.

The LCIA characterisation comparison results comparing the baseline BD production scenario with that of a set-up with co-products economic allocation consideration, is one of the most interesting outcomes of this study. Fossil fuels resource use impact category showed -12 MJ surplus (-33%), with regards to heat/electricity energy obligation for the proposed BD production process model (with co-product allocation consideration). Similar negative percentage reduction values were indicated for ozone layer impact category (-35%), climate change impact category (-16.9%), and respiratory organics impact category (-23.2%) respectively. The negative percentage values are common with bio-

based products (Consultants, 2008) and depicts the energy credit required to compensate for future resource use. Likewise, there were positive reduction outcomes indicated for mineral use impact (52.7%), land use (56.6%), and across all other characterised impact categories on account of co-product/avoided products allocation consideration. These results show the importance of allocation outcomes consideration to LCA as a decision support tool. Also, uncertainty analysis run using Monte Carlo function in SimaPro comparing the differences between the two production scenarios showed that, aside the absolute uncertainty value for land-use impact category which depicted considerably high uncertainty score. Uncertainty values for other impact categories confirm that there is significant difference between the baseline model and the scenario with co-product economic allocation consideration.

In this study the potential of increasing algae lipid yield during cultivation, via biochemical engineering strategy, as a possible prospect for enhancing the economics and environmental sustainability of the algae biofuel production process was analysed. LCIA results indicated negative impact values across all impact categories due to a 70% increase in lipid yield compared to the baseline BD production scenario. With the most contrasting results being due to mineral use impact category, which indicated higher impact value for the set-up with 70% induced increase in lipid yield. Even though the LCI data value for nutrients (0.09kg N, and 0.21kg P<sub>2</sub>O<sub>5</sub>) were lowered relative to the baseline set-up (0.33kg N, and 0.71kg P<sub>2</sub>O<sub>5</sub>) as a consequence of nutrient limitation. The discrepancy in impact results for this scenario is thought to be due to the use of soya oil production data as representative data for microalgae. As microalgae oil production data is currently not captured in SimaPro ecoinvent data base. In addition LCIA at single score impact characterisation levels excluding infrastructural processes/excluding long-term emissions was carried out using eco-indicator 99 method in SimaPro, in order to show in clearer details the distribution share of each individual materials. Results show fossil fuels use impact increased by 3.9% from 1.29 pts for the baseline scenario to 1.34 pts for the proposed scenario with 70% induced increase in algae lipid yield. Land use

impact also indicated an unusually high score value increase, from 0.31 pts for the baseline scenario to 1.44 pts on account of 70% induced increase in algae lipids yield. This amounts to over 360% increase in land use requirement as a consequence of 70% induced increase in lipid yield via nutrient limitation. It is apparently far too high range of value to be practicable. However, this brings to light the significance and implications of previous result outcomes. Additionally, uncertainty analysis comparing the difference in results outcome between the proposed set-ups with 70% induced increase in lipid yield and that of the standard BD production scenario, showed significant differences between both processes. For example, the absolute uncertainty value for mineral use requirement of the production scenario with 70% induced increase in lipid yield, is clearly higher than its LCI data value. This is inconsistency with nutrient reduction practice, a standard requirement for induced increase in algae lipid yield.

Comparison results of the prospective of a 70% induced increase in algae lipid yield for the algae-to-biogas AD production system, on the other hand indicated better outcomes. Screened results indicated reductions across all impact categories as a consequence of induced increase in algal lipid yield strategy, relative to the baseline production scenario. With the most significant reductions being for land use impact category 96.39% reductions, carcinogens impact category 92.61%, and a 71.6% reduction in mineral use obligations respectively. Also, single score impact characterisation comparison of both scenarios showed that 70% induced increase in algae lipid yield brought about 0.12 pts reductions in fossil fuels use impact, and 0.01 pts reductions in climate change impact relative to the baseline model.

The results for the LCIA characterisation comparison of all the proposed processes for each impact category with that of the baseline model, indicated that BD production process (with co-products allocation consideration) showed the least impact score of 12 MJ surplus (- 31.9 %) for fossil fuels use impact category. The BD process options with the use of wastewater/sea water and the set-up incorporation 100% recycling of production harvest water, indicated the

same impact score values of 32 MJ surplus (85 %) for fossil fuels use. However, the proposed model with a 70% induced increase in lipid yield strategy, impacted the most on fossil fuels use impact category relative to the baseline BD production scenario. On the other hand, the set-up proposing the potential use of wastewater/sea water as culture media for the AD of algae-to-biogas process apparently offered the best result value for fossil fuels use impact, with a score of 13.4 MJ surplus (75.8 %) compared to the baseline model. The production process integrating a 70 % induced increase in algae lipid yield closely follows this results, indicating 14.4 MJ surplus (81.4 %) score for fossil fuels use impact category. However, the AD production scenario with 100% recycling of production harvest water showed the least impact reductions in terms of fossil fuels use relative to other production scenarios, showing 15.7 MJ surplus (88.7 %) impact score.

With regards to mineral use requirement for BD production process, the set-up with the use wastewater/sea water as culture medium clearly brought about the largest reduction of 4.81 % (best results) in mineral use obligation. This is closely followed by the BD production scenario with co-products allocation consideration, with impact score reduction of 2.89 % for mineral use. For LCIA characterisation comparison of all the AD production processes, the potential use of wastewater/sea water to grow algae offered the best reductions results, with regards to mineral use obligation relative to other proposed parallel production scenarios. With reductions impact results outcome of 92.65 % for mineral use requirement, a result outcome that is very close to the 94 % reductions in mineral usage reported recently by Yang et al., (2011) as a consequence of wastewater/sea water application. However, wastewater/sea water tolerant microalgae species are very few in algae-based biofuel application.

With regards to climate change impact category, the proposed BD production scenario with co-products allocation consideration showed the best results impact score of -14.2%. Followed by the production scenario integrating 100% recycling of production harvest water, with analysed chart value of 65.9% and

the set-up proposing the use of wastewater/sea water indicating 74.5%, which is 9% lesser than the climate change impact value of the baseline scenario. However, for all the processes analysed, the worst climate change impact outcome was indicated for the scenario assuming a 70% induced increase in algae lipid yield. Similarly, the proposed BD production scenario with 70% induced increase in algae lipids yield also showed worst impact results across other impact and damage categories screened relative to the baseline BD production model.

The single score LCIA comparing processes for the AD of algae biomass to biogas showed that the production scenario proposing the exploitation of wastewater/sea water to culture algae offered the best results outcomes with regards to lowered material/energy use and its impact to environment. Bringing about 24.17% reductions in fossil fuels use impact category, and 19.8% reductions in climate change burden as a result of the alternative use of wastewater/sea water in place of freshwater-use as culture media. Closely following these results is the production scenario integrating a 70% induced increase in algae lipid yield via nutrient limitation, providing an 18.64% offset in fossil fuels use and a 16.2% reductions in climate change footprint relative to the baseline production model. 100% recycling production harvest water would bring about 11.37% reductions on fossil fuels use impact and 9.2% offset in climate change footprint of the traditional AD production model.

Overall, it is important to note that the LCA results presented in this study suggest that suitable reduction alternatives can be developed, by cross-comparing LCIA results based on different criteria using LCA method. This is because it allows for evaluating alternative production pathways and identifying integration opportunities with greater economic and environmental benefits, relative to existing microalgae biofuel production models. This study therefore represents a necessary step at quantitatively assessing the potential for commercial microalgae based biofuel production.



### **5.3 Limitations and recommendations for further research**

This study as with any research project, the achievement of set objectives is usually limited by time, availability of research tools and funding constraints, which are conditions for drawing up a rational scope of work. Thus, it has not been possible to explore and exhaustively investigate all areas of interest related to microalgae biofuel production optimization, reported on here.

In this regard, the LCA report in this study has been limited to evaluating energy consumption of individual materials, environmental impacts of the production system, and developing necessary alternative reduction options aimed at significantly improving the microalgae biofuel production process. Impact assessment is adapted to the assumptions excluding infrastructure processes/excluding long-term emissions. Thus, in future work is recommended to extend the consideration of additional resources such as the algae production facility and its construction, feedstock processing logistics and transport infrastructure.

Analysis conducted in this study suggests that a significant amount of on-site energy (electrical/heat) could to be recovered from the LEA biomass in order to compensate and keep energy consumption within the production system down. Hence, a more detailed engineering analysis of on-site power (CHP) generation is key consideration to resolving the high energy demand limiting commercial microalgae biofuel production.

In addition, the assessment of the effect of 100% recycling of production harvest water in this study has been based on a hypothetical production scenario, without the consideration that recirculation may lead to excess nutrient concentration. Thus, further research is required, which should include a concrete site layout to evaluate the energy and environmental cost of routinely flushing the algae pond. A standard practice employed to control the accumulation of salts or growth inhibitors, in order to increase microalgae nutrient consumption.

In this study, the potential use of wastewater/sea water application as algae growth medium brought about the largest reduction value with regards to mineral use obligation of the production system relative to the baseline BD model. With mineral use impact chart value of 0.68% compared to the baseline BD production scenario analyses chart value of 5.49%. However, wastewater/sea water tolerant algae species are very limited in microalgae biofuel applications, due to usually high nutrient/salt concentration, presence of heavy toxic metals and prevalence of invasive bacteria and zooplanktons in wastewater/sea water. Therefore, future work must consider these issues and determine how they affect the product system.

Furthermore, the LCIA comparison results of the baseline BD production scenario with that of the setup integrating 70% induced increase in algae oil yield showed differing outcomes, particularly with regards to mineral use and land use impacts categories. This is attributed to the apparent use of soya oil production data as representative data, due to the absence of microalgae oil production data in SimaPro ecoinvent data base currently. Consequently, there is need to update the ecoinvent data base by including sector specific data for microalgae bio-production processes, as it does not fulfil all sector needs for foreground data at present.

Overall, evidence in this study shows that the technical and economic viability of microalgae biofuel production system hinges on sustained commitment towards the development of technologies to optimise production system. Particularly, with regards to algae culture conditions, biomass harvesting/oil extraction techniques and downstream biomass conversion processes. Priority research is desirable in these directions for the long-term sustainability of industrial-scale algae biomass-based energy production.

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