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SCHOOL OF CHEMICAL AND BIO ENGINEERING

CULTIVATION OF MICROALGAE FOR BIOFUEL PRODUCTION: COUPLING WITH SUGAR FACTORIES

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A PhD dissertation Presented to the School of Chemical and Bio Engineering in partial fulfillment of the requirements of the Degree of Doctor of Philosophy (Process Engineering Stream)

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DECLARATION

I declare that this dissertation has been composed by the author alone. Whole or any part of the work has not been submitted before for any other degree or professional qualification. I confirm that the work submitted is my own, it contains no material previously published or written by other persons. I have adequately cited and referenced the original sources.

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This is to certify that the dissertation entitled as **"Cultivation of Microalgae for Biofuel Production: Coupling with Sugar factories** submitted to Addis Ababa Institute of Technology, School of Chemical and Bio engineering for partial fulfilment of the requirements for the degree of Doctor of Philosophy (Process Engineering Stream) complies with the regulations of the university and meet the accepted standards with respect to originality and quality.

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ABSTRACT

Due to many important characteristics of microalgae including high oil content, ability to overcome harsh conditions and high photosynthetic efficiency the production of biofuels from algal biomass has gained interest. However, large scale production of biofuel from microalgae is still uncertain primarily due to a lack of feasibility of the process and that it proves to be capital and energy-intensive. Therefore, an integration of microalgal cultivation with other processes for achieving an inexpensive nutrient and a reduced energy use has paramount importance. Coupling of microalgal biofuel production with other process such as wastewater treatment, industrial and power plants has been found helpful in increasing feasibility of the process. The main objective of the present study was to evaluate the potential of sugar factories' wastes to support the growth of microalgae for biofuel production. A case study approach was followed considering a sugar factory, Metahara sugar factory, with annexed distillery plant. Two scenarios were considered. In the first scenario the wastewater from the sugar mill was used as the only nutrient source for the growth of the microalgae. In the second scenario the vinasse from the ethanol production plant was used as an additional nutrient source along with the wastewater from the sugar mill. Economic feasibility was also performed for the second scenario. The first scenario of the study shows that 12 mg of total nitrogen (TN) and 7.4 mg of total phosphorus (TP) per liter of wastewater could be transferred to algal growth ponds, and approximately 121 tons/year algal biomass would be produced from the integrated process. By applying all the underlined assumptions reductions of COD (mg O_2/L) from 2200 to 447, BOD_5 (mg O_2/L) from 1200 to 207, total nitrogen, TN (mg/L) from 15 to 0.6 and total phosphorus, TP (mg/L) from 10 to 1.5 were found from the coupled process. The results from second scenario of the study showed three products: biodiesel production of 188 ton/year, biogas production of 2011000 m^3/year, and bio-fertilizer production of 42

tons/year. Economic evaluation of the coupled process for the biofuel production showed that in order for the biodiesel from microalgae to be competitive with the current petroleum its minimum selling price (MBSP) needs to be reduced at least by half. Sensitivity analysis on the MBSP shows that oil content of the microalgae and nitrogen content in the waste effluents are the two dominant factors which significantly affect the feasibility of the process. This study investigated the potential of a future possible bio-refinery and environmental pollution reduction concept by coupling microalgae biomass production with sugarcane-processing factory wastes and by-products. It was found that the factory wastes and by-products have a significant potential for a viable biofuel production from microalgae.

Study on the biology of the microalgae to get a robust strain with high oil content, the development of energy-efficient and cost-effective harvesting technology, and study on the development of selective, sensitive and inexpensive control methods, etc. are essential future research works for boosting viability of biofuel production from microalgae.

Keywords: wastewater, flue gas, vinasse, process coupling, process evaluation, TEA, MBSP, sensitivity analysis

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List of symbols and Abbreviations

Symbols	Description
A_{SUR}	Surface area of a pond
A_{TFF}	Total facility foot print
A_{TOT}	Total cultivation area required
B_P	Total algal biomass production rate
$CO_{2_{FED}}$	Total amount of CO_2 in the flue gas added to the cultivation pond
$CO_{2_{TOT}}$	Total amount of CO_2 to be converted to microalgae
C_{CD}	Disruption factor
C_{DAF}	Energy requirement for the dissolved air floatation
$C_{E,AD}$	Electricity requirement for the anaerobic digester
$C_{E,ext}$	Electricity requirement for the extraction
$C_{E,tr}$	Electricity requirement for the transesterification
$C_{E,upg}$	Electricity requirement for the upgrading
C_{FI}	Energy requirement for flue gas injection to ponds
C_{FI}	Energy requirement for flue gas injection to ponds

C_{PF}	Energy requirement for pumping effluents from ponds
C_{PP}	Energy requirement for pumping primary treated wastewater to cultivation ponds
C_{PW}	Energy requirement by paddle wheel
$C_{TH,AD}$	Thermal energy requirements for the anaerobic digester
$C_{TH,ext}$	Thermal energy requirements for the extraction
$C_{TH,t}$	Thermal energy requirements for the extraction
C_{TR}	Transesterification efficiency
C_{cent}	Energy requirement for the centrifugation
C_{filt}	Energy requirement for the filtration
C_{filt}	Energy requirement for the filtration
E_{DAF}	Total energy requirement for the dissolved air floatation
$E_{E,AD}$	Electricity requirement for the anaerobic digester
$E_{E,ext}$	Total electricity requirement for the extraction
$E_{E,tr}$	Total electricity requirement for the transesterification
$E_{E,upg}$	Total electricity requirement for the upgrading

E_{FI}	Total energy requirement for flue gas injection to ponds
E_{PF}	Total energy requirement for pumping effluents from ponds
E_{PP}	Total energy requirement for pumping primary treated wastewater to cultivation ponds
E_{PW}	Total energy requirement by the paddle wheel
$E_{TH,AD}$	Total thermal energy requirements for the extraction
$E_{TH,ext}$	Total thermal energy requirements for the extraction
$E_{TH,tr}$	Total thermal energy requirements for the transesterification
E_{cent}	Total energy requirement for the centrifugation
E_{filt}	Total energy requirement for the filtration
f_n	Mass fraction of microalgae in concentrate stream, C_n
H_{RXN}	Amount of hydrogen which is obtained from the water reaction in the photosynthesis process
H_{TOT}	Total amount of hydrogen to be incorporated in to microalgae
K_{CO2}	Carbon dioxide utilization factor in the raceway ponds
K_N	Nitrogen utilization factor in the raceway ponds
K_P	Phosphorus utilization factor in the raceway ponds

$M_{CO_{2FED}}$ Total amount of CO_2 in the flue gas added to the cultivation pond

$M_{CO_{2TOT}}$ Total amount of CO_2 to be converted to microalgae

M_{C_n} Mass flow rate of microalgae in the more concentrated exiting stream from the harvesting operation ’n’

$M_{D,F_{CD}}$ Disrupted biomass in the feed stream to the extraction unit

M_{D_n} Mass flow rate of microalgae in the more dilute exiting stream from the harvesting operation ’n’

$M_{F_{CD}}$ Algae biomass in the feed stream to the extraction unit

$M_{F_{PS}}$ Biomass feed to the phase separation unit

$M_{F_{n+1}}$ Mass flow rate of microalgae in the feed stream to the harvesting operation ’n + 1’

$M_{N,R_{NUT}}$ Total amount of nitrogen in the recycle nutrient added to the cultivation step

$M_{N_{FED}}$ Total amount of nitrogen in the wastewater added to the cultivation step

$M_{N_{TOT}}$ Total amount of nitrogen to be converted to microalgae

$M_{P_{FED}}$ Total amount of phosphorus in the waste water added to the cultivation step

$M_{P_{TOT}}$ Total amount of phosphorus to be converted to microalgae

$M_{Q_{EFF}}$ Total algal biomass production rate

M_{BD} Mass of the biodiesel

M_{BF} Production rate of bio-fertilizer

M_{GLY} Mass of the glycerol

M_{LEA} Mass of lipid extracted algae in the residue

$M_{P,R_{NUT}}$ Total amount of phosphorus in the recycle nutrient added to the cultivation step

M_R Mass of biomass in the residue

$M_{algaefilt}$ Amount of algae removed in the filtration

$M_{algaefilt}$ Amount of algae removed in the filtration

$M_{wt\ CO_2}$ Molecular weight of CO_2

$M_{wt\ C}$ Molecular weight of carbon

N_{FED} Total amount of nitrogen in the wastewater added to the cultivation step

N_{FER} Total amount of nitrogen in the makeup nutrient added to the cultivation step

$N_{TOT,AD}$ Total nitrogen feed to the anaerobic digester

N_{TOT}	Total amount of nitrogen to be converted to microalgae
N_p	Number of ponds
P_B	Productivity of algae in the ponds
P_{FED}	Total amount of phosphorus in the wastewater added to the cultivation step
P_{FER}	Total amount of phosphorus in the makeup nutrient added to the cultivation step
P_{TOT}	Total amount of phosphorus to be converted to microalgae
Q_{D_n}	Volumetric flowrate of dilute stream
$Q_{BIOMASS}$	Water changed to microalgal biomass
Q_{EFF}	Water in the effluent coming out from the ponds
Q_{EVAP}	Water lost by evaporation from the pond
Q_{FILT}	Water going with the filtrate
Q_{RXN}	Water lost during reaction
Q_{WW}	Flow rate of the primary treated water
Q_{wcent}	Amount of water removed in the centrifugation
$q_{blowdown}$	Blow down water
q_{recy}	Recycled water from the filtration unit to the ponds

q_{super} Water going with the supernatant

$r_{N,AD}$ Reduction rate of nitrogen in the anaerobic digester

r_n Microalgal recovery rate for the harvesting operation 'n'

R Ratio of total area to total facility foot print

V_A Methane production from the whole algae

V_{LEA} Methane production for the lipid extracted algae

W_{C_n} Mass flow rate of water in concentrate stream

X_N Concentration of total nitrogen in the wastewater

X_P Concentration of total phosphorus in the wastewater

Abbreviations

AD Anaerobic digester

AF Anaerobic filter

BD Biodiesel

BF Bio-fertilizer

BG Biogas

BOD	Biochemical oxygen demand
C	Carbon
CEPCI	Chemical engineering plant cost index
COD	Chemical oxygen demand
CPI	Consumer price index
CSTR	Continuous stirred-tank reactor
DAF	Dissolved air floatation
FCI	Fixed capital investment
GHG	Greenhouse gas
GTP	Growth transformation plan
H	Hydrogen
IRR	Internal rate of return
ISBL	Inside battery limit
LEA	Lipid extracted algae
MBSP	Minimum biodiesel selling price
NPV	Net positive value

OLR	Organic loading rate
OSBL	Outside battery limit
P	Phosphorus
PBR	Photobioreactor
TC	Total capital
TCI	Total capital investment
TDC	Total direct cost
TDS	Total dissolved solid
TEA	Techno-economic analysis
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solid
UASB	Up-flow anaerobic sludge blanket
VS	Volatile solids
WW	Wastewater

WWT	Wastewater treatment

CHAPTER ONE

1. Introduction

1.1. Background

Energy crisis is a big challenge almost in every part of the world because of rapid industrialization and population growth. Though the current energy demand is being fulfilled mostly by fossil fuel in the near future it seems extremely difficult due to the depletion of fossil fuels (Abdullah et al., 2007; Lam et al., 2012). The instability of price of petroleum based fuels; energy protection; global warming due to GHG emissions, for example between 1970 and 2004 annual GHG emissions increased by 80% (Greenwell et al., 2010) resulted in focusing in renewable energy sources such as wind, solar, hydro, geothermal and biomass worldwide (Dragone et al., 2010; Rajkumar et al., 2014; Chowdhury & Loganathan, 2019). There are several activities in the developed countries which promote the share of renewable energy sources (Laurens, 2017). The promotion of the share of renewable energy is not limited only to the developed nations; it is also the concern of developing African countries including Ethiopia, which fundamentally depends on the imported energy. Among the sustainable renewable energy sources are biofuels that are made mostly from different plant materials.

Higher plants, oil crops, and lignocellulosic biomass are among the alternative renewable biofuel sources. The losses of ecosystems & biodiversity, global warming and increase in food prices are the main problems associated with production of biofuels from higher plants (Williams, 2007; Patil et al., 2008). Though crops such as soy, palm oil, corn, and sugar cane are widely used as raw materials for feasible biofuel production at large scale because of well-established farming practices, simple and cheap processes for the release of starches, sugars and oils they are also beautiful food crops that their utilization for biofuel production results in an increase in food prices. Hence it may not be possible to meet the full demand of renewable fuel energy from such crops (Somma et al., 2010). Lignocellulosic biomass is proposed as the second-generation feedstock for biofuel production particularly for bioethanol production. Though it is the most abundant biomass and does not compete with food supplies (Alvira et al., 2010) the requirement of an additional pretreatment step to break down the complex structure of lignin is a big challenge (Cardona & Sánchez, 2007). In this regard third generation biofuel production, which essentially refers to microorganism-based options for biofuel production, has got growing interest (Brennan & Owende, 2013). Under this category are the microalgae which have tremendous potential to produce several biofuels including microalgal lipids, bioethanol or biobutanol from microalgal sugars, biohydrogen, long-chain hydrocarbons, methane, and crude oils from the pyrolysis of microalgal biomass (Chisti, 2007; Torzillo et al., 2009; Kraan, 2010).

Microalgae are photosynthetic microorganisms which lack true roots, stems, and leaves that characterize terrestrial plants and represent a complex and diverse array of life forms that vary greatly in their metabolic capabilities, environmental adaptations, and morphology. Microalgae are characterized by their small size, ability to grow autotrophically (i.e. they use carbon dioxide to produce their own carbon compounds for metabolic purposes in sunlight) though some are hetero/mixotrophic (they can assimilate a variety of carbon compounds in the absence of sunlight), and their ability to produce varying amounts of oil in the form of diglycerides and triglycerides (Lyon et al., 2015). Several important advantages associated with microalgae including their economic potential as a feedstock for biofuel production coupled to bioremediation, high biomass and oil productivity, diverse biodiversity, high photosynthetic efficiency, and hetrotrophic/mixotrophic capabilities (Lyon et al., 2015) are causing the interest in microalga-derived biofuels to rise. This is again activated by crude oil price fluctuation, political instability, energy security, greenhouse gas effects, and competition for food-oriented agricultural commodities (Laurens, 2017). Moreover the international & national agreements in shifting towards more secured renewable energy system are also other driving factors to make use of algae as renewable energy sources (Laurens, 2017).

Ethiopia is abundant in its natural resources as could be evident from the phytoplankton and other aquatic community which could bring about significant and sustainable economic changes in the country. Among the aquatic biodiversity, algae are the most important ones with many biotechnological applications. The tropical sun light and suitable weather conditions makes the country a potential site for the algal biomass production (Asmare et al., 2013). At a larger scale, this could bring about a meaningful transformation in energy system based on the microalgae resources.

In spite of the tremendous microalgae potential for production of different biofuel, still industrial microalgal biofuel production is an important challenge; the overall process is energy and capital intensive (Ugwu et al., 2008; Farrell & Sarisky-Reed, 2010; U.S. DOE, 2010). In this regard several approaches have been identified by the scientific and industrial community. These includes improvement of algal strain (Khraiwesh et al., 2015), developing material and energy efficient harvesting/fuel conversion technologies (Ghosh & Das, 2016), and coupling of algal cultivation with other processes to reduce nutrient and fresh water requirement (Broberg, et al., 2011).

According to data from the Ethiopian sugar corporation, it is estimated that when all the sugar and ethanol projects are completed and start to operate with their full capacity, together with the already operating factories the country will have the potential of producing more than 3 million tons of sugar per year and about 400000 metric tons of ethanol per year. Metahara Sugar factory, located in the South-eastern part of Ethiopia, is one of the major sugarcane processing factories in Ethiopia. The total cane cultivation area of the factory is 10230 ha and its sugar and ethanol production capacities are 130000 tons/year and 12000 m^3/year respectively. These figures suffice the opportunity to couple algal biomass cultivation with sugar factories by utilization of resources including CO_2, and wastewater generated from these factories. This integrated approach reduces both the cost of production of biofuel and the environmental pollution (Broberg et al., 2011). This integration can provide microalgae with required nutrients. Furthermore wastewater treatment systems using microalgae represent a low-cost and environment-friendly wastewater treatment alternative when compared to conventional wastewater treatment processes (Liu et al., 2013). Other advantages include effective utilization of CO_2 rich exhausts, production of biofuel and other value added products (α-Linolenic acid, docosahexaenoic acid, and docosahexaenoic acid) (Hwang et al., 2016).

Most algal related researches in Ethiopia focused on the phytoplankton biomass spatial distribution, seasonal variation and identification of algae at different taxonomic level (Kebede and Belay, 1994; Broberg et al., 2011; Fayissa, 2015). However studies related to the algae cultivation, algae extraction technology, algal biofuel production, environmental pollution reduction, and the techno-economic feasibility for the commercial production of different products from algae still remain and needs to be explored. This present study aims to propose a viable technology for production of biofuel from microalgae through conceptual coupling of microalgae cultivation with sugarcane processing factory wastes and byproducts. It focuses on production of algal biofuel, environmental abatement and evaluation of the integrated process. The algae would be cultivated inexpensively using the waste streams and CO_2 from sugar factories. A conceptual microalgae cultivation coupled with a sugar factory has been developed and evaluated. A case study approach was used, Metahara Sugar factory, with annexed distillery, in the South-eastern part of Ethiopia, was selected for the adoption of the presented strategy.

1.2. Objectives

1.2.1. General objective

The general objective of this dissertation was to investigate the potential of a future possible bio-refinery and environmental pollution reduction concept by coupling microalgae biomass production with the sugar factory's wastes and byproducts. To evaluate the potential synergies between the factory and algal biomass cultivation for biofuel production, process modeling was utilized to couple microalgal biofuel production into the operation of the factory which has a capacity of about 130,000 metric ton of sugar per year and 12,000 metric ton of ethanol per year respectively.

1.2.2. Specific objectives

Under the frame work of the general objective the following specific objectives were designed:

- To identify the current challenges and advances in microalgal biofuel production
- To assess the potential of the sugarcane processing factories' wastes, byproducts and flue gases to support the growth of microalgae
- To investigate possible process options for coupling of the microalgae cultivation with sugarcane processing factories
- To evaluate the potential coupling of microalgal biofuel production with sugar cane factories with regard to product outputs, energy requirements, and environmental pollution reduction

- To study the economic feasibility of coupling of microalgal biofuel production with sugar factories

1.3. Research questions

The following fundamental questions were considered in the initial of the dissertation work.

- What are the drivers, current challenges, advances and future prospects in the technology for production of biofuel from algae? State-of -the -Art review
- What potential do sugarcane processing factories' wastes, byproducts and flue gases have to support growth of algae?- Resources assessment and modelling
- To what extent do the algae reduce the environmental pollution in the factories? Process modelling
- How much area of land is required for the microalgae cultivation? Process modelling
- What process options are there? And which ones are appropriate to adopt the proposed idea? Process choice, design and integration
- What is the potential of the integrated process for biofuel production? And is it techno-economically feasible? Evaluation of the integrated process

1.4. Structure of the dissertation

The present dissertation is composed of five chapters as described below.

Chapter 1: it provides a short background, the objectives, and research question of the dissertation. Moreover the general overview of each chapter of the dissertation is included in this chapter.

Chapter 2: this chapter presents the literature review on related works. In this section the challenges and advances in technology for microalgal biofuel production are investigated and future research direction are identified.

Chapter 3: this section describes utilization of sugarcane mill wastes and flue gases for cultivation of microalgae. This is case I of the dissertation in which the sugar mill wastewater is used as the only nutrient source for the algae growth in ponds. The potential of the wastewater to support the algae growth and the potential of the algae to reduce environmental pollution were studied using material energy balance approach.

Chapter 4: case II of the dissertation is presented in this chapter where the vinasse from the ethanol production plant is used as an additional nutrient source for the cultivation of the algae after it is anaerobically digested along with other inputs. It is also here that the result for further processing of the microalgae for production of biofuel and bio-fertilizer is reported. The process was evaluated with regard to product output and energy requirement

Chapter 5: this part is concerned on the techno-economic analysis of the coupled process. The economic feasibility of the process was investigated in this section. Discounted cash flow analysis approach was used to determine the minimum selling price of the biodiesel by fixing the price of the biogas and the bio-fertilizer at the current market price.

Chapter 6: here the main conclusions of the dissertation are presented concisely. Notable areas for future research works were also pointed out in this chapter.

CHAPTER TWO

2. Literature review

2.1. Important aspects of microalgae for biofuel production

Several important advantages associated with microalgae including their economic potential as a feedstock for biofuel production coupled to bioremediation, high biomass and oil productivity, diverse biodiversity, high photosynthetic efficiency, and hetrotrophic/mixotrophic capabilities (Lyon et al., 2015) are causing the interest in microalga-derived biofuels to rise. This is again activated by crude oil price fluctuation, political instability, energy security, greenhouse gas effects, and competition for food-oriented agricultural commodities (Laurens, 2017). Moreover the international & national agreements in shifting towards more secured renewable energy system are also other driving factors to make use of algae as renewable energy sources.

Microalgae are promising and potent source of oil. Though the oil (triglycerides) content of microalgae, in most cases, varies from 20–50% it sometimes may reach up to 86% by weight of dry biomass which is tremendously higher than other agricultural crops (Chisti, 2008; Yi-Feng & Wu, 2011). The comparison of microalgae with other feedstocks for biofuel production is shown in Table 2.1.

Table 2. 1 Comparison of microalgae for biofuel Production with other feedstocks (Chisti, 2007; Mata et al., 2010)

Plant type	Seed oil content (%/wt. biomass)	Oil yield (L/ha.year)	Land use (m^2 /year.kg biodiesel)	Biodiesel productivity (kg/ha. year)
Soybean (*Glycine max L.)*	18	636	18	562
Sunflower (*Helianthus annus L.*)	40	1,070	11	946
Corn/maize (*Zea mays L.*)	44	172	66	152
Jatropha (*Jatropha curcas L.*)	28	741	15	656
Camelina (*Camelina stativa L.*)	42	945	12	806
Castor (*Ricinus communis*)	48	1,307	9	1,158
Palm (*Elaeis guineensis*)	36	5366	2	4,747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	126,900	0.1	121,104

Microalgae are generally considered as more efficient converters of solar energy compared with the conventional energy crops due to the simplicity in their cellular structure and function (they exist in single cells or simple clusters of a few cells); it has been reported that they can convert 2-5% of the total sun's energy into biomass (Lyon et al., 2015). However only 1% solar energy conversion is recorded by conventional energy crops such as corn or sugarcane (Gouveia, 2011; Yi-Feng & Wu, 2011). Under normal conditions, autotrophic microalgae use sunlight and fix inorganic carbon from the atmosphere for assimilation in the form of carbohydrates and lipids, which can be exploited for biofuel production while under stress condition such as nitrogen deficiency and high light intensity they can accumulate more neutral lipids (particularly triglycerides) (John et al., 2011). It has been known that some heterotrophic microalgae such as *Chlorella protothecoides* can accumulate over 50% of neutral lipids (Yi-Feng & Wu, 2011). Since microalgae exhibit high photosynthetic efficiencies they can give higher biomass yields per hectare, e.g 37 tonnes $ha^{-1}yr^{-1}$ have been recorded (Weissman et al., 1989), which is up to twice that of terrestrial plants. Unlike oil crops they grow at high rates (1–3 doublings/day); some microalgae can double their biomass in as little as 3.5 h in the laboratory and 24 h in outdoor ponds, which makes microalgae an ideal renewable source for biofuel production (Lee, 2012; Yi-Feng & Wu, 2011). Since microalgae grow more rapidly they have short harvesting cycle. This short harvesting cycle is the key advantage of their importance which is better than other conventional crops with harvesting cycle of once or twice a year (Chisti, 2007; Schenk et al., 2008). Microalgae can be harvested batch wise all year round; their production is not seasonal showing that they have high photosynthetic productivity providing the rationale for developing microalgae based bioenergy supply chains to displace significant quantities of fossil energy (Wu et al., 2012; Laurens, 2017).

Microalgae have adopted various metabolic routes viz., autotrophic, heterotrophic and mixotrophic for their growth and survival, which are interchangeable under different nutrient and sunlight conditions (Devi et al., 2012). As these metabolic path ways directly determine algal biomass formation and lipid accumulation they are considered as important advantages of the microalgae (Yi-Feng & Wu, 2011).

Through photosynthesis light energy is converted to chemical energy and from this stored chemical energy all forms of biofuels including biodiesel from oils, bioethanol and bio-butanol from sugars and starch, bio hydrogen through synthesis of protons and electrons, and bio-methane can be produced (Hankamer et al., 2007; Costa et al., 2011). Biodiesel and bioethanol are the most technically feasible and commercialized alternative renewable fuel sources which can both replace diesel and gasoline with slight or no modifications to vehicle engines. Production of biodiesel and bioethanol does not need developing of new technologies since both biofuels can be produced using the existing technologies; transesterification (for biodiesel) and fermentation (for bioethanol) (Gouveia, 2011). In the case of biodiesel production from microalgae the glycerol, which is the main byproduct, has several industrial applications thus increasing the economic viability of the process (Chisti, 2008). Also the biomass resulting after oil extraction can be processed using a biorefinery concept into ethanol or methane to be used also as biofuel, livestock feed or as organic fertilizer (Brennan & Owende, 2013). For conversion of biofuels from microalgae, several potential path ways exist including chemical reaction, thermochemical conversion, biochemical conversion and direct combustion (Wang et al., 2008). Important properties of microalgal biofuel such as a high caloric value, low viscosity and low density as compared with plants' biofuel make microalgae more suitable for biofuel production than lignocellulosic materials (Miao et al., 2004). Microalgae require only water (with some nutrients such as nitrogen phosphorus and potassium), atmospheric CO_2 and sunlight to synthesize biomass, which is available at essentially, low cost (Nigam & Singh, 2011). Also as microalgae cells grow in aqueous suspension, they have more efficient access to water, CO_2 and other nutrients (Chowdhury & Loganathan, 2019). Microalgae utilize about ten times lesser land compared to terrestrial plants (Table 2.2). For example, low lipid containing microalgae land requirement is 0.2 m^2 /kg biodiesel per year whereas palm oil land requirement is estimated about 2 m^2 /kg biodiesel per year (Mata et al., 2010). Comparison of microalgae for land and water requirements with other feedstocks is shown in Table 2.2.

Table 2. 2 Comparison of microalgae for land and water requirements, energy and biofuel yield with other feed stocks, adapted from (Singh et al., 2011)

Plant source	Land use (m^2GJ^{-1})	Water footprint(m^3GJ^{-1})	Energy ($GJha^{-1}yr^{-1}$)	Biofuel yield ($L\ ha^{-1}yr^{-1}$)	
				Biodiesel	Bioethanol
Rice	85	212	47	-	2250
Corn	50	133	75	-	3571
Cassava	148	79	126	-	6000
Potatoes	105	114	88	-	4167
Sugarcane	50	81	124	-	5882
Jatropha	396	162	62	1896	-
Palm oil	75	52	192	5906	-
microalgae	<379	2-13	793-4457	24355-136886	-

Microalgae can be grown in a number of environments that are unsuitable for growing other crops such as fresh, brackish or salt water and on non-arable lands (e.g. desert and seashore lands) that are unsuitable for conventional agriculture (Chen & Wu, 2011). Furthermore the ability of micro algae to grow in extreme environment of temperature and salt avoids the requirement for herbicides or pesticides in algal cultivation as no other competent cannot exist in such environment (Chisti, 2007).

Since microalgae can utilize various wastewaters, seawater, and other forms of produced water which cannot be introduced into the agricultural system competition for limited land and freshwater resources will be reduced (Chisti, 2007). Microalgae can potentially utilize CO_2, organic carbon, & nutrients from waste streams. Some microalgae species may utilize high doses of CO_2 present in flue gases compared to terrestrial biomass, indicating they have the potential to reduce greenhouse gas emission (about 1.83 kg of CO_2 is utilized per kg of dry algal biomass yield) (Chisti, 2007; Raeesossadati et al., 2015). Microalgae can utilize growth nutrients from wastewater sources (such as industrial and municipal wastewaters agricultural run-off, concentrated animal feed operations) providing additional environmental benefits (Muylaert et al., 2015).

The ecology, morphology, biochemistry, and physiology of microalgae are more diverse than other higher plants. The number of algal species is estimated to range from 350,000 and 1,000,000 (Bux & Chisti, 2016; Guiry, 2012) though a limited number of approximately 30,000 have been isolated and characterized (Radmer & Parker, 1994; Richmond, 2004).

In addition to biofuel production, this huge reservoir of biodiversity supports potential commercial exploitation of many value added products, e.g. vitamins (tocopherols, vitamin B12 and provitamin A), carotenoids (β-carotene, astaxanthin, canthaxanthin and lutein), other pigments (phycocyanin and phycoerythrin) and w-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) (Lorenz & Cysewski, 2000; León et al., 2003). These compounds may favorably contribute to making biofuel manufacturing from microalgae more competitive, based on a biorefinery approach.

Since they are single-celled organisms that duplicate by division, high-throughput technologies can be used to rapidly evolve strains. Furthermore when compared to conventional agricultural crops, microalgae have a high content of proteins and lipids, a low content of structural carbohydrates such as cellulose, hemicellulose, and lignin (Lam & Lee, 2012). This is an attractive property of microalgae, because it implies that depolymerization efficiency is increased and most of the biomass can be valorized (Saqib et al., 2013). Due to the low content of structural carbohydrates such as lignin or cellulose, microalgal biomass has a high content of nitrogen (N) and phosphorus (P) (about 10 % N and 1 % P per unit dry weight).

In the light of the outlined advantages of microalgae research and development efforts focused on the development of algal biofuels have grown significantly in recent years (Brennan & Owende, 2013; Laurens, 2017). Though several challenges are waiting to be overcome for viable biofuel production from microalgae, there are appealing advances in technology which show the bright future towards using microalgae as a substitute feedstock for viable biofuel production (Ogbonna et al., 2015). The next section discusses the challenges and advances in technology for algal biofuel production.

2.2. International biofuel policies and commitments as drivers for biofuel production from microalgae

There is a consensus that on an international level, renewable energy must play a fundamental role in the transition towards a more competitive, secure and sustainable energy system. This transition will not be possible without a much larger contribution of renewable energy to the current infrastructure. The production and use of biofuels has been mainly driven by governmental policies in order to reduce oil dependency and in turn increase the share of renewable energy contributing to carbon dioxide (CO_2) emissions mitigation (Laurens, 2017).

Numerous policies have been put in place since the 1975 oil crisis in order to promote the use of renewable fuels in several countries such as the United States, Canada, Europe, and Asia. In Canada, for example, these include implementation of excise taxes exempting propane and natural gas which was extended to ethanol made from biomass and methanol in 1992. Development of renewable fuel strategy which proposed four components: increasing availability of renewable fuels through regulation, supporting the expansion of Canadian production of renewable fuels, assisting farmers to seize new opportunities in this sector and accelerating the commercialization of new technologies (Kedron, 2015).

In general the main mechanisms for governments supporting biofuel policies are blending mandates and tax exemptions. Other polices which can support biofuel production can be grants to support the installation of production facilities, farmer premiums for the production of energy crops, and supporting research and development (R&D) funding.

The following are some of the commitments for bio energy.

- The UK energy strategy with a commitment of emission reduction to zero by 2050 (BBC news as of June 12, 2019)
- The European Union (EU) energy strategy with a commitment of achieving, by 2030, at least 27% share of renewables and 40% greenhouse gas (GHG) emissions reduction relative to emissions in 1990 (Knopf et al., 2015)
- The Energy Independence and Security Act of 2007 (EISA), which set a target for the use of 136 billion litters (36 billion gallons) of renewable fuels, including advanced and cellulosic biofuels and biomass based diesel, by 2022
- Research and development projects, which were to be in effect in 2019, in South Korea: The projects include the Marine Bioenergy Development Project, Green Growth via Marine Algal Biomass Project, Global Frontier project which includes Algal Technology, and the Carbon Capture and Sequestration 2020 project.
- China's commitment to renewable energy which is defined by the 13th Five Year Plan (FYP) which covers the years 2016-2020. And energy from microalgae is one target.
- Other countries which have biofuel program include Japan, Ethiopia, India, Taiwan, and Brazil
- Support from commercial entities: Along with numerous government-supported projects, a large number of commercial entities are supporting algae (both micro-and macroalgae) production and research.

The above commitments clearly show how much efforts have been done and to be done so that there will be a substantial transformation of energy system which is based on more secure, sustainable and low-carbon economy. In, Ethiopia there is a target put to increase the share of renewable energy, and thus, the aforementioned policies and commitments are highly encouraged by the country. For example the activity of blending of ethanol with gasoline (with 5% ethanol) shows its efforts towards increasing share of sustainable energy.

2.3. Challenges, approaches and advances in microalgal biofuel production

Although microalgae have huge potential for biofuel production and there are international driving policies for production of biofuel from microalgae, there are several challenges which have delayed the industrial production of microalgal biofuel. During microalgal biofuel production challenges are associated with each step; algal cultivation (e.g. strain biology, cultivation strategy, resources and siting) (Ugwu et al., 2008), algal harvesting and processing (e.g. harvesting/dewatering technologies, fractionation/ extraction technologies) (Hirano et al., 1998), and conversion of algal components into biofuels and other products (e.g. fuel synthesis, conversion and upgrading technologies, infrastructure, market of fuel product) (Hirano et al., 1998; Pienkos & Darzins, 2009). The fundamental questions for each step to be raised and need to be addressed are: is it economically and environmentally sustainable? And can the system be scaled up to the necessary scales to fuels?

2.3.1. Challenges in Microalgae cultivation

The following important questions need to be addressed when cultivating microalgae. Can one access all necessary inputs to cultivate algae and still maintain a cost effective and sustainable process? Is system recycling of water, nutrients, and energy feasible and necessary? Is it possible to keep the system and the system productivity stable? What about proximity, sustainable availability, and cost of all resources? All these directly or indirectly will affect price of the biofuel (Pienkos & Darzins, 2009; U.S. DOE, 2010).

System stability is among the important challenges during cultivation of algae. Presence of other communities such as microalgal predators and pathogens along with the required algae may undesirably affect the microalgal growth by competing for resources, thus causing the culture to disrupt though some may have desirable effect as they can scavenge and recycle nutrients or manufacture essential vitamins for the algae growth. Furthermore microalgal predators and pathogens are prevalent and only little is known about them (Cheng et al., 2004). Particularly when wastewater is used as nutrient source the required microalgae strain are more likely to be outcompeted by faster growing species of microalgae or cyanobacteria (Ogbonna & Moheimani, 2015). In such cases in addition to selecting stable culture which is robust to environmental changes and predators/pathogens it is important to explore the use of naturally occurring mixed cultures in wastewaters such as mixed cultures of selected strains of microalgae and mixed cultures of microalgae and yeasts (Ogbonna & Moheimani, 2015). Moreover to overcome challenges associated to culture stability sensitive, selective, and inexpensive control methods are required (U.S. DOE, 2010).

Real-time control of some important parameters is also a big challenge (Ugwu et al., 2008); it is among the major difficulties which can cause low biomass productivity (Suh & Lee, 2003). The pH of the culture is among such parameters. Bioavailability of CO_2, and use of the medium nutrients by the algae are affected by the pH values of the culture (Borowitzka, 2013). If pH is not maintained in the required range the cellular processes may be affected by the extreme pH (Liu et al., 2007). The control of culture pH can be performed by addition of alkaline solution to the culture and such control mechanism must be integrated with the aeration system (Wang et al., 2012). For outdoor microalgae cultivation high pH tolerant strain are preferred since high pH condition inactivates pathogenic microorganisms and other microalgae (Kumar et al., 2010). The concentration of CO_2 in the culture dominantly affects the pH, and the demand of this gas by the microalgae depends on the balance between the transfer of CO_2 to the liquid and CO_2 consumption by the microalgae (Wang et al., 2012). When molecular CO_2 from the gas phase is transferred into the culture medium, some of the CO_2 gas will dissolve and become soluble phase, HCO^-_3 which is utilized by the microalgae, and this conversion highly depends on the pH of the culture (Miller et al., 1990). Thus carbon dioxide can be supplied in response to pH signal. pH-static control via direct CO_2 sparging into the culture media is the best and most convenient method of pH control, and at the same time supplying CO_2 for high yield in mass algal cultures (Moheimani & Borowitzka, 2006a).

Temperature is an important environmental factor for microalgae growth and target-product production (Kumar et al., 2015). The control of temperature is a big challenge particularly when raceways are used since temperature is governed by the sunlight regimen, evaporation, and the local air temperature. Moreover some studies show that the lethal temperature is usually only a little higher than the optimum temperature (Borowitzka, 1998). Exceeding the optimum temperature by only 2–4ºC may result in the total culture loss (Moheimani & Borowitzka, 2006b). There are some temperature control mechanisms in a closed photobioreactors. Incorporating a heat exchanger with the photobioreactor, immersion of the culture in water, spraying with water, and shading are some of the mechanisms (Kotzabasis et al., 1999). Temperature in raceways is typically not monitored, as doing so is impractical (Chisti, 2013). However if cultivation is in greenhouse temperature can be controlled using greenhouse by keeping the greenhouse closed at low temperature and erecting the sides of the greenhouse at high temperatures. In open systems using microalgal strains adapted for the local conditions is an important approach (Chisti, 2013).

Photo inhibition is among the main challenges. In natural system, all incoming sun radiations are not available for photosynthesis as part of it is absorbed in the atmosphere (gases in the atmosphere absorb 30% of incoming radiation). Growth in microalgae is driven by photosynthetically active radiation, or PAR, the component of the sunlight that is within the wavelength range of 400–700 nm. This PAR is absorbed by chlorophyll in microalgae (Rashid et al., 2014). In a tropical location at solar noon the peak PAR value may reach a value of 2000 $\mu Em^{-2}s^{-1}$ but photosynthesis saturates at roughly 10–20% of the peak PAR value. Thus in raceway cultivation of microalgae photosynthesis does not increase beyond a PAR value of about 100–200 $\mu Em^{-2}s^{-1}$; all the excess light is wasted (Chisti, 2013). Once the PAR value exceeds the saturation threshold algal cultures become photo inhibited and in a photo inhibited raceway culture, the rate of photosynthesis actually decreases with a further increase in irradiance. Aside from inhibiting photosynthesis, photo damage during photo inhibition needs to be repaired and this has a metabolic cost to the algae (Raven, 2011) reducing net productivity. Photoinhibition during the day is inevitable at least to some degree (Kromkamp et al., 2009; Richmond, 2004). Thus selection of species showing less photoinhibition can be of advantage. Also it is important to understand that oxygen supersaturation can result in photo inhibition (Raven, 1997). If there is an actively photosynthesizing dense algae culture the medium rapidly reaches oxygen supersaturation and this time photosynthesis is significantly inhibited by the high O_2 concentration (Raven, 1997). Since high O_2 concentration is the major limiting factor in both open ponds and photobioreactors it need to be removed from the culture. Oxygen removal in open ponds is poor. The main mechanism in which oxygen can be removed in raceways is during agitation by paddlewheel but it is mostly insufficient (Chisti, 2013; Mendoza et al., 2013). Sometimes the culture may be sparged with air to control buildup of oxygen so that inhibition of photosynthesis is reduced (Mendoza et al., 2013).

It is estimated that microalgal biomass contains about 50% C by dry (Mirón et al., 2003; Grobbelaar, 2004) and it has been estimated that the cost of carbon accounts for 8–27 % of the total production cost of the microalgal biomass (Li et al., 2013). Hence CO_2 diffusion losses may significantly affect the overall cost of the microalgal biofuel production. It has been reported that there may be up to 15% total loss of CO_2 in open ponds (Nappa et al., 2015). To reduce this problem the gas distribution and the contact of the gas with the liquid need to increase, and gas transfer equipment such as propellers, blades, perforated piping, jet aerators, and U-tubes need to be added in the design of the cultivation system (Brennan & Owende, 2013). Also solubility of CO_2 can be improved to some extent by controlling its bubble size. Large sized bubble (1–2μm) has high velocity and reach to the top of water surface (in a photobioreactor) immediately. Thus CO_2 is neither fully mixed nor consumed by microalgae (Mata et al., 2010). On the other hand small sized bubbles (<1μm move slowly and burst within the water surface (Ho et al., 2010; Lam & Lee, 2012).

Challenges associated with water conservation, management, and recycling should be considered. In microalgal biomass production water provides a growth environment for algae to live and multiply. Furthermore it serves as a medium for nutrients delivery, waste removal and temperature regulation. Natural /evaporation losses from the system affects water requirement for microalgal cultivation. A study on evaporation estimates on tropical areas shows that there is a loss of 0.47 cm/day (Vallet-coulomb et al., 2001). It has been also estimated that to fill 1 ha of land with 20 cm depth, 530,000 gallon of water is required and in desert areas 13,000 gallon of water per day can be lost from 1 ha of pond (U.S. DOE, 2010). The water lost through evaporation needs to be replaced for sustainable production. Use of fresh water for replacing may not be economically viable and is often unsustainable option. Other low quality water such as municipal wastewater, industrial wastewater may be used economically. However use of such low quality water may be problematic as it may concentrate salts, toxins, and other materials in the culture. Thus for viable microalgal production these problems need to be addressed. Use of closed systems may decrease evaporative loss but the added cost to use such reactors needs to be balanced. However it should be considered that evaporative may help to cool the system in hot climates. GIS analysis of water resources, developing cultivation systems with minimal water consumption, studying water recycle, assessing non-traditional water use for fuel application, etc. are some of the future focus areas to overcome these challenges (U.S. DOE, 2010).

Another challenge in microalgal cultivation is associated with the system productivity (U.S. DOE, 2010). Improving productivity at minimized cost is the main challenge. High Productivity involves both high yield of a desired product, e.g., biodiesel and high growth rate/ biomass yield. These parameters in turn are functions of many factors such as nutrient supply, and growth environmental conditions (e.g., temperature, pH). For example it has been known that nitrogen starvation and high salt and high light stress (in some marine phytoplankton) can result in increases in lipid content (Azachi et al., 2002).

There are also challenges associated with sustainable availability of resources such as land, water, CO_2 or sugar, nutrients, and electricity (Pienkos & Darzins, 2009). The productivity of microalgae on non-arable land and ability of many microalgae to grow in saline and marine water may ease land and fresh water requirement.

In microalgae cultivation nutrient supplies have a considerable impact on cost, sustainability, and production siting (U.S. DOE, 2010) and the major nutrients (nitrogen, phosphorous, iron, and silicon (in the case of diatoms)) need primary focus. Since the world's supply of phosphate is in danger of running out (Abelson, 1999) phosphorous appears to be an especially important issue in developing sustainable nutrient supply. In the case of nitrogen costs are tied to fossil fuel prices since its fixation process requires fossil fuel, particularly natural gas. Thus energy inputs in this process needs to be considered in the life cycle analysis (U.S. DOE, 2010). None of these major nutrients (nitrogen, phosphorus, and iron) are contained in the final fuel product. Thus nutrient recycle may prove to be more valuable than using the spent biomass for products such as animal feed from sustainability perspective. For example most of these nutrients can be returned back to the growth system with the sludge after anaerobic digestion of the residue obtained after extraction (Benemann et al., 2003). However the processes through which these nutrients are re-mobilized and made available for microalgal growth are not well understood (U.S. DOE, 2010). To avoid problems related to nutrient level it is important to have careful control. Though limitation of nutrients such as nitrogen and phosphorus are desirable to increase liquid accumulation in the microalgal cells it may have also negative impact by decreasing the biomass productivity. If excess nutrients are used the unused nutrients may create a problem in the wastewater disposal. Thus appropriate control should be designed to keep the nutrient at the desired level.

From the perspective of using low cost resources it has been found that utilizing the nutrient content of municipal, agricultural, or industrial waste streams is a very attractive alternative in order to have cost effective production system. However related problems such as toxicity, the facility siting and disposal issues may be challenging and needs to be considered carefully.

Regarding nutrient supply research areas such as techno-economic and life cycle analysis to understand the energy cost and sustainability; studies on the mechanisms of nutrient recycling; and Geographic Information System (GIS) analyses to understand wastewater resources have been identified (U.S. DOE, 2010).

The economic viability of producing algal biomass for low-value products such as biofuels is also a challenge (Lyon et al., 2015). The cost for microalgal biomass production is currently much higher than from other energy crops (Laurens, 2017). Another challenge is the decline in the price of petroleum, coupled with ongoing low prices for natural gas and absence of consistent policies on carbon pricing. This causes a significant challenge in the development of cost-competitive production algae based bioenergy products like gaseous and liquid fuels (Laurens, 2017).

2.3.2. Coupling of microalgal cultivation with other processes

Previously it has been mentioned that nutrients, particularly nitrogen & phosphorus, and CO_2 supplies have a considerable impact on cost, sustainability, and production siting in microalgae cultivation (U.S. DOE, 2010). Nitrogen is an essential constituent of all structural and functional proteins in algal cells accounting for about 7–10 % of cell dry weight (DCW) (Aishvarya et al., 2015). Compounds such as nitrate, urea, and ammonium are used as nitrogen sources. Though urea and ammonia show similar growth of microalgae, nitrate is mostly used (Aishvarya et al., 2015). Currently, algal cultivation predominantly uses nitrogen fertilizer produced from the Haber–Bosch process (Singh & Das, 2014). Fertilizer-grade nutrient inputs and freshwater accounts for 50% of energy inputs associated with algal cultivation (Clarens et al., 2011). Thus it is important to find alternative methods to reduce this cost. Recycling algal-biomass nutrients via anaerobic digestion and/or thermochemical conversion techniques (Rösch et al., 2012) and utilizing the nutrient content of municipal, agricultural, or industrial waste streams (Clarens et al., 2011) is a very attractive alternative in order to have cost effective production system.

Although algal biomass contains less than 1% P, it is often one of the most important growth limiting factors as it is easily bound to other ions (e.g., CO_3^{2-} and iron) resulting in its precipitation and consequently rendering this essential nutrient unavailable for algal uptake (Grobbelaar, 2013).

Phosphorus is used to carry out many cellular processes such as energy transfer, biosynthesis of nucleic acids, DNA, etc (Grobbelaar, 2013). The preferred form of phosphorus in which it is supplied to algae is as orthophosphate (PO_4^{2-}) and its uptake is energy dependent (Grobbelaar, 2013). Since world P reserves, phosphate rock, is being depleted (Cordell et al., 2009) for sustainable implementation of algal biofuels on a large scale, P requirement has to be obtained from sources other than mineral rock phosphate. The P content of the microalgal-waste left after the oil-extraction or conversion process must be recycled into growing the next batch of microalgae. As in the case of N, utilization of municipal, agricultural, or industrial waste streams can help minimize requirements of fertilizer P input.

Microalgal biomass contains about 50% C by dry weight (Mirón et al., 2003; Grobbelaar, 2004), and this carbon is derived from carbon sources. It has been estimated that the cost of carbon accounts for 8–27 % of the total production cost of the microalgal biomass (Li et al., 2013). Thus use of less expensive carbon sources is important for viable production of microalgal biomass. Under natural growth conditions, microalgae assimilate CO_2 from the air (ca. 360 ppmv CO_2). Due to the very low concentration of CO_2 in the air (0.04%) together with its low solubility in water, using atmospheric air as the only CO_2 source for microalgal biomass cultivation may lead to low biomass yield due to CO_2 limitation, and insufficient mixing increases the problem due to reduced mass transfer. In open pond cultivation atmospheric air provides to the pond surface only 5% of the CO_2 required for photosynthesis (Stepan et al., 2002). Thus continuous pumping is required to ensure the availability of CO_2 over entire cultivation period, though pumping poses additional cost on cultivation. Therefore, targeting industrial C emissions as a source is attractive and can increase the overall sustainability of algal cultivation. Microalgae can also assimilate CO_2 from other sources such as discharge gases from industrial processes and soluble carbonates (Wang et al., 2008). Using of CO_2 coming from flue gas of power plants and industrial activities as a substrate for biofuel production could help to achieve a net-zero CO_2 emission. Furthermore Photoautotrophic growth of microalgae represents an ideal model of reutilization of CO_2 coming from flue gas (Packer, 2009), as microalgae biomass can be further utilized to produce biofuels or other value-added products (Mirón et al., 2003; Hsueh et al., 2007). It should be considered that if flue gas is used as carbon dioxide source, a well-designed supply system is required to control the loss of CO_2 to atmosphere (Chisti, 2013; de Godos et al., 2014).

2.3.3. Sugarcane processing factories' waste products, and flue gases for cultivation of algae

Waste products produced from sugarcane processing factories include solid and liquid constituents that can unfavorably affect the flora and the fauna of the surrounding environments. These waste products include bagasse, filter cake, ash slurry, wastewaters, vinasse, and flue gases. The wastewater from the different sections of the sugar mill, and the vinasse from the ethanol production plant can be used as inexpensive nitrogen and phosphorus sources for growth of algae. Whereas the flue gases from both the boiler house in the sugar mill and from the fermenters in the ethanol production plant are possibly used as CO_2 sources. Utilization of these resources can potentially help to increase the feasibility of production of biofuel from algae by reducing the high nutrient costs which are one of the bottlenecks to the feasibility of largescale algal culture. Nutrient costs and CO_2 addition costs of \$407 ton^{-1} and \$ 40 ton^{-1} respectively have been reported (Ryan Davis et al., 2011; Sun et al., 2011). In this regard using of the existing ponds and construction of new ones in sugar factories can be favorable candidates to support large scale microalgal growth as no additional CO_2 or nutrients are added to support the growth. In addition to this pumping requires low energy because of the existing infrastructures and as well the production cost can be offset by the revenue generated from the biofuels and other valuable byproducts. In Ethiopia sugar factories both the operating ones and the ongoing mega projects are considered as the main economic sources of the country. These factories and projects include Metahara sugar factory, Fincha sugar factory, Wonji Shoa sugar factory, Tendaho sugar factory, Beles sugar factory, Kessem project, Arjo Dediessa project, Omo-Kuraz development project, and Welkait sugar development project. The wastes and byproducts generated from Metahara sugar factory (Fig 2.1) can be inexpensive nutrient and CO_2 sources for cultivation microalgae in open ponds and it is the focus of the present study.

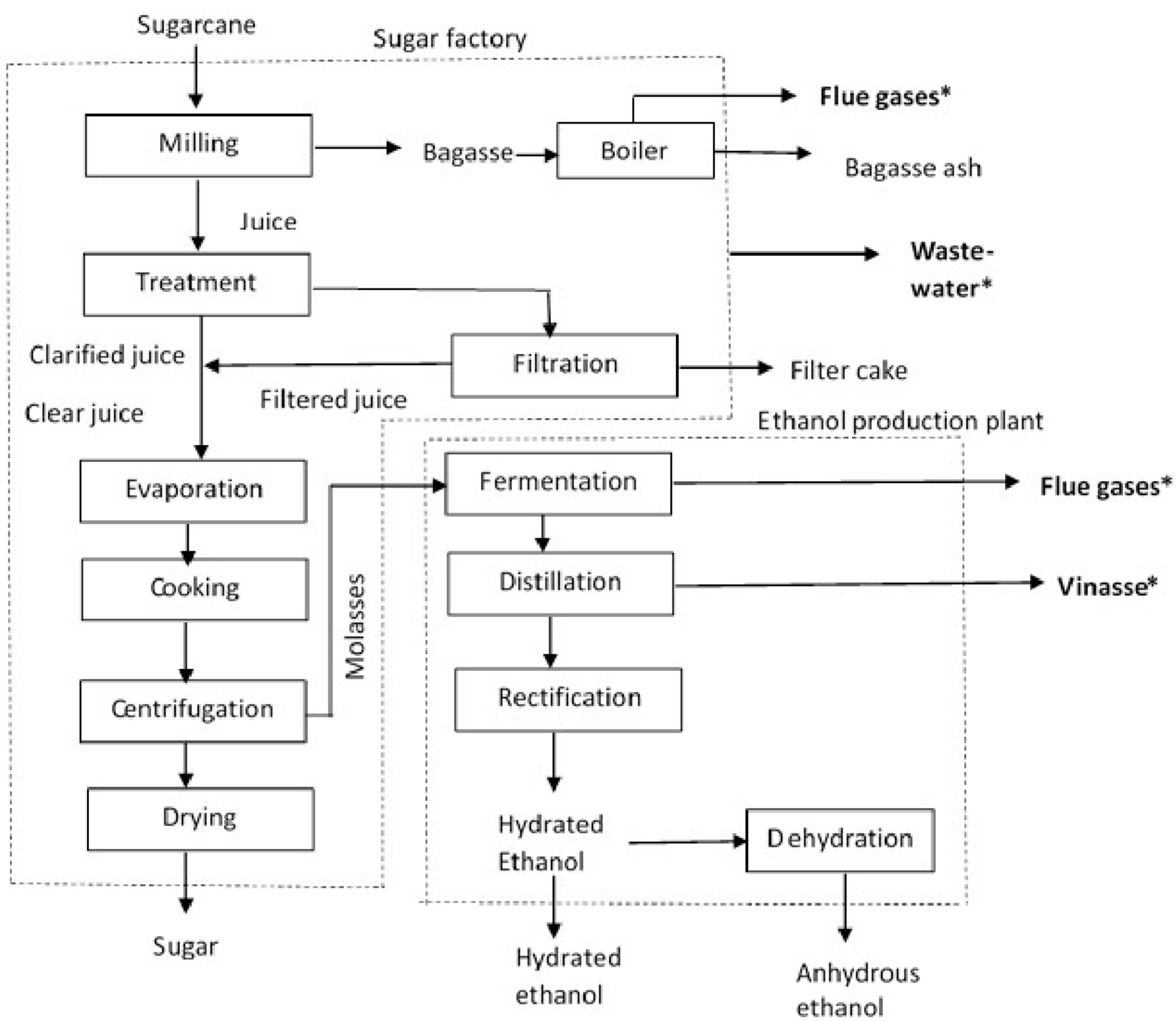

Fig. 2. 1 Sugar and ethanol production flow chart for the Metahara Sugar Factory (* the wastewater from the sugar mill, the flue gases, and the vinasse would be used to support the algae growth)

2.3.4. Harvesting and dewatering

Some microalgae to biofuel pathways require pre-processing steps such as harvesting and dewatering prior to the conversion of microalgae to biofuels. As moisture in the biomass will negatively interfere with the downstream processing and greatly influence the cost of product recovery the microalgal biomass needs to be concentrated prior to further processing (Show & Lee, 2014).

The microalgal cells in the broth are characterized by having small size (5–50μm in diameter) (Uduman et al., 2010), carrying negative surface charges (predominantly carboxylic (-COOH) and amine (-NH_2) groups) (Ghosh & Das, 2016) and excess extracellular polymeric substances they produce during metabolism (Amaro et al., 2011). These characteristics makes them to be stable being loosely suspended in the aqueous phase. Indeed separation of these tiny and loosely suspended microalgal particles from the broth can be very problematic. In addition, since the concentration in a culture broth is low (about 0.5 g/L dry biomass in open pond and 5 g /L in photobioreactors) (Ghosh & Das, 2016), large volumes of culture need to be processed in order to recover biomass in a feasible approach (Cooney et al., 2009; Ramanan et al., 2010). In general harvesting such dilute microalgal suspension with dry matter content of 0.02-0.06% is highly challenging, energy-and capital intensive and it accounts for a significant portion of the overall production cost of microalgal biofuels (20%–30%) (Rawat et al., 2011, 2013). Indeed, energy-efficient and cost-effective harvesting are two major challenges in the commercialization of biofuels from microalgae (Cooney et al., 2009; Reijnders, 2008).

The most common harvesting processes include filtration, coagulation, screening, flocculation, flotation, sedimentation, cell immobilization, and centrifugation. Other harvesting techniques such as ultrasound, electrophoresis, and electro-flotation, are used to lesser extents (Chen et al., 2011).

Factors such as strain, cell density, culture condition, growth media and value of target products determine both the cost of and ease of harvesting (Kumar et al., 2010).

The choice of technology for algae harvesting is basically governed by energy-efficiency and low cost for viable biofuel production. Also the characteristics of microalgae and the state in which they thrive can greatly affect the choice of algae-harvesting technology and its performance (Cooney et al., 2009).

Continuous centrifugation is the most rapid and effective method for total biomass separation. However the main drawback is that it is energy intensive and not economically feasible for most low-value products such as biofuels (Muylaert et al., 2015; da Silva & Reis, 2016).

Harvesting cost can be substantially reduced using other approaches such as flocculation (Vandamme, Foubert, & Muylaert, 2013). Flocculation process aggregates the microalgal cells to increase the overall cluster sizes (Mata et al., 2010), and is generally used as a preparatory step for other harvesting methods (Grima et al., 2003). It may be successfully used to enhance the settling characteristics by increasing particle density of culture, particularly for those with low particle density. Using flocculation, the biomass can be concentrated from a dilute culture with a dry matter content of about 0.05 % to a sludge with a dry matter content of 0.5–5 % (Muylaert et al., 2015). The remaining extracellular water can be removed by using techniques such as centrifugation and filter press so that a thick paste with a dry matter content of 20 % can be obtained (Muylaert et al., 2015). Flocculation involves the addition of multivalent cations and cationic polymers to the culture medium to neutralise the negative charge that is carried on the surface of many microalgal cells (Packer, 2009). Flocculation may also physically link one or more aggregates in a process referred to as bridging (Grima et al., 2003). Flocculation process can be enhanced by using membrane separator and sedimentation tank. Filtration and sedimentation techniques in combination with flocculation may be used successfully for dewatering of microalgae. Vacuum filtration for larger microalgae sizes (greater than 30 μm) and microfiltration or ultra-filtration for smaller sizes has been found to be effectively applied to harvest microalgae (da Silva et al., 2016). Since chemical flocculants such as high concentration of metal salts are used, flocculation process is unsuitable for food-grade products (Venkatesan et al., 2015).

Gravity sedimentation is one of the most commonly used harvesting processes because of its capability to handle large volumes of culture, and suitability for low-value biomass (Nurdogan & Oswald, 1996). However, it is highly affected by the density and radius of microalgal cells; it can only be applied to species with large cell size (>70 µm) like *Spirulina* (Muñoz & Guieysse, 2006).

Ultrasonic aggregation may be used as precursor to enhance sedimentation. The main advantages are that it is a non-fouling technique, therefore can be used for food-grade products, and it can also be operated continuously without inducing shear stress on the microalgal cells which causes cell destruction (Bosma et al, 2003).

It was found that dissolved-air flotation was more economical, but, if the recovered algae were to be incorporated into animal feed, the use of coagulants such as alum could have undesirable effects on the growth rate of the animal. The problem could be overcome by the use of nontoxic coagulants such as chitosan (Show & Lee, 2014).

Conventional filtration processes with micro-strainers or rotating screen filters with backwash may be the most appropriate for harvesting of large cell algae (>70 mm) such as Spirulina (Mata et al., 2010), but they cannot be used for species of bacterial dimensions like Scenedesmus, Nannochloropsis and Chlorella. Membrane filtration and ultra- filtration are possible alternatives to conventional filtration for the recovery of smaller microalgae cells (<30 mm) (Petrusevski et al., 1995), however they are more expensive processes due to the need for membrane replacement and pumping (Mata et al., 2010).

Cell immobilisation has been taken as an option for harvesting microalgae biomass in wastewater treatment systems. This process prevents cells from moving independently of its neighbours to all parts of the aqueous phase of the system (Mallick, 2006). However assessment for large-scale applications is still limited (Kumar et al., 2010) and more research is needed on the effects of immobilisation on algal cell physiology and biochemistry (Mallick, 2006).

Drying is sometimes required after the harvesting steps to prevent fouling of the final biomass product. Different drying methods including freeze-drying, drum drying, spray drying, oven drying, and solar drying, fluidised bed-drying, and low-pressure shelf-drying can be employed (Brennan & Owende, 2013; da Silva & Reis, 2016). Sun-drying is the most popular method due to the low cost, but disadvantages include long drying times, the requirement for large surface areas, and high material loss (Prakash et al., 1997). Spray-drying and freeze-drying are expensive processes commonly used for the high-value products and oils (Desmorieux & Decaen, 2005; Grima et al., 1994). Fig 2.2 show the possible processing pathway for production of biofuel from microalgae.

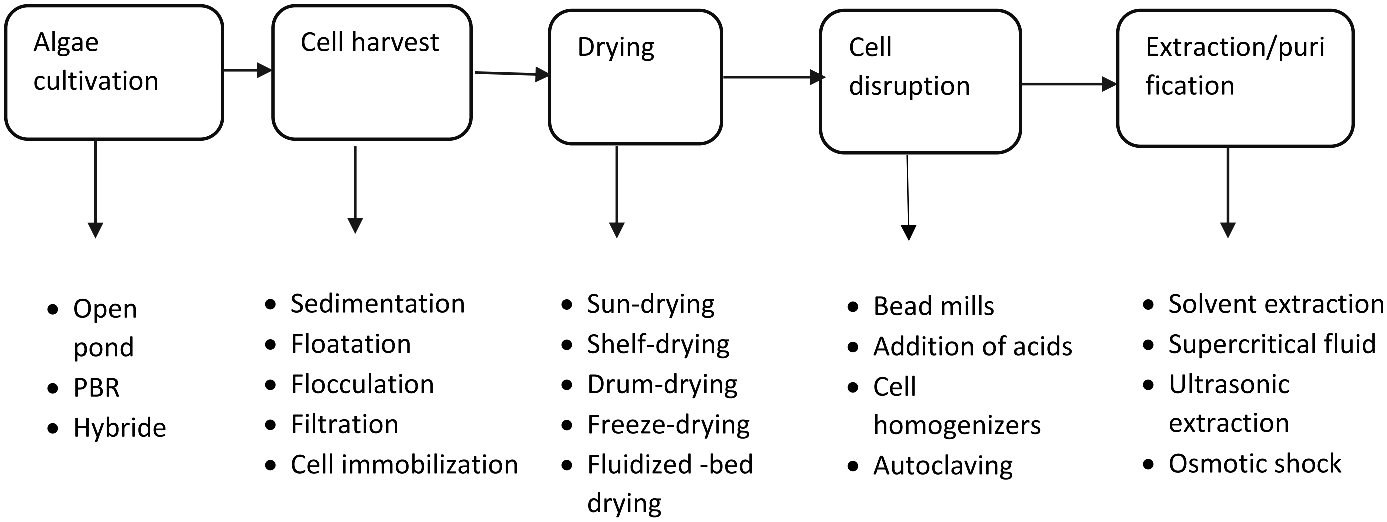

Fig. 2. 2 Processing path way for microalgal-directed biofuels (Brennan & Owende, 2013)

2.3.5. Extraction and other conversion technologies

Microalgae biomass can be converted to renewable fuels through different pathways that can be used to extract and convert microalgae wet biomass (20 % solid) into bioenergy.

Many technology options for converting algal biomass into different biofuels economically and favorably should be investigated. The technology should also consider production of co-products that will aid in cost-effectiveness of entire system. In the development of the technology issues to be considered include catalysts, energy intensity, GHG emissions, and conversion rates (Pienkos & Darzins, 2009). The major bottle neck in microalgae biomass extraction/conversion process is that the conversion processes can result in negative energy balance (Hirano et al., 1998), and thus the input costs are high. However recently there are encouraging efforts in this area. For example, wet extraction and oil conversion technologies (SRS Energy Solutions, Inc., Dexter Mich.; Genifuels, Inc., Salt Lake City, Utah) are in demonstration phase (Lyon et al., 2015).

It is also believed that the integrated biorefinery concept can overcome the technical and economic constraints. Recent technology developments in biorefinery concept facilitate utilization of all microalgal biomass components; no longer focusing the biomass production solely on achieving high lipid production (Laurens, 2017); there are promising process operations for future commercial development such as biomass conversion and fractionation into lipids, protein and carbohydrates, thermochemical hydrothermal liquefaction and biogas production from whole algal biomass (Laurens, 2017). Microalgal biorefinery could potentially integrate several different conversion technologies to produce biofuels including biodiesel, green diesel, green gasoline, aviation fuel, ethanol and methane, as well as valuable co-products, such as fats, polyunsaturated fatty acids, natural dyes, sugars, pigments (mainly β-carotene and astaxanthin), antioxidants and polyunsaturated fatty acids (EPA, DHA) (Das, 2015).

Currently hydrothermal liquefaction is considered to be the most energetically positive method for biofuel production from microalgae (de Boer et al., 2012). However, extensive research and development is still required to determine the most energetically favourable and economically feasible process for extracting and converting the algal biomass for renewable bioenergy (Moheimani et al., 2015).

In the case of production of biodiesel the biomass needs to be extracted and the extraction process is highly affected by the upstream and downstream unit operations. The presence of water is one problem that water can either promote the formation of emulsions in the presence of ruptured cells or participate in side reactions when present in the bulk solution. It is possible to disrupt the solute matrix interactions and to reduce the viscosity and surface tension of the water by increasing the temperature, thereby improving the contact between the solvent and the solute.

It is possible to disrupt the solute matrix interactions and to reduce the viscosity and surface tension of the water by increasing the temperature, thereby improving the contact between the solvent and the solute. The transport of the solvent to the analytes, which have been trapped in pores, can be enhanced by increasing the pressure. Also pressure helps to increase the contact between the solvent and the matrices. For example using supercritical CO_2 as an extracting solvent under high pressure it is possible to achieve near 100% recovery of lipids (Yen et al., 2015; Patel et al., 2020), but it is a very energy-intensive and therefore may not be economical for biofuel extraction (Scott et al., 2010). To reduce the temperature and pressure requirements during extraction cell disruption can be applied. Mechanical disruption can reduce the pressure and temperature requirements (Brennan & Owende, 2013).

2.4. Concluding remarks

The two big concerns associated with the use of fossil fuels are sustainability due to depletion of fossil fuels and global warming due to GHG emissions. This leads the scientific community in the world to think about sustainable and environmentally alternative energy sources. First generation biofuel from food crops have being used for decades. However there is a substantial controversy to sustainably use food crops for biofuel production due to food security issues. Lignocellulosic materials are other easily available sources to be used as renewable energy sources. But the high energy intensive pretreatment step is the major bottleneck to use these materials as a viable substitute feedstock for biofuel production. In this regard production of biofuel from microalgae has received a substantial interest due to the important characteristics associated to the microalgae, such as high solar energy conversion efficiency, high oil productivity, and possibility to grow microalgae in non-arable land. To this end there have been made a substantial effort to commercially produce microalgal biofuel. In spite of this effort production of biofuel from microalgae is still limited to only laboratory scale, only rare production plants are found, as economic analysis depicts that large scale production is not found viable. Thus some problems associated to microalgal biofuel production are waiting to be addressed so as to use microalgae as future possible substitute feedstock for biofuel production. Overall to realize large scale production of biofuel from microalgae it will be important to use the technological opportunities such as advances in algal biology, advances in new rector development, possibility of cultivating microalgae heterotrophically so as to overcome problems associated with phototrophic microalgae, use of an integrated biorefinery approach so as to exploit every component of the microalgal biomass raw material to produce a wide range of chemicals and biofuels in a cost-effective and environmentally sustainable manner, and coupling of microalgal cultivation with other processes so as to utilize inexpensive nutrient and CO_2 sources. As mentioned in section 2.3.1.4 coupling of microalgal cultivation with other processes for the inexpensive nutrient and CO_2 utilization is one of the identified approach towards increasing the feasibility of microalgal biofuel production. In the section 2.3.1.5, it is discussed that waste streams and byproducts from sugarcane processing factories could have potential to support the growth of algae. And such coupling approaches are plausible for countries like Ethiopia where sugar factories are among the major economic sources. Furthermore the tropical weather condition, the abundant natural resources including algae, the cheap labors highly stimulate such activities. Thus the present study was devoted for integrated biorefinery approach i.e. the coupling of algal cultivation with sugar factories' wastes streams and evaluation of the coupled process.

CHAPTER THREE

3. Utilization of Sugarcane Factories' Wastes as Inexpensive Source of Nutrients and CO_2 for Microalgal Biomass Production

3.1. Introduction

Several important advantages associated with microalgae, such as their economic potential as a feedstock for biofuel production coupled to bioremediation, high biomass and oil productivity, diverse biodiversity, high photosynthetic efficiency (Lyon et al., 2015) are causing the interest in microalga-derived biofuels to rise. Furthermore the international & national agreements in shifting towards more secured renewable energy system are also other driving factors to make use of microalgae as renewable energy sources (Laurens, 2017).

Nitrogen, phosphorus, and CO_2 supplies have a considerable impact on cost, sustainability, and production siting in microalgae cultivation. Use of less expensive CO_2 and nutrient supplies by coupling microalgae production with large scale facilities is, therefore, important for viable production of microalgal biomass. Since microalgae can utilize various wastewaters, seawater, and other forms of produced water which cannot be introduced into the agricultural system, competition for limited land and freshwater resources will be reduced (Chisti, 2007; Klein et al., 2018). Several studies are found on the utilization of wastewater for microalgae cultivation (Guldhe et al., 2017). Microalgae belonging to the *Scenedesmus* genus, for example, are broadly explored as they can accumulate large amount of carbohydrates, lipids and proteins and can grow under non-optimal conditions, including, for example, the application of culture media enriched with wastewaters (Di Caprio et al., 2018). The wastewater effluent and the flue gases of sugar factories can be used as inexpensive nutrient (nitrogen and phosphorus) and CO_2 sources for the growth of microalgae (Klein et al., 2018). The possibility of coupling microalga cultivation to existing sugarcane processing factories is being currently declared in the scientific, industrial, and environmental community. Several studies are found on the matter. The possibility of collocating microalgae cultivation to sugarcane mill in Colombia have been assessed through process simulation (Lohrey & Kochergin, 2012). Similar analysis on the integration of an existing sugar mill with microalgal production in Louisiana was also performed (Moncada et al., 2014). Likewise a number of studies are found on the environmental benefits of integrating microalgal production and sugarcane processing in Brazilian context (Souza, et al., 2015; Chagas et al., 2016; Maranduba et al., 2016).

This chapter aims on studying production of biomass from microalgae through the coupling of microalgae cultivation with sugarcane processing factory in Ethiopian context, and focuses on both production of algal biomass and environmental abatement. The algae would be cultivated inexpensively using the wastewater and CO_2 from the sugar factory. Metahara Sugar factory has been selected for the adoption of the presented strategy.

3.2. Materials and methods

This chapter involves coupling of a conceptual microalgal cultivation with an actual sugar factory (Metahara Sugar factory) (Fig 3.1) and then evaluation of the coupled process for its outputs and environmental abatement. Three data sources viz. literature data, factory data and experimental data were employed.

3.2.1. Experimental works

3.2.1.1. Materials

The microalgae *Scenedesmus* (genus level) used for the experimental work was obtained from Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.

3.2.1.2. Wastewater sampling

Wastewater sampling from Metahara sugar factory was carried out at the beginning, in the middle and at the end of the milling season of 2018/2019. Samples were taken from a focal point where wastewater from milling house, boiling house, factory laboratory, and factory garage mixes together. The chemical oxygen demand (COD), the biological oxygen demand (BOD_5), the total nitrogen (TN), the total phosphorus (TP) and the oil and grease content of the wastewater was determined for the samples collected in the three seasons. The TN and TP were determined using UV/VIS Spectroscopy (Bridgewater, 2017; Ferree & Shannon, 2001). All the other parameters were also determined according to standard methods for the examination of water and wastewater (Bridgewater, 2017; Strickland & Parsons, 1972). The result for the average values is shown in Table 3.1.

Table 3. 1 Characteristics of wastewater effluent from Metahara sugar factory, Addis Ababa, Ethiopia

Parameter	**Value** [a]
BOD_5 (mg/L_{WW})	1200 ± 163.30
COD (mg/L_{WW})	2200 ± 108.01

Nitrogen (mg/L_{WW})	15 ± 0.41
Phosphorus (mg/L_{WW})	10 ± 0.33
TSS (mg/L_{WW})	362 ±2.16
Oil and grease (mg/L_{WW})	60 ± 4.67
TDS (mg/L_{WW})	210 ±3.74
pH	6.6 ± 0.65
Temperature (°C)	29.7 ± 0.65
Average flow (m^3/day)	1074 ± 6.89

[a] The average value ± standard deviation estimated for the wastewater during the three milling seasons

3.2.1.3. Testing the growth of microalgae in the wastewater

The growth of microalgae (*Scenedesmus*) in the wastewater was tested in the laboratory. The productivity of the algae, the reductions in COD and BOD_5 of the wastewater and the utilization of the nutrients (TN and TP) in the wastewater were studied.

3.2.1.4. Inoculum preparation

The cells of *Scenedesmus* were cultured in deionized water using BG-11 broth (Blue-Green Medium) containing the following chemicals (g/L): 0.075, $MgSO_4 \cdot 7H_2O$; $K_2HPO_4 \cdot 3H_2O$, 0.04; $CaCl_2 \cdot 2H_2O$, 0.036; $C_6H_8FeNO_7$, 0.006; $C_6H_8O_7$, 0.006; $C_{10}H_{16}N_2O_8$, 0.001; $NaNO_3$, 1.5; Na_2CO_3, 0.02; trace metal mixture which consisted of (µg/L) $ZnSO_4 \cdot 7H_2O$, 0.222; H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $Na_2MoO_4 \cdot 2H_2O$, 0.391; $Co(NO_3)_2 \cdot 6H_2O$, 0.05; and $CuSO_4 \cdot 5H_2O$, 0.079

(Grobbelaar, 2013). Each 1ml media in 1L of deionized water was sterilized at 15 psi pressure for 30 min in autoclave after adjusting its pH to 7.5. Using this media *Scenedesmus* was incubated under continuous artificial light (5000 lux) at 25 °C for 8 days. The fresh cultures from the BG-11 were preserved at 4 °C and were used in further study.

3.2.1.5. Cultivation of the microalgae in the wastewater sample

Sugar industry effluent was obtained from Metahara sugar factory, and the sample was transported to laboratory and kept in a cold room maintained at 4 °C prior to cultivation (Chinnasamy et al., 2010). The wastewater sample was then filtered with 50 μm mesh and sterilized at 15 psi pressure for 20 min using autoclave and allowed to cool to room temperature. The experiment was conducted in duplicate using 2 L conical flasks with 1000 mL treated wastewater as growth medium with the following culture conditions: temperature 25 °C, artificial light 5000 lux (12 h. light/ 12 h. dark) and CO_2 supply. 1 mL of *Scenedesmus* suspensions grown in the media (8 days old) (10 mg/mL) were taken and inoculated in the flasks containing 1000 mL of wastewater sample as the growth medium. Before the inoculation the pH of the samples were adjusted to 7.5 using a 1 molar KOH. The wastewater samples in the flasks were assigned as sample one, S1, and sample two, S2, both samples in duplicate. 2 mL of ammonia solution (NH_4OH) with density of 0.91 g/mL (25% w/w) was added to S2 at the beginning of the third day to study the effect of the nutrient supply on the growth of algae, reductions in COD, BOD_5 TN and TP in the wastewater. The algal growth was monitored for 20 days (Craggs et al., 2011; Broberg et al., 2011).

3.2.1.6. Biomass determination

Sampling and measurements were carried out once in every two days interval at the same time of day. To determine the dry cell weight of the microalgae, 40 mL of the sample were withdrawn and centrifuged at 1500 rpm for 15min. Then the centrifuged samples were washed with distilled water and dried in an oven at 105 °C for 15 h. The productivity of the biomass was determined by using Equation 3.1.

$$C_B = \left(\frac{C_t - C_{to}}{t - to}\right) \qquad (3.1)$$

Where C_B is net biomass productivity (mg/mL.h), C_t is biomass concentration at time t (mg/mL), and C_{to} is biomass concentration at time t_o (mg/mL).

3.2.1.7. Removal of COD, BOD$_5$, TN, and TP in the wastewater

Samples (20 mL) were taken every two days of interval to measure the removal of COD, BOD_5, TN, and TP in the wastewater. Reductions in COD and BOD_5 were determined according to standard methods for the examination of water and wastewater (23rd edition), part 5000 of sections 5210 and 5220 (Bridgewater, 2017). Likewise the removal of TN and TP were determined by using UV/VIS Spectroscopy (Bridgewater, 2017; Ferree & Shannon, 2001). The procedure for the determination of TN used 2nd derivative of the absorption spectrum for NO_3^- that has a peak at 224 nm proportional to NO_3^- concentration. Samples were 1st oxidised to NO_3^- by persulphate digestion. Organic nitrogen standards were prepared from reagent urea, and distilled water was used as a blank. Blanks, standards and samples were scanned on the spectrophotometer between 190 and 250 nm to measure the value of NO_3^- peak at 224 nm. The detail procedures used for the TN determinations were according to Ferree and Shanoon (Ferree & Shannon, 2001). In the same way TP was determined after digesting all phosphorus forms in the wastewater to the orthophosphate forms using sulfuric acid. The absorbances were read at 880 nm using reagent blank (distilled water) to zero spectrometer (Bridgewater, 2017). The removal efficiencies were determined using the following equation.

$$Percentage\ removal = \frac{Initial\ concentration - Final\ concentration}{Initial\ concentration} \times 100 \qquad (3.2)$$

3.2.2. Coupling of the sugar factory with the conceptual microalgae cultivation

Important literature and actual factory process data used for the modelling and design of the process are found in Table 3.2. The process is intended to produce microalgal biomass which is to be used for production of biofuel by using the factory wastes as inexpensive nutrient and CO_2 sources. The process is assumed to involve primary treatment of the wastewater from sugar factory before entering the cultivation pond, selection of the microalgae, culturing of the algae in a closed system photo bioreactor (PBR), phototrophic cultivation of the algae in a pond, and harvesting of the algae.

Table 3. 2 Design process parameters for the sugarcane factory used in the modelling

Parameters	Value	References
Sugarcane crop area (ha)	10230	Factory data
Cane production (ton/ha .yr)	144	Factory data
Days of operation of factory	250	Factory data
Mill Capacity (tons/day)	5000	Factory process data
Bagasse production, dry wt. (% on cane)	14%	Factory process data
Excess Bagasse (% total bagasse)	15.5% (14-17%)	Factory process data

Heat content of Bagasse (BTU/lb dry wt.)	7893	(Tsai et al., 2006)
Mass of flue gasses produced (dry wt.) (kg of flue gases /kg of bagasse)	7.41	(Abdalla et al., 2018)
CO_2 Produced, (kg of CO_2/kg bagasse dry)	1.72	(Abdalla et al., 2018)
Surplus water produced at mill (% on cane)	20%	Factory process data
Molasses produced (% on cane)	3.2%	Factory process data
Ethanol produced (m^3/tons molasses processed)	0.23	Factory process data
CO_2 produced from EtoH plant, (tons CO_2/tons of molasses used)	0.21	Factory process data
Vinasse produced, (m^3 of vinasse/ tons of molasses processed)	2.3	Factory process data

3.2.2.1. Primary treatment of the wastewater from the sugar factory

It is assumed the wastewater effluent from the factory would be treated in the primary treatment plant before entering the cultivation pond to avoid sedimentation for the next treatment steps by reducing solids and increase photosynthetic efficiency in the following algae cultivation. The nutrient concentration of wastewater from the primary treatment effluent is obtained by applying the pollution reduction factors as shown in Table 3.3. Retention time and over flow rate are the two main design parameters for a clarifier in a primary treatment of wastewater and usually a retention time of 1.5-2.5 h. and overflow rate of 30-50 m/day are used (Lundquist et al., 2010). For the present study over flow rate of 40 m/day (1.67 m/h.) with typical depth of 4 m and retention time of 2.4 h is assumed. With a wastewater flow of 1074 m^3/day, a total of ten 107.4 m^3 of clarifiers, with surface of 26.85 m^2 are needed. Settled solids are assumed to be removed from the bottom of tanks by sludge rakes. Scum which mainly contains the oil and grease is assumed to be swept across the tank surface by water jets. The primary treatment effluent is sent to the microalgae cultivation pond, while the sludge is removed from the bottom. The estimated compositions of the effluent and sludge after the primary treatment are shown in Table 3.4.

Table 3. 3 Pollutant reduction during primary treatment of sugar factory wastewater (WW)

Parameter	Reduction factor	References
BOD_5	0.31	(Pescod, 1992; Broberg et al., 2011)
COD	0.30	(Marani et al., 2004; Broberg et al., 2011)

Total N	0.20	(Pescod, 1992; Broberg et al., 2011)
Total P	0.26	(Broberg et al., 2011)
TSS	0.6	(Pescod, 1992; Broberg et al., 2011)
Oil & grease	0.65	(Pescod, 1992; Broberg et al., 2011)
TDS	0.63	Estimated (average of TSS and oil & grease)

Table 3. 4 Estimated composition of effluent and sludge (bottom product) after primary treatment

Parameter	Values	
	Effluent	Sludge
BOD_5 (mg O_2/L_{ww})	828	372
COD (mg O_2/L_{ww})	1540	660
TN (mg/L_{ww})	12	3

TP (mg/L_{ww})	7.4	2.6
TSS (mg/L_{ww})	145	217
Oil & grease (mg/L_{ww})	21	39
TDS (mg/L_{ww})	78	132
Flow rate (m^3/day)	1042	32

3.2.2.2. The characteristics of the microalgae

Selection of strain is an important step in the cultivation of microalgae. Productivity is among the important factors which need to be considered in choice of microalgae strain. Productivity involves both cell content of the desired product and growth rate (Griffiths & Harrison, 2009). In this study the biomass produced is intended to be used for production of biodiesel and biogas. Thus lipid productivity is considered to be the key factor. Several number of microalgae, including *Scenedesmous* and *Chlorella* have been identified by the Ethiopian biodiversity institute (Table 3.5). Literature review shows that *Scenedesmous,* and *Chlorella* have been identified as potential microalgae for biodiesel production (Abou-Shanab et al., 20011; Cai et al., 2013; Sydney et al., 2011). As presented in section 3.2.1.3, *Scenedesmus* was selected and experiments were performed to test its growth potential on the wastewater from the sugar mill. The parameters for reduction of COD, BOD_5, TN, and TP obtained in the experiment were considered in the conceptual modelling. Taking into account that there are several local microalgae strain other than *Scenedesmus,* the present study was not limited on a specific strain. Thus, the modelling followed a generic approach based on modelling without experimental data of a specific strain.

Table 3.5 Common microalgae identified by Ethiopian biodiversity institute, Addis Ababa, Ethiopia from 2015-2018

R.N	Identified microalgae	Source
1.	*Scenedesmus*	Killole (Debrezeyit) and Hawassa, Fikr hayk
2.	*Chlorella*	Killole
3.	*Chalmydomonas*	Killole, Beseka (Metahara)
4.	*Chrococcus*	Arenguade (Debrezeyit) and Killole
5.	*Haematococcus*	Chitu (Shashemene)
6..	*Monoraphidium*	Hardibu (Wollo)
7.	*Anabaena*	Abaya
8..	*Coccomyxa*	Hawassa
10..	*Cryptomonad*	Killole
11.	*Arthrospira*	Arenguade (Debrezeyit)

Microalgae, for their growth, need carbon, nitrogen, phosphorus, trace elements (such as sulphur, potassium, sodium, iron, and magnesium) and vitamin sources (Grobbelaar, 2013). However, for large scale production, it would not be feasible to provide all these requirements. The three most important nutrients for phototrophic growth are carbon (C), nitrogen (N), and phosphorus (P) and their supply is central to algal biotechnology. Algal biomass contains about 50% of C, 1-10% of N and less than 1% of P (Borowitzka, 2013). It was proposed that carbon would be provided in the form of CO_2 from the flue gases, nitrogen in the form of nitrates (NO_3^-), and ammonia (NH_4^+) from wastewater, and phosphorus in the form of orthophosphate (PO_4^{2-}) from the wastewater. Nutrient deficiency in the wastewater would be supplied by makeup nutrient. It was assumed that other micronutrients such as calcium, potassium and manganese would not be limiting for the growth of microalgae and iron would be naturally present in the wastewater. The microalgae used in this study were suggested to have the elemental formula of ($C_{106}H_{181}O_{45}N_{15}P$) (Broberg et al., 2011; Davis et al., 2011) with molecular wt. % of components as follows: C, 52.69; H, 7.5; O, 29.83; N, 8.7; and P, 1.28. From the elemental analysis of the microalgae (Table 3.6) the C:N:P mass ratio in microalgae is approximately 41.03:6.77:1, and this composition was used to evaluate the potential of the wastewater from factories to support the microalgae growth.

Table 3. 6 Elemental composition of the microalgae ($C_{106}H_{181}O_{45}N_{15}P$)

	C	H	O	N	P
No. of atoms	106	181	45	15	1
Atomic wt.	12	1	16	14	31
Mass per mole of algae	1272	181	720	210	31

% mass	52.69	7.50	29.83	8.70	1.28
C:H:O:N:P			41.03:5.84:23.23:6.77:1		

3.2.2.3. Cultivation of the microalgae in the pond/secondary wastewater treatment

All the underlying assumptions considering operating conditions and nutrient uptake by microalgae are shown in Table 3.7. A two-stage cultivation system, the first stage involves inoculation of the desired algae and the second stage involves cultivation of the algae in open pond raceways, was proposed. The inoculated microalgae was supposed to be obtained from the inoculation system which would use photo bioreactors (PBRs), covered pond, and the open lined pond (Lundquist et al., 2010). The inoculation stage is used to establish a monoculture of microalgae within the ponds and ensure the dominance of the desired species. For the second stage phototrophic cultivation was proposed where microalgae would use sunlight as energy source. Microalgae can be cultivated either in open or closed systems and each has its own advantages and drawbacks; usually an important choice of factor is economic viability. It was considered that the microalgae would be cultivated in open pond systems as they are the most commonly used systems in microalgae cultivation due to their low investment and maintenance costs, which results in lower production costs (Ugwu et al., 2008; Pires et al., 2012). For the present study the land requirements to cultivate the microalgae would be based on the productivities of the algae in the raceways and the amount of nutrients contained in the wastewater. Realistic values for productivities of microalgae in open ponds are mostly in the ranges of 20-30 g/m^2/day (Frank et al., 2011; Nappa & Karinen, 2015). In the present study a value of 25 g/m^2/day was suggested.

A study on evaporation estimates of Metahara region based on monthly average values of temperature, air humidity and sunshine duration shows that there is water evaporation rate of 6.91

mm/day. Hence in the present study evaporation of water from the ponds was presumed to occur at a rate of 6.91 mm/day.

Oxygen to satisfy BOD_5 is obtained from photosynthesis of algae, oxygen in flue gases and oxygen diffusion from the atmosphere. Since there would be sufficient supply of CO_2 to the pond from the sources, it was considered that the ponds would at all times be oversaturated with regard to CO_2; the ponds lose CO_2 rather than their absorption. Thus absorption of atmospheric CO_2 was supposed to be negligible. Utilization efficiency of CO_2 was expected to be 85% (Nappa et al., 2015). It was assumed that the flue gas would be pressurized and injected along the ponds through PVC pipes. The electricity consumption required for the CO_2 distribution was assumed to be 0.0222 kWh/kg-CO_2 (Kadam, 2001; Broberg et al., 2011). Ethiopian sugar factories operate on average for 250 days per year since they are under maintenance for the remaining days and it was considered that the facility for the microalgae cultivation would operate for 250 days per year. The experimental values for the BOD_5, COD, TN, and TP reductions were used in the modelling.

In the raceways a paddle wheel mixer is used to prevent settling of the algae, ensure that all the algae are exposed to sunlight and that there is no clumping. Since the paddle wheel has a low clearance with the sides and the bottom of the pond, this low clearance avoids backflow and allows all of the power of the paddle wheel to mix the pond (Rosenberg et al., 2011). Several sources are found on power consumption of paddle wheel (Kadam, 2001; Lundquist et al., 2010). The mixing velocity typically varies between 20 and 30 cm/s and depends on the alga species (Frank et al., 2011). In this study power consumption of 2 kW/ha with 25 cm/s mixing velocity was supposed (Lundquist et al., 2010). Water would be pumped from the primary clarifiers into the ponds to handle continuous distribution of water flow. Some rules of thumb show that water effluents with solid suspensions below 2% may be treated as water (Frank et al., 2011). Frank et al., 2011 in their study mentioned that pumps that consume 2.4×10^{-5} kWh/L and move up to 1.14 m^3/min under the mentioned constraints could be found (Frank et al., 2011). For the present study pumping power consumption of 2.4×10^{-5} kWh/L was assumed to pump the primary treated water to the ponds. The flow out of slurry from the ponds to the harvesting (bio-flocculation unit) also requires pumping. Algae suspensions can meet the criteria for intermediate water pumping (low solids, relatively clean, amendable to more efficient pumps), which require power consumption of 4.8×10^{-5} kWh/L with a total head of 4.57 m (Franket al., 2011). For the present study pumping power consumption of 4.8×10^{-5} kWh/L was assumed to pump the algal suspension from the pond to the harvesting section. Part of the separated water in the harvesting section would be recycled to the pond when it would be found necessary. It was also suggested that the recycle pump would require a power consumption of 2.5×10^{-5} kWh/L (Franket al., 2011).

In the cultivation of microalgae pond depth is one of the important factors which affect cell growth. Thus an appropriate pond depth should be selected. Ponds with depths shallower than 30 cm limit the size (area) of the ponds due to pond hydraulics, CO_2 outgassing, and CO_2 storage, and also exhibit greater diel temperature fluctuations. Whereas ponds with depths much greater than 30cm have the disadvantages of more water handling during the initial harvesting step, though they can improve the temperature regime that the algae experience, improve CO_2 storage, etc. (Lundquist et al., 2010). Taking these in to account, for the present study, the depth of the ponds is assumed to be 30 cm. The pond design is assumed to have dimensions as used by Lardon et al., 2009 such that its dimensions are 10 m wide, 100 m long, and 30 cm deep oval shaped built in concrete blocks. The concrete needs to be covered by a thick sole PVC liner so as to decrease roughness and avoid biomass attachment (Lardon et al., 2009) but it would be made so at the expense of high capital investment (Davis et al., 2016). Thus, in the present study unlined ponds built in concrete blocks would be used to minimize the capital cost. It was supposed that the algal biomass would contain 30% extractable triglycerides (Lundquist et al., 2010).

Table 3. 7 General assumptions for microalgae cultivation in the pond

Parameter/description	Value	References
Assumed dimensions of a pond (m)	10, 100 and 0.3 for width, length and depth respectively	(Lardon et al., 2009; Lundquist et al., 2010)
Fraction of area occupied by photo bioreactor (PBR) (%)	0.1	(Lundquist et al., 2010)
Temperature (°C)	29.7	Characteristics of the waste water
pH	6.6	Characteristics of the waste water
Algae growth rate/ productivity ($g/m^2/day$)	25	(Delrue et al., 2012; Davis et al., 2016)
Mixing velocity in the pond (cm/s)	25	(Lundquist et al., 2010; Frank et al., 2011)
Electricity demand by paddle wheel (kW/ha)	2	(Lundquist et al., 2010)

Electricity demand to pump WW to pond (kWh/L)	2.4×10^{-5}	
Electricity demand to pump from pond to bio-flocculation (kWh/L)	4.8×10^{-5}	(Frank et al., 2011)
Electricity demand for recycle pump (kWh/L)	2.5×10^{-5}	
Electricity consumption for flue gas injection (kWh/kg-CO_2)	0.0222	(Kadam, 2001)
% of flue gases captured	90	Estimated
CO_2 utilization (% converted to algae)	85	(Lundquist et al., 2010; Frank et al., 2011)
CO_2 required for algal growth (g-CO_2/g algae dry)	1.92	From the microalgae composition
Nitrogen required for algal growth (g N/g algae dry)	0.087	From the microalgae composition
Phosphorus required for algal growth (g P/g algae dry)	0.0128	From the microalgae composition
BOD_5 reduction (%)	75	Experimental data

COD reduction (%)	71	Experimental data
Total N reduction (%)	95	Experimental data, (Fagerstone, 2011; Carter et al., 2012)
Total P reduction (%)	80	Experimental data, (Lundquist et al., 2010)
Water loss by evaporation (m/day)	0.00691	Average evaporation rate data for Metahara ("Ethiop sugar corporation," 2019)
Algae Oil Content (wt %)	30	(Lundquist et al., 2010; Mata et al., 2010)
Culture density (g/L)	0.5 (0.1-2)	(Fagerstone, 2011; Davis et al., 2016)
Ratio of total pond area to total facility foot print (%)	84	(ANL;NREL; PNNL;, 2012)

3.2.2.4. The stoichiometric model for predicting nutrient requirements by the microalgae in the ponds

The cultivation model (Fig.3.2 & Fig.3.3) requires input data for the carbon, nitrogen, oxygen, hydrogen, and phosphorus based on the composition of the algae. The elemental composition of the microalgae is entered in the mass balance equations (Equations 2.3 to 2.8) in the form of the mass fraction of each element. The derivation of the mass balance equations in the cultivation model is based on the following assumptions:

- Photoautotrophic microalgae cultivation is assumed in the ponds that the flue gas from the boilers of the sugar mill is the only carbon dioxide sources; the microalgae do not get their carbon source from the wastewater. Though the microalgae could get organic carbon from the wastewater, in the present study it is considered insignificant for the simplicity of the model. The CO_2 uptake efficiency factor in the ponds is entered in the mass balance equations (Equation 2.8) to account for any losses due to off gassing from the ponds.
- The nitrogen and phosphorus requirements of the microalgae are obtained from the primary treated wastewater of the sugar mill, and it was assumed that any nitrogen and phosphorus deficit is supplied by makeup nutrient.
- All macronutrient elements (C, N, P) feeding the ponds are converted quantitatively into microalgal cells, and the recycle water from the harvesting operations contains no usable C, N, and P
- The utilization efficiency of nitrogen and phosphorus is entered to account for any losses of nitrogen and phosphorus

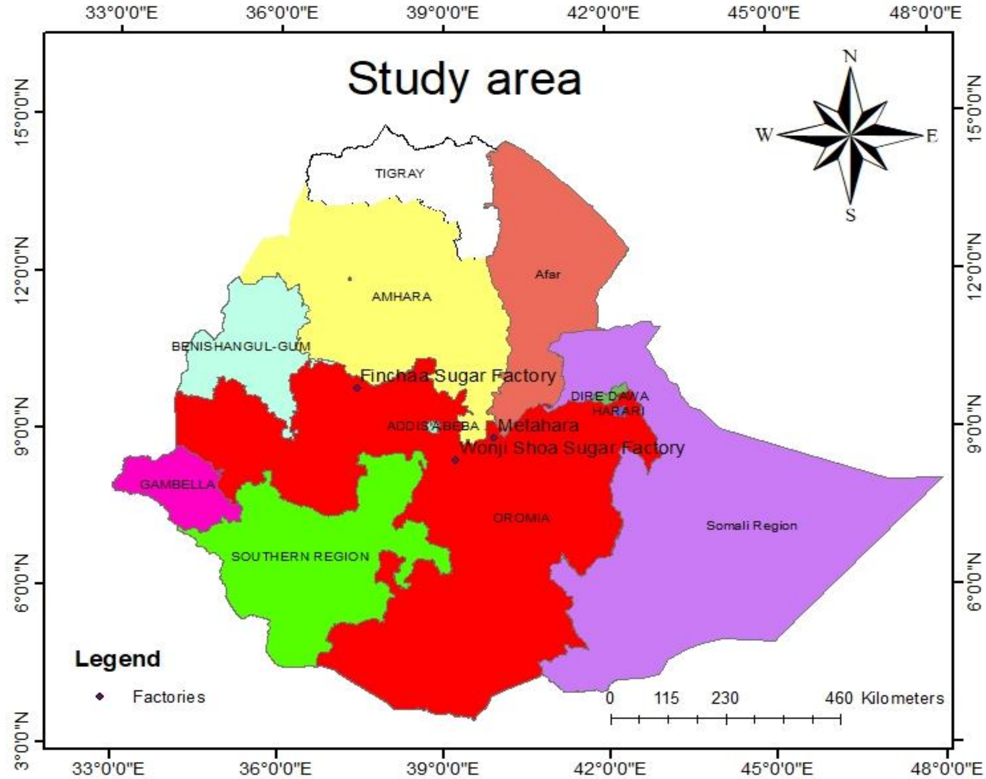

Fig. 3. 1 Location of Metahara sugar factory on Ethiopian Map

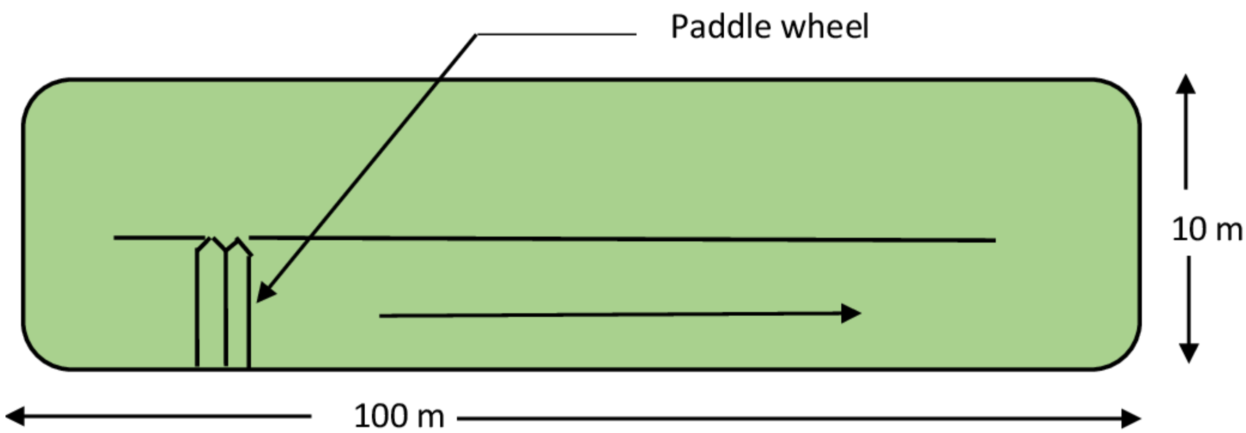

Fig. 3. 2 Schematic of the cultivation pond

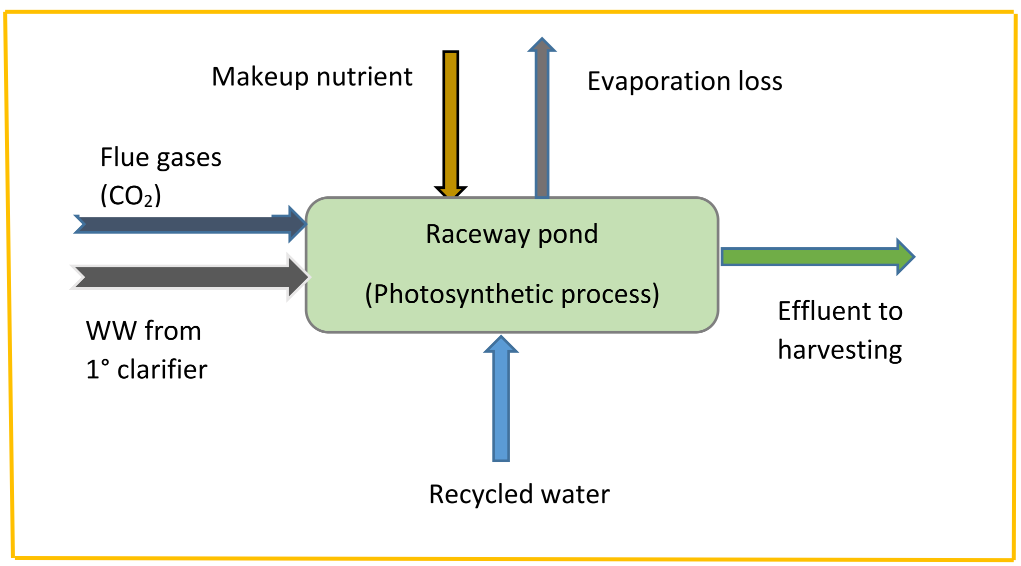

Fig. 3. 3 Simplified mass balance model around the pond

Equations (3.3) to (3.8) represent mass balance calculations around the cultivation/growth step.

$$N_{FED} = X_N Q_{WW} + N_{FER} \tag{3.3}$$

$$P_{FED} = X_P Q_{WW} + P_{FER} \tag{3.4}$$

$$CO_{2_{FED}} = \left(\frac{M_{wt\,CO_2}}{M_{wt\,C}}\right)\left(\frac{\%C}{\%N}\right) N_{FED} \tag{3.5}$$

$$N_{TOT} = K_N N_{FED} \tag{3.6}$$

$$P_{TOT} = K_P P_{FED} \tag{3.7}$$

$$CO_{2_{TOT}} = K_{CO2} CO_{2_{FED}} \tag{3.8}$$

where:

N_{FED} is total amount of nitrogen in the wastewater added to the cultivation step [kg/day]

N_{FER} is total amount of nitrogen in the makeup nutrient added to the cultivation step [kg/day]

Q_{WW} is the flow rate of the primary treated water [m^3/day]

X_N is the concentration of total nitrogen in the wastewater [-]

P_{FED} is total amount of phosphorus in the wastewater added to the cultivation step [kg/day]

P_{FER} is total amount of phosphorus in the makeup nutrient added to the cultivation step [kg/day]

X_P is the concentration of total phosphorus in the wastewater [-]

$CO_{2_{FED}}$ is total amount of CO_2 in the flue gas added to the cultivation pond [kg/day]

$M_{wt\,CO_2}$ is molecular weight of CO_2 [kg/k-mol]

$M_{wt\,C}$ is molecular weight of carbon [kg/k-mol]

$\%C$ is mass fraction of carbon in the microalgae [-]

$\%N$ is mass fraction of nitrogen in the microalgae [-]

N_{TOT} is total amount of nitrogen to be converted to microalgae [kg/day]

P_{TOT} is total amount of phosphorus to be converted to microalgae [kg/day]

$CO_{2_{TOT}}$ is total amount of CO_2 to be converted to microalgae [kg/day]

K_N is nitrogen utilization factor in the raceway ponds [-]

K_P is phosphorus utilization factor in the raceway ponds [-]

K_{CO2} is CO_2 utilization factor in the raceway ponds [-]

From elemental analysis of the microalga, considering one mole of microalgae, mass percentages for carbon (% C), hydrogen (% H), oxygen (% O), nitrogen (% N), and phosphorus (% P) in the microalgae are =52.69, 7.5, 29.83, 8.7, and 1.28 respectively. Thus the C:H:O:N:P mass ratio in the microalgae is 41.03:5.84:23.23:6.77:1. The N:P ratio in the microalgae is compared with the ratio of mass of nitrogen utilized by the microalgae in the wastewater and the mass of phosphorus utilized by the microalgae in the wastewater, i.e., $X_N Q_{WW} : X_P Q_{WW}$. Then the satisfactory condition for the microalgae to grow by utilizing all the nitrogen and the phosphorus in the wastewater was considered $4:1 \leq K_N X_N Q_{WW} : K_P X_P Q_{WW} \leq 40:1$ (Broberg et al., 2011). If $K_N X_N Q_{WW} : K_P X_P Q_{WW} \leq 4:1$ the microalgae cannot grow by utilizing all the nitrogen and phosphorus implying that there should be added make up nitrogen, N_{FER} while if $K_N X_N Q_{WW} : K_P X_P Q_{WW} \geq 40:1$ there should be added a phosphorus source, P_{FER} to avoid excess nitrogen which may retard the algal growth in the pond. In the light of this:

$$N_{FER} = [K_P P_{FED}\left(\frac{\%N}{\%P}\right) - K_N N_{FED}]/K_N \tag{3.9}$$

$$P_{FER} = [K_N N_{FED}\left(\frac{\%P}{\%N}\right) - K_P P_{FED}]/K_P \tag{3.10}$$

Once the above parameters are determined the total biomass production rate, in in the raceway ponds, the total cultivation area required, total facility foot print, and the number of ponds are determined as:

$$B_P = K_N(X_N Q_{WW} + N_{FER})/\%N \tag{3.11}$$

OR

$$B_P = K_P(X_P Q_{WW} + P_{FER})/\%P \tag{3.12}$$

$$A_{TOT} = B_P/P_B \tag{3.13}$$

$$A_{TFF} = A_{TOT}/R \tag{3.14}$$

$$N_P = A_{TOT}/A_{SUR} \tag{3.15}$$

where:

B_P is total algal biomass production rate [kg/day]

P_B is productivity of algae in the ponds [kg/m^2.day]

A_{TOT} is total cultivation area required [m^2]

A_{TFF} is total facility foot print [m^2]

R is ratio of total area to total facility foot print [none]

A_{SUR} is surface area of a pond [m^2]

N_p is number of ponds [none]

The amount of water consumed in the photosynthesis reaction can also be predicted using the stoichiometric model. To do so an elemental balance on hydrogen around the ponds is performed with following assumptions:

- The hydrogen content of the nitrogen and phosphorus containing compounds in the wastewater and in the makeup nutrient is negligible

- Sufficient water is oxidized in the photosynthesis reactions to exactly satisfy the balance around the ponds

Having these assumptions the following equations can be written:

$$H_{TOT} = H_{RXN} \tag{3.16}$$

where:

H_{TOT} is total amount of hydrogen to be incorporated in to microalgae [kg/day]

H_{RXN} is amount of hydrogen which is obtained from the water reaction in the photosynthesis process [kg/day]

The H_{RXN} is obtained from Equation (3.16) and substituted in Equation (3.17).

$$H_{RXN} = \left(\frac{\%H}{\%N}\right) N_{TOT} \tag{3.17}$$

3.2.2.5. Water balance model around the cultivation step to predict water requirements

The mass balance around the cultivation step is completed by performing a water balance around the ponds (Fig 3.4). Q_{WW} is the flow rate of the primary treated water, Q_{EVAP} is estimated using the evaporation rate given in Table 3.7 and the total area of the ponds which is calculated in Equation (3.13), and Q_{RXN} is obtained from the stoichiometric model in Section 3.2.2.4. $Q_{BIOMASS}$, $Q_{EFF,}$ and Q_{FIL} are known since the concentrations of the biomass are known at the corresponding points. Hence the unknowns: q_{SUPER}, q_{RECY}, and $q_{blowdown}$ can be calculated (Equations (3.18-3.20).

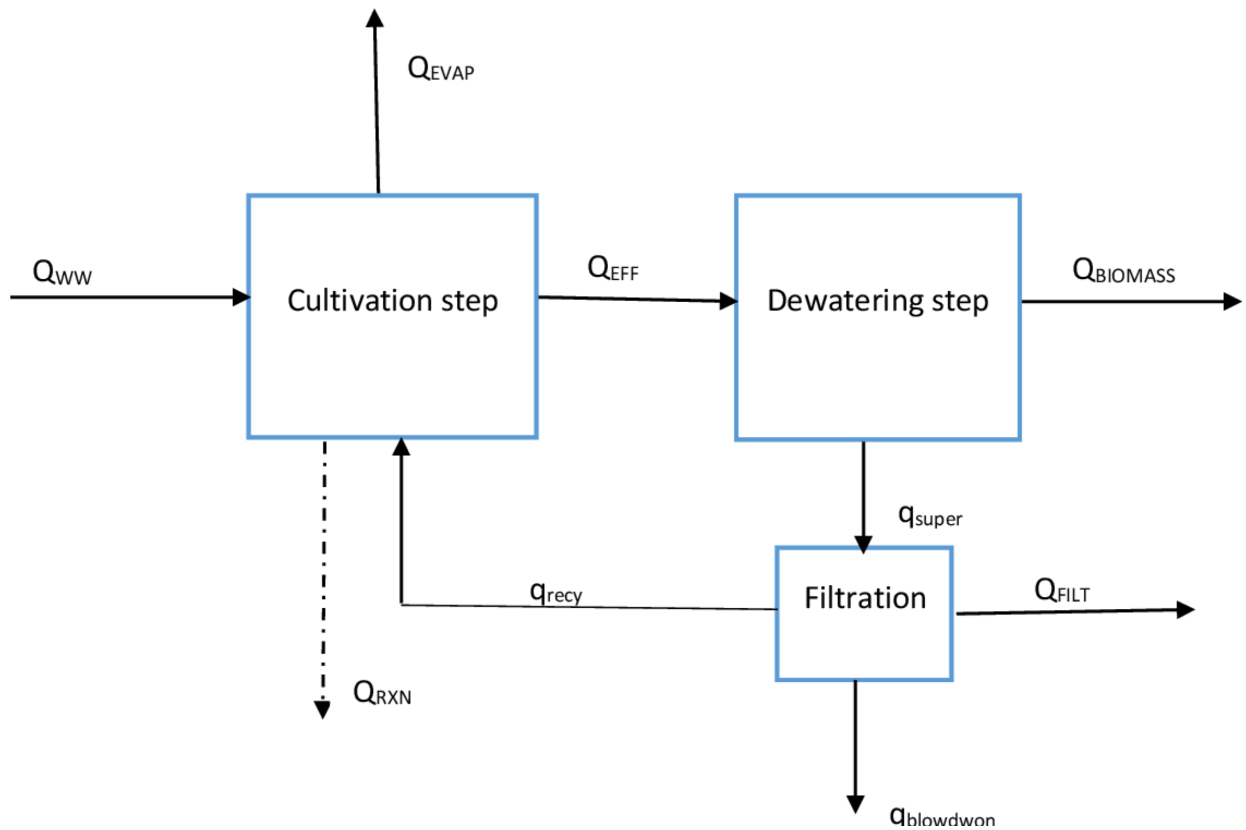

Fig. 3. 4 Schematic of the water balance

$$q_{blowdown} = Q_{WW} - (Q_{EVAP} + Q_{RXN} + Q_{BIOMASS} + Q_{FILT}) \quad (3.18)$$

$$q_{super} = Q_{EFF} - Q_{BIOMASS} \quad (3.19)$$

$$q_{recy} = (Q_{EVAP} + Q_{EFF} + Q_{RXN}) - Q_{WW} \quad (3.20)$$

where:

$q_{blowdown}$ is the blow down water [m^3/day]

Q_{WW} is the primary treated water [m^3/day]

Q_{EVAP} is the water lost by evaporation from the pond [m^3/day]

Q_{RXN} is the water lost during reaction [m^3/day]

$Q_{BIOMASS}$ is the water changed to microalgal biomass [m^3/day]

Q_{FILT} is the water going with the filtrate [m^3/day]

q_{super} is the water going with the supernatant [m^3/day]

Q_{EFF} is the water in the effluent coming out from the ponds [m^3/day]

q_{recy} is the recycled water from the filtration unit to the ponds [m^3/day]

3.2.2.6. Energy Requirements for mixing paddle wheel, flue gas injection, and wastewater pumping

The energy requirements are determined by using Equations 3.21 to 3.24. The units of A_{TOT}, Q_{WW}, and Q_{EFF}, $CO_{2_{FED}}$ should be in ha, L/day, L/day, and kg/day respectively, and their values are known from our previous assumptions and calculations.

$$E_{PW} = C_{PW} A_{TOT} \tag{3.21}$$

$$E_{PP} = C_{PP} Q_{WW} \tag{3.22}$$

$$E_{PF} = C_{PF} Q_{EFF} \tag{3.23}$$

$$E_{FI} = C_{FI} CO_{2_{FED}} \tag{3.24}$$

where:

E_{PW} is total energy requirement by the paddle wheel [kW/day]

C_{PW} is energy requirement by paddle wheel [kW/ha] (Table 3.7)

E_{PP} is total energy requirement for pumping primary treated wastewater to cultivation ponds [kWh/day]

C_{PP} is energy requirement for pumping primary treated wastewater to cultivation ponds [kWh/L] (Table 3.7)

E_{PF} is total energy requirement for pumping effluents from ponds [kWh/day]

C_{PF} is energy requirement for pumping effluents from ponds [kWh/L] (Table 3.7)

E_{FI} is total energy requirement for flue gas injection to ponds [kWh/day]

C_{FI} is energy requirement for flue gas injection to ponds [kWh/kg-CO_2] (Table 3.7)

3.2.3. Microalgal harvesting

Algae harvesting is an important factor in the whole microalgae production. It accounts about 20-30% of the total production cost (Rawat et al., 2011, 2013). The final concentration of the biomass required in this step depends on the final product output. For example for biodiesel production (wet extraction) the biomass needs to be concentrated to about 25 wt.% (Davis et al., 2011) and 4–12 wt.% biomass concentration is required in the case of biogas production (Lundquist et al., 2010). It is assumed that the microalgae would grow to the steady-state concentrations of 0.5 g/L_{WW} (0.05% wt.) in the ponds (Pienkos & Philip, 2009; Craggs et al., 2011) and supposed that the biomass would need to be concentrated to 25% in the harvesting step. Inexpensive and efficient technologies have to be applied to perform the harvesting process. Three steps of harvesting/dewatering viz. auto-flocculation without addition of any chemical, dissolved air floatation (DAF), and centrifugation were proposed. Auto-flocculation was selected because it has an advantage of lower energy requirement over other harvesting methods. It was assumed that dilute algal biomass from pond with concentration 0.5 g/L_{WW} (0.05%) would be directed to auto-flocculation process where it would be concentrated to 10 g/L_{WW} (1% concentration) via auto-flocculation (Benemann and Oswald, 1996; Davis et al., 2011). A continuous below ground clarifier that can remove 95% of the algae biomass was assumed (Lundquist et al., 2010). No electricity demand was accounted for mixing during coagulation. Since flocculation alone would not be able to make the biomass reach the required concentration, the dewatered solids were then assumed to go to dissolved air floatation (DAF) which would again have an assumed capture efficiency of 90% with 60 g/L_{WW} (6% solids concentration) as output (Frank et al., 2011). In the DAF it was supposed that biodegradable flocculant would be used to make the flocculation process faster. Thus chitosan (440 mg/m^3) was selected for this purpose (Divakaran, 2003). It was assumed that power consumption of 0.1 kWh/m^3 would be used in the DAF (Nappa and Karinen, 2015). DAF was selected because it is considered as the most preferred method of wastewater treatment lagoons that harvest microalgal biomass (Hwang et al., 2016).

In order for the biomass to reach the required final concentration it was supposed that biomass from the DAF would again be sent to a centrifugation unit. Centrifugation is considered as one of the rapid, efficient and practical algae harvesting methods (Sharma et al., 2013). Two type of centrifuge are common; self-cleaning disc stack centrifuges and decanter bowl. A disc stack centrifuge is among the most common used in industry (Uduman et al., 2010). Hence for the present study self-cleaning disc stack centrifuges with energy consumption of 5 kWh per m^3 of water removed and 95% algae retention was assumed (Pienkos & Philip, 2009; Delrue et al., 2012). Here the concentration of the culture was expected to increase to 250 g/L_{WW} (25%) (Shelef et al., 1984). The underlined assumptions in the harvesting model are shown in Table 3.8. In all steps of the harvesting the lost biomass was presumed to be filtered for further use. Filtration is considered as highly efficient, simple and lower cost alternative when compared to centrifugation (Sharma et al., 2013; Hwang et al., 2016). Microfiltration with a pore size of 0.1-10μm is most suitable for harvesting algae, and the same method was assumed to be used in the present study (Milledge & Heaven, 2013).

3.2.3.1. Mass balance model equations for the microalgal biomass harvesting

The schematic of the harvesting step is displayed by Fig. 3.5. The mass balance on microalgae is modelled as shown by Equations (3.25) to (3.27). From these equations the mass flow rate of biomass in all dilute, concentrate and feed stream can be calculated.

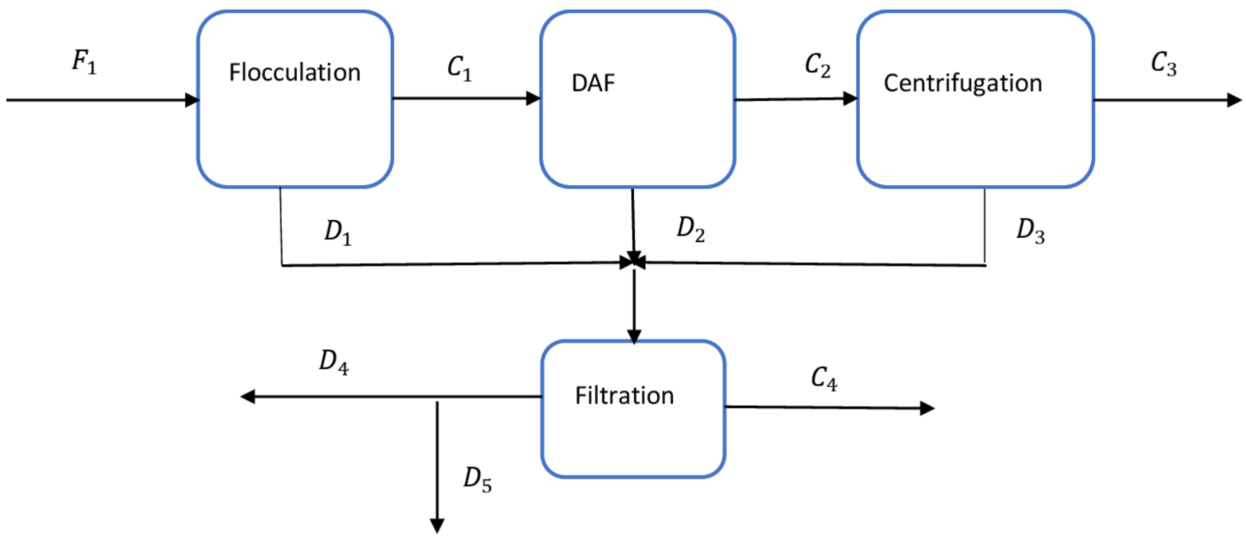

Fig. 3. 5 Schematic of the harvesting step

$$M_{F_n} = M_{C_n} + M_{D_n} \tag{3.25}$$

$$M_{C_n} = r_n M_{F_n} \tag{3.26}$$

$$M_{C_n} = M_{F_{n+1}} \tag{3.27}$$

where:

M_{F_n} is mass flow rate of microalgae in the feed stream to the harvesting operation 'n' [kg/day]

$M_{F_{n+1}}$ is mass flow rate of microalgae in the feed stream to the harvesting operation 'n + 1' [kg/day]

M_{C_n} is mass flow rate of microalgae in the more concentrated exiting stream from the harvesting operation 'n' [kg/day]

M_{D_n} is mass flow rate of microalgae in the more dilute exiting stream from the harvesting operation 'n' [kg/day]

r_n is microalgal recovery rate for the harvesting operation 'n' [kg/kg]

The mass flowrate of water in each concentrate stream is calculated from the following equations.

$$f_n = M_{C_n}/(M_{C_n} + W_{C_n}) \tag{3.28}$$

where:

f_n is mass fraction of microalgae in concentrate stream, C_n [kg/kg]; and

W_{C_n} is mass flow rate of water in concentrate stream [kg/day].

By rearranging Equation (3.28) can be written as:

$$W_{C_n} = M_{C_n} \frac{(1-f_n)}{f_n} \tag{3.29}$$

Then flow rate of water in the dilute stream can be calculated from the following relations:

$$W_{F_n} = W_{C_n} + W_{D_n} \tag{3.30}$$

Since the mass concentration of biomass in the effluent from the ponds is specified, W_{F_1} is known. Thus W_{D_1} can be calculated. It is also possible to calculate the flow rate of every concentrate and dilute stream by using the following equation:

$$W_{F_{n+1}} = W_{C_n} \tag{3.31}$$

Then the mass flowrates are converted to volumetric flowrates using the following relations:

$$Q_{D_n} = \frac{W_{D_n}}{\rho} \tag{3.32}$$

where:

Q_{D_n} is volumetric flowrate of dilute stream D_n [m^3/day]

ρ is the density of the water [kg/m^3]

3.2.3.2. Energy requirements in the harvesting step

In general microalgae in culture are relatively dilute- most of the water must be removed before the microalgae can be processed into fuel. In the present study, the energy requirement in the harvesting step is estimated by using equations 2.33, 2.34 and 2.35.

$$E_{DAF} = C_{DAF} Q_{wDAF} \tag{3.33}$$

$$E_{cent} = C_{cent} Q_{wcent} \tag{3.34}$$

$$E_{filt} = C_{filt} M_{algfilt} \tag{3.35}$$

where:

E_{DAF} is total energy requirement for the DAF [kWh]

C_{DAF} is energy requirement for the DAF [kWh/m^3] (See Table 3.6)

Q_{wDAF} is amount of water removed in the DAF [m^3]

E_{cent} is total energy requirement for the cent [kWh]

C_{cent} is energy requirement for the centrifugation [kWh/m^3] (See Table 3.8)

Q_{wcent} is amount of water removed in the centrifugation [m^3]

E_{filt} is total energy requirement for the filtration [kWh]

C_{filt} is energy requirement for the filtration [kWh/kg-algae] (See Table 3.8)

$M_{algaefilt}$ is amount of algae removed in the filtration [kg]

Table 3. 8 General assumption for the harvesting process

I. Auto-flocculation/gravity settling		
Parameters	Value	Reference
Algae removal rate (%)	95	(Broberg et al., 2011; Craggs et al., 2011; Lundquist et al., 2010)
Retention time (h)	6	(Lundquist et al., 2010)
Output solids concentration (g algae/L_{WW})	10	(Lundquist et al., 2010; Broberg et al., 2011)
II. Dissolved Air floatation (DAF)		
Electricity demand (kWh/m^3)	0.1	(Nappa et al., 2015)

Algal removal rate (%)	90	(Frank et al., 2011)
Output solids concentration (g algae/L_{ww})	60	(Shelef et al., 1984)
III. Centrifugation		
Electricity demand (kWh/m^3)	5	(Lundquist et al., 2010; Delrue et al., 2012)
Algal removal rate (%)	95	(Grima et al., 2003; Broberg et al., 2011)
Output solids concentration (g algae/L_{ww})	250	(Shelef et al., 1984; Davis et al., 2011; Wiley et al., 2011)
IV. Filtration		
Electricity demand (kWh/kg-algae)	0.01	Estimated

3.2.4. Evaluation of the coupled process

The coupled process was evaluated with regard to product output, energy requirement, and pollution reduction. Material and energy balances were performed using a spread sheet to determine the material and energy flows in the process.

3.3. Results and discussion

3.3.1. Microalgae cultivation in the wastewater

The growth of the microalgae in the wastewater, the reductions in BOD_5 and COD in the wastewater by the microalgae, and the utilizations of the TN, and TP under different treatments have been studied. Biomass yield increased sharply up to the 16th day and then decreased due to the depletion of the nitrogen in the media (Fig. 3.6). It was also observed that biomass productivity significantly increased in the nutrient supplemented media by 200 mg/L.h, proving the deficit of nitrogen in the wastewater. The percentage reductions in BOD_5 and COD increased in the nutrient supplemented media (Fig. 3.7). Percentage reductions in BOD_5 and COD increased respectively by 4.5% and 4% when the media was supplemented with nutrient. Increase in reductions of BOD_5 and COD possibly attributes to the increase in the biomass due to the addition of nutrient to the media. The increase in biomass in turn increased the rate of production of oxygen during the photosynthesis which eventually decreased the BOD_5 and COD in the wastewater. The depletion of phosphorus in the media increased by about 25% when the media was supplemented with the nutrient. This is due to the increase in biomass which requires more phosphorus (Fig. 3.8).

Percentage reductions of COD, BOD_5, TN, and TP in the wastewater were 75, 71, 79, and 63% respectively. In other previous studies on the cultivation of microalgae (*C. vulgaris*) in industrial wastewater (brewery effluent) removal of 88% BOD, 82% TN and 54% TP has been reported (Mohsenpour et al., 2020). The experimental values of the percentage reductions in BOD_5 (75%), and COD (71%) were used in the modelling. The experimental removal efficiencies of both nitrogen (79%) and phosphorus (63%) (Fig. 3.9) were modified before used in the modelling. As the wastewater from the sugar factory would pass through a primary treatment plant, it is expected that total solids, BOD_5, and COD decrease while photosynthesis increases in the ponds. Hence the above experimental values for the consumptions of nitrogen and phosphorus were modified; a loss of 5% of total nitrogen (Fagerstone, 2011; Carter et al., 2012) and a loss of 20% of phosphorus was supposed in the ponds (Lundquist et al., 2010).

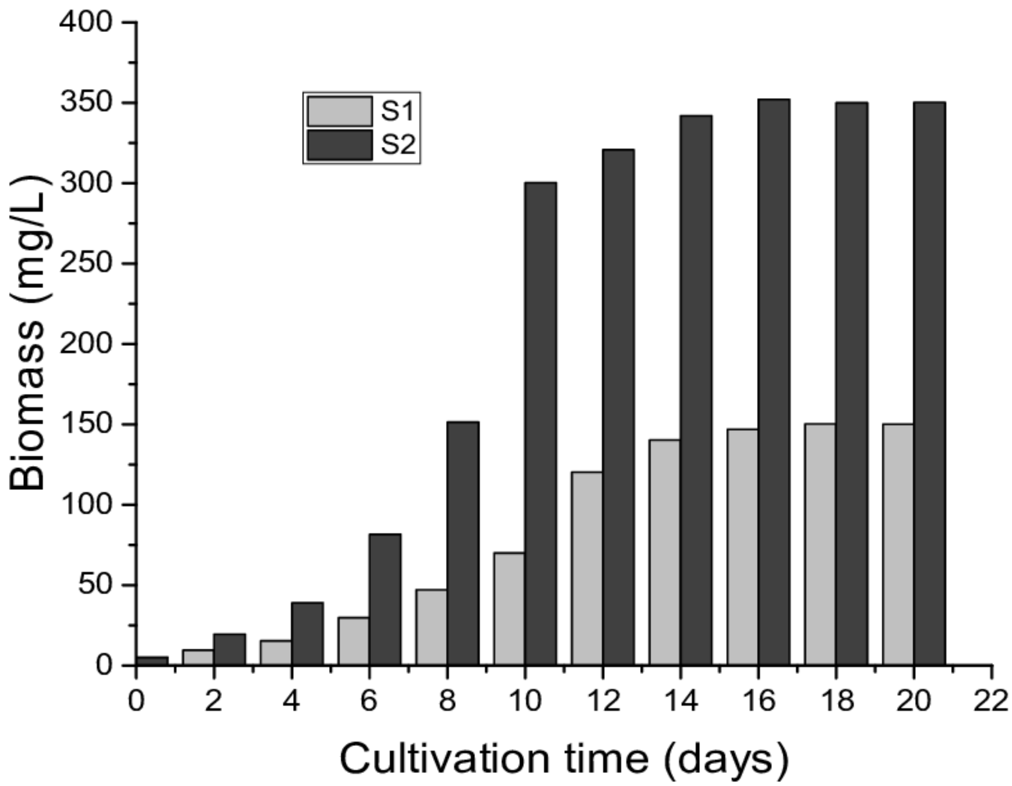

Fig. 3.6 Biomass concentration (mg/L) in the wastewater samples (S_1 is non-nutrient supplemented wastewater sample and S_2 is nutrient supplemented wastewater sample)

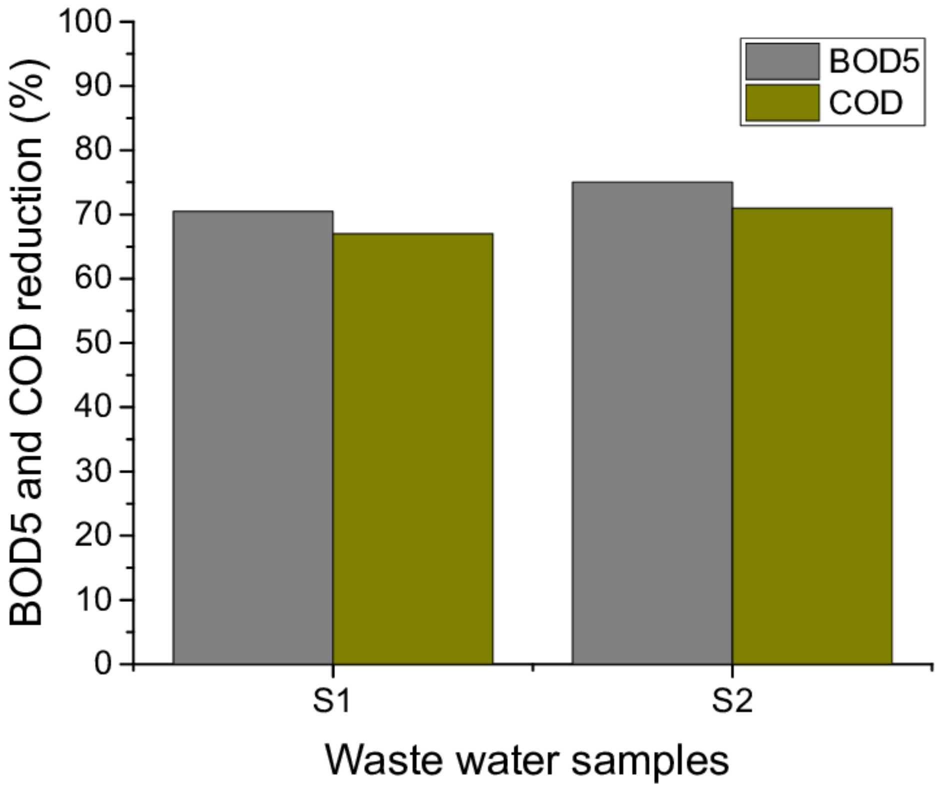

Fig. 3.7 COD and BOD_5 reductions (%) in the wastewater (S_1 is non-nutrient supplemented wastewater sample and S_2 is nutrient supplemented wastewater sample)

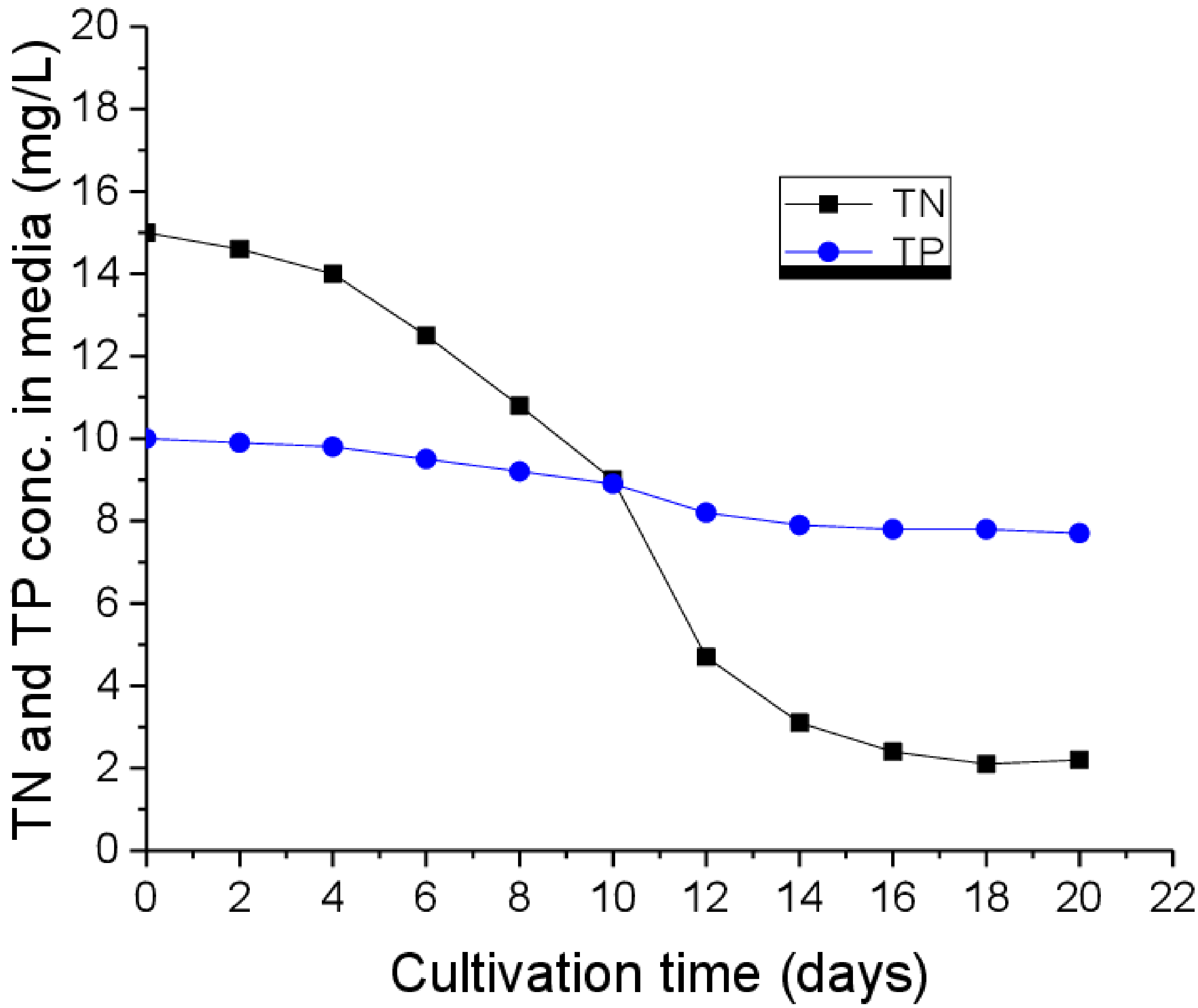

Fig. 3.8 TN and TP utilization (mg/L) in non-nutrient supplemented wastewater

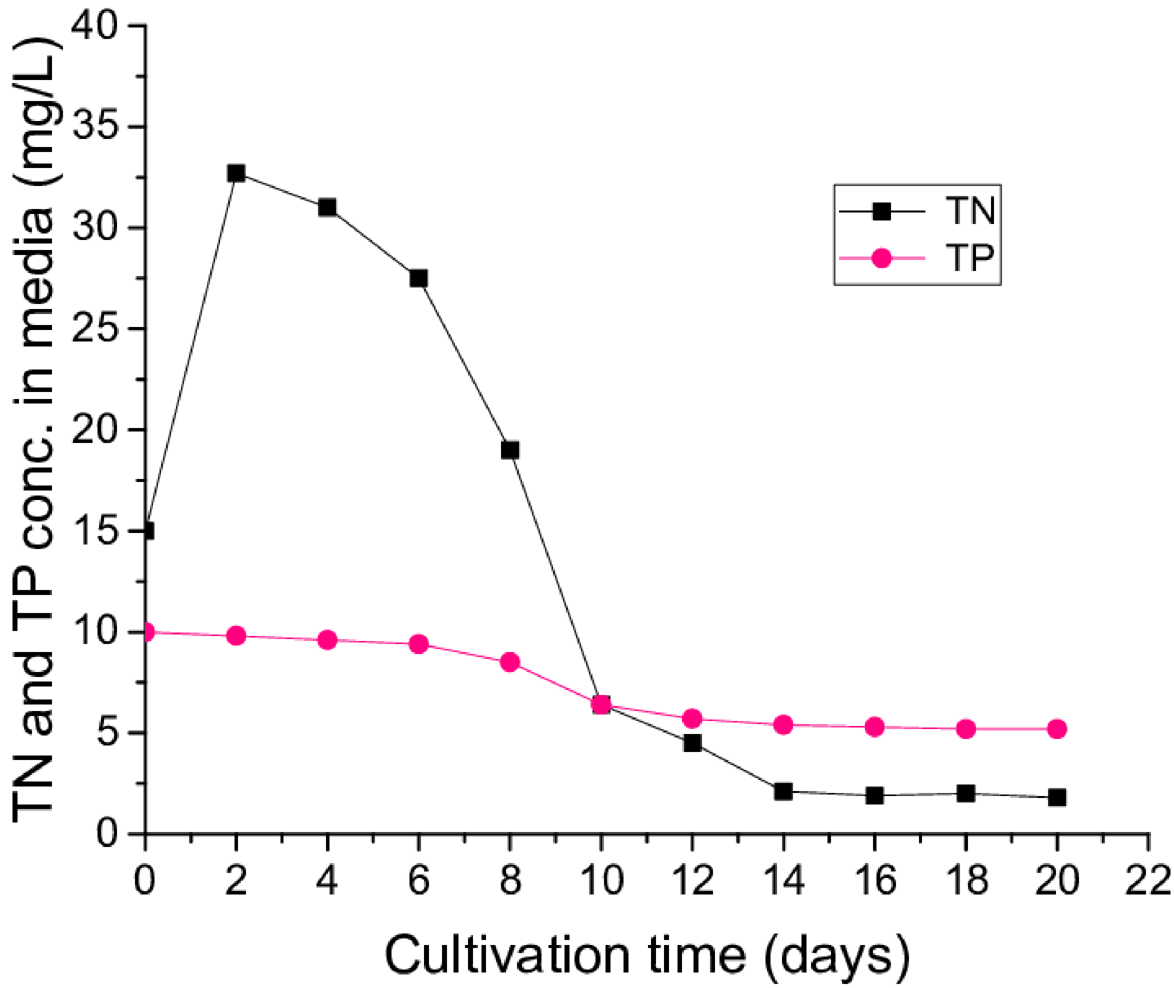

Fig. 3.9 TN and TP utilization (mg/L) in nutrient supplemented wastewater

3.3.2. Evaluation of the coupled process

The coupled process is shown in Fig.3.10. The wastewater from the sugar factory is treated in the primary wastewater treatment plant and then sent to the ponds where the microalgae are cultivated. The treated wastewater is used as a source of nutrients. The nitrogen deficit is supplied by the makeup nutrient. Photo bioreactor (PBR) is used to grow the selected algae strain before being transferred to the open pond so that the risk of contamination would be reduced. The CO_2 is supplied from the sugar factory; its amount is based on the nutrient supply. The main product from the process is the algal biomass from the harvesting section. The algae lost due to the inefficiency of the harvesting is recovered by filtration. As it is found from the harvesting model no recycle water is required, hence, the whole water from the filtration unit is blown down. The sludge from the primary water treatment can be used for a production biogas in a downstream process.

The product outputs were estimated using a spread sheet by material and energy balance approach while the wastewater after treatment in the primary treatment plant and the pond is compared with a standard in order to identify whether it meets the criteria or not.

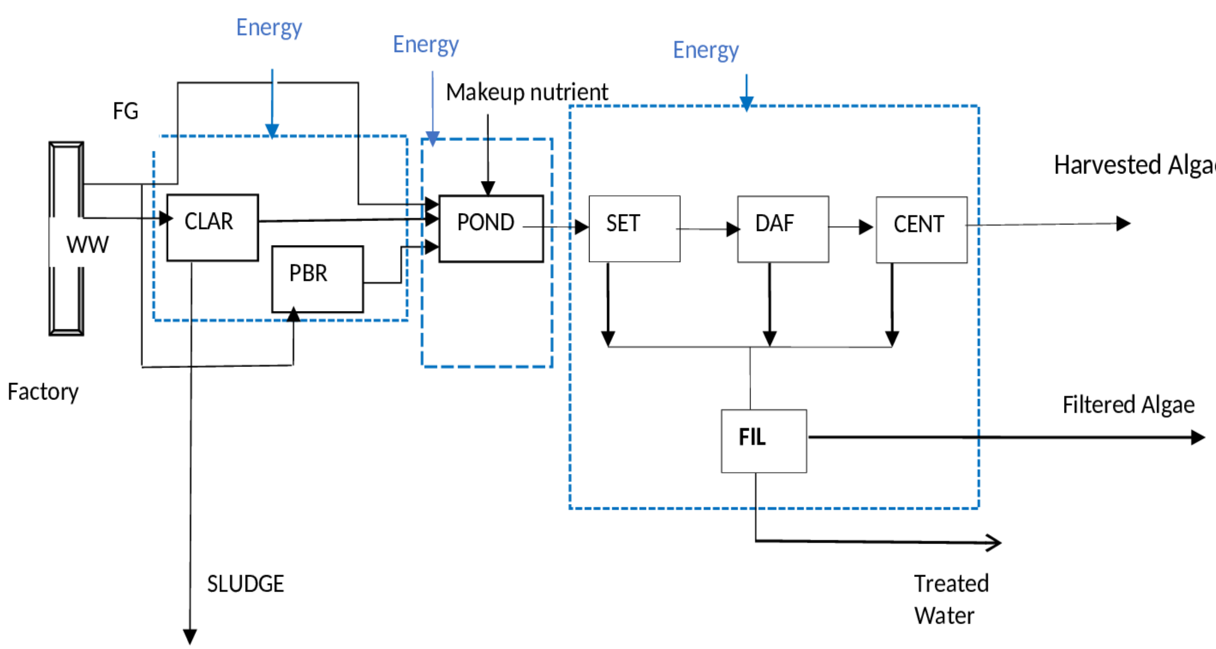

Fig. 3.10 Process flow diagram for the coupled process (WW- Wastewater, FG- Flue gas, CLAR- Clarification, PBR-photo-bioreactor, SET-settling, DAF-dissolved air flotation, CEN-Centrifugation, FIL-filtration, TW-treated water)

3.3.3. Microalgae cultivation in the pond

As presented in Section 3.2 and shown in Fig. 3.10 the Metahara sugar factory is coupled with conceptual microalgae cultivation. The wastewater from the sugar factory is assumed to be used as nitrogen and phosphorus sources, and the makeup nutrients would be used to avoid deficit. Based on underlying assumptions presented in Section 3.2 using spread sheet and material and energy balance approach the cultivation model gives the results as shown in Table 5 for the fundamental parameters including the nutrients flow, algae biomass output, algae concentration, and CO_2 requirement.

The N:P mass ratio in microalgae can vary from 4:1 to 40:1 (Broberg et al., 2011). For example in a modelling study, Lardon, 2009 has used N:P ratio of 4.54:1 as low nitrogen content (nitrogen starvation condition) for *C. vulgaris* (Lardon et al., 2009). It has been reported that cultivation under nitrogen starvation conditions leads to a marked increase in the oil/lipid content (70–85 % of dry weight), depending on the microalgae species utilized (Mandal & Mallick, 2009). On the other hand , nitrogen limitation to increase intracellular lipid content may have negative effects on the cell growth and lipid productivity on some microalgae strains (Hsieh & Wu, 2009). Commonly nitrogen to phosphorus ratio of 15-16:1 in microalgae composition (mass ratio of 6.8-7.25:1) is taken to be the appropriate composition for the proper growth of most of the microalgal species (Davis et al., 2011). As shown in Table 3.9 the cultivation model gave N:P mass ratio of 1.62:1 in the wastewater which is below the minimum ratio 4:1 and thus less than the optimum ratio. 6.8:1 is considered in this study. The nutrient and CO_2 inputs to the ponds with corresponding outputs, and CO_2 reduction by the algae in the ponds are shown in Fig. 3.11, Fig. 3.12 respectively. Likewise, Fig. 3.13 clarifies the biomass production if all the CO_2 from the factory would be used, and Fig. 3.14 displays the land requirement for the cultivation of the algae. The experimental result for this study proves that the nutrient supplemented wastewater gave better result for the biomass productivity and phosphorus uptake.

Therefore, it is required to use a makeup nitrogen in order to meet the optimum ratio, as it is shown in the cultivation model. A makeup nitrogen (N) of 7.91 tons/year is required. Either ammonia or nitrate can be used as nitrogen sources. Since ammonia is lost from the growth media due to volatilization (Michael A. Borowitzka, 2013), nitrate could be better used as a nitrogen source.

From the cultivation model, it is also estimated that the total CO_2 required for the microalgae cultivation is 231.3 tons/year. The flue gases either from the sugar mill or from ethanol plant can provide this CO_2. If the whole flue gas had been considered to be used for microalgae cultivation it would have a potential of producing 136850 tons/year (huge amount) but with a need of meeting the nutrient deficit not provided by the wastewater from the sugar factory. The net CO_2 reduced in the process would be equal to the amount required by the algae in the ponds, which is 231.3 tons/year.

The cultivation model result gives that the total pond area for the microalgae cultivation is 1.93 ha, which is only 0.02% of the total land required for sugarcane cultivation.

As could be observed from Table 3.10, in the wastewater treatment (WWT) model, the wastewater from the sugar factory after primary treatment meets the requirement by Ethiopian Environmental Authority standards with respect to all the determined parameters except TSS (EEAS, 2003) and could be discharged to the land. The wastewater after algae treatment meets the requirements for discharge in land for TN and TP. It does not meet for COD, BOD_5, TSS and oil and grease. This implies that the algae treatment should be employed after biological treatment step, where activated sludge oxidizes the organic matter. Since the treated water meet the requirement to discharge to land, it could be taken that the algae have potential for bioremediation along with biomass growth. Similar analysis on environmental mitigation by integration of an existing sugarcane processing factory with microalgal production in Brazil shows that the life cycle greenhouse gas emissions could be improved by 10% (Souza et al., 2015).

The result of the cultivation model on energy requirement shows (Table 3.11) that mixing is the most energy intensive unit operation followed by pumping for harvesting; mixing shares 49% of the total energy demand (Fig. 3.15). It has been reported that one important challenge in mixing is that it would require a substantial amount of energy input to improve mixing so as to avoid poor mixing (Chiaramonti et al., 2013). However there are improvements in the design of raceway ponds which minimizes energy consumption while achieving sufficient mixing to prevent sedimentation and dead zones (Hadiyanto et al., 2013; Huang et al., 2015).

Table 3.9 Total nutrients entering to the pond, algal biomass output, algae concentration, and CO_2 required

S.N	Parameters	Value	S.N	Parameters	Value
1.	TN flow rate to ponds from primary clarifier (tons/year)	3.13	11.	TP required if all the CO_2 would be used (tons/year)	1750
2.	TP flow rate to ponds from primary clarifier (tons/year)	1.93	12.	Total water flow to pond (m^3/year)	260500
3.	Make up N required (tons/year)	7.91	13.	Water loss from pond by evaporation (m^3/year)	1730
4.	Total CO_2 required (tons/year)	231.3	14.	Algae concentration in pond (g/L)	0.5

5.	Algal biomass output (tons/year)	120.5	15.	Land required for algae cultivation (ha)	1.93
6.	CO_2 emission reduction (tons/year)	231.3	16.	Percentage of land used for algae cultivation from the total land (%)	0.02
7.	CO_2 released from boiler (tons/year)	254350	17.	Total foot print area (ha)	2.3
8.	CO_2 released from fermenter (tons/year)	8400	18.	Number of ponds required	19
9.	Algae produced if all the CO_2 would be used (tons/year)	136850	19.	Surface area per pond (m^2)	1000
10.	TN required if all the CO_2 would be used (tons/year)	11910	20	Excess bagasse production (tons/year)	27125

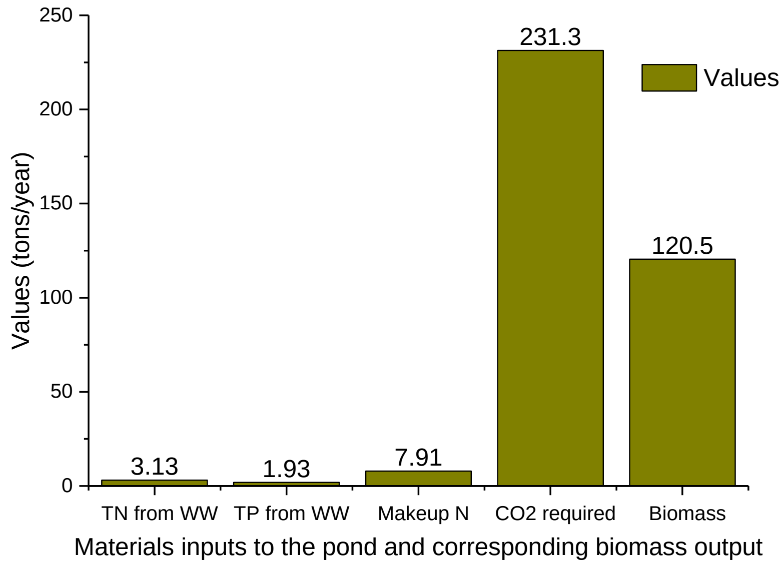

Fig. 3.11 Nutrient and CO_2 flow to pond and the corresponding biomass production

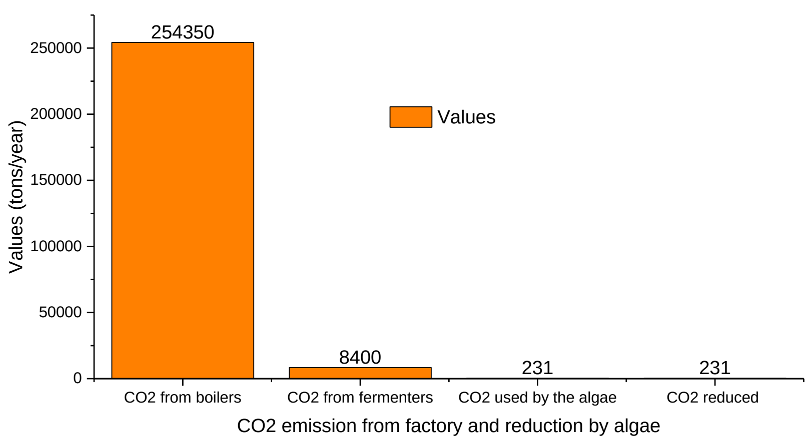

Fig. 3.12 CO_2 Reduction from the factory by the algae in the ponds

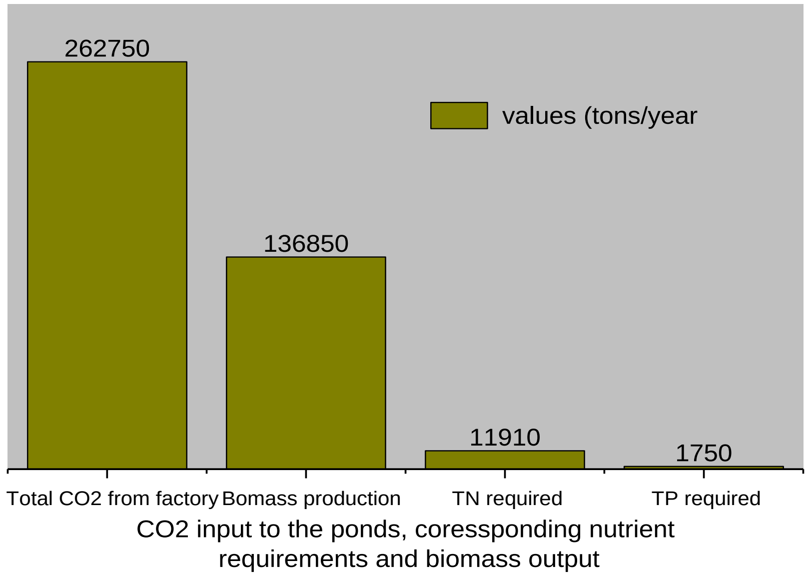

Fig. 3.13 Biomass production if all the CO_2 from the factory would be used

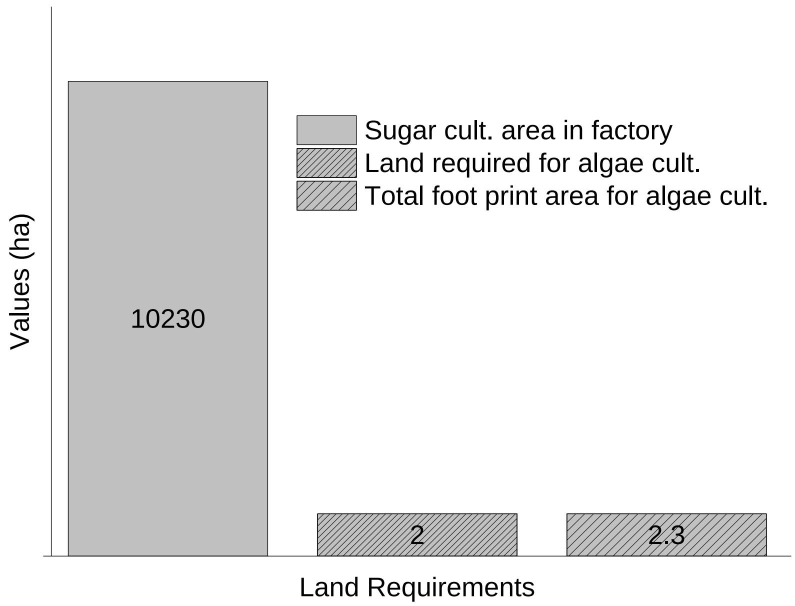

Fig. 3.14 Land requirement for the cultivation of the algae near the factory

Table 3.10 Wastewater (WW) after cultivation as compared to Ethiopian Environmental Authority standards (EEAS, 2003) emission limits of effluents

Parameters	WW after cultivation	EEAS to apply to water	EEAS to apply to land
COD (mg O_2/L_{ww})	447	250	2100
BOD_5 (mg O_2/L_{ww})	207	60	500
TN (mg/L_{ww})	0.6	15	nd*
TP (mg/L_{ww})	1.5	5	nd
TSS (mg/L_{ww})	<145	50	100
Oil &grease (mg/L_{ww})	<21	15	30
TDS (mg/L_{ww})	<78	3000	nd

*nd refers to 'no data'

Table 3.11 Energy requirements for the cultivation in the ponds

Process unit	Value
Paddle wheel/Mixing (MWh/year)	23.16
Pumping from clarifier to pond (MWh/year)	6.25

Pumping from pond to harvesting (MWh/year)	12.5
Pumping for recycling from harvesting to pond (MWh/year)	-
Flue gas injection (MWh/year)	5.13
Total (MWh/year)	**47.04**

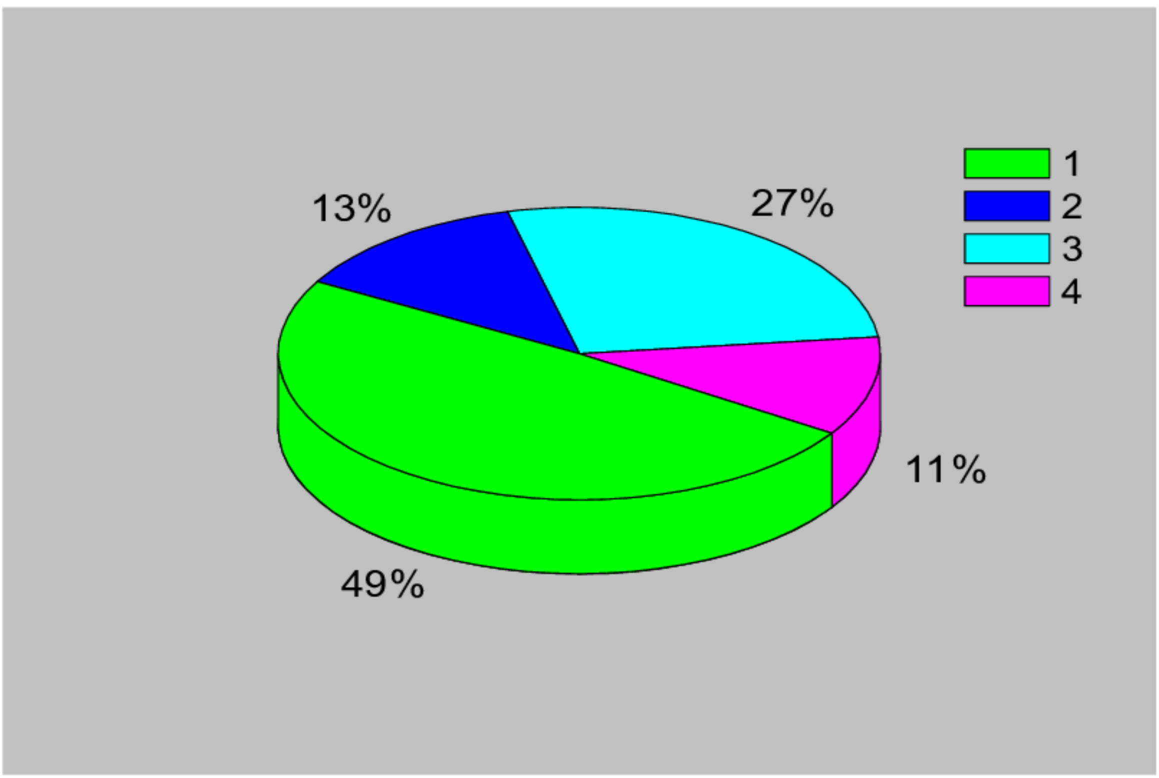

Fig. 3.15 Energy requirements for the algae production process expressed in % (1, 2, 3, and 4 refers to mixing, pumping to clarifier, pumping to harvesting, and flue gas injection)

3.3.4. Microalgae harvesting

It is assumed that the microalgae suspension from the pond would go to auto-flocculation and then it would successively pass through DAF and centrifugation units. In the DAF, it is assumed that biodegradable flocculant, chitosan, would be used to help flocculation. Also, after dewatering, the remaining microalgae in water is assumed to be completely removed using filter. Based on the underlying assumptions given in Table 3.8 the main results from modelling of the different harvesting process steps are given in Table 3.12.

With the biomass concentration of 250 g algae/L_{WW} (0.25g algae/g wastewater), dry content of microalgae in the centrifugation step amounted to 98.14 tons/year (Fig. 3.16). And the corresponding water content in the microalgae was 400 m^3/year. In the filtration unit, 22.61 tons/year of unrecovered algae due to inefficiencies are recovered. Fig. 3.17 illustrates the water flow to the ponds and its removal in the harvesting process, likewise Fig. 3.18 displays the corresponding water remained in the biomass. In the harvesting model the most energy intensive operation was found to be the centrifugation step; about 67% of the energy requirement by the harvesting process is required by the centrifugation section (Fig. 3.19). It has been recommended that harvesting cost can be substantially reduced using other approaches, such as flocculation (Vandamme et al., 2013).

Also it needs to consider that there is 15.5% (Table 3.2) excess bagasse of the total bagasse produced in the sugar factory, which is equal to 27125 tons/year, as shown in Table 3.9. This bagasse has an energy content of 7893 BTU/lb, implying that it would have a potential of 14611 MWh/year energy production. Thus the use of the excess bagasse for energy production in the process integration is significant.

Table 3.12 Outcomes from the harvesting section

Parameters	Harvesting		
	Bio-flocculation	DAF	Centrifugation
Biomass concentration after harvesting (g/L_{ww})	10	60	250
Dry content of algae after harvesting (tons/year)	114.48	103.03	98.14
Algae over flow (tons/year)	6	11.45	5.16
Total (tons/year)		22.61	
Filtered algae (tons/year)	6	11.45	5.16
Total filtered algae (tons/year)		22.61	
Total water required in the pond (m^3/year)		241000	
Total water feed to the pond from sugar factory (m^3/year)		260500	
Water loss by evaporation (m^3/year)		11730	

Water recycle to the pond		Not required	
Treated water (m^3/year)	229550	9720	1320
Total treated water (m^3/year)		240590	
Water in the microalgae (m^3/year)	11450	1720	400
Electricity demand (MWh/year)	-	0.972	2
Total electricity (MWh/year)		2.972	
Electricity demand for filtration (MWh/year)	0.06	0.115	0.056
Total electricity for filtration (MWh/year)		0.231	
Total electricity (MWh/year)		3.203	

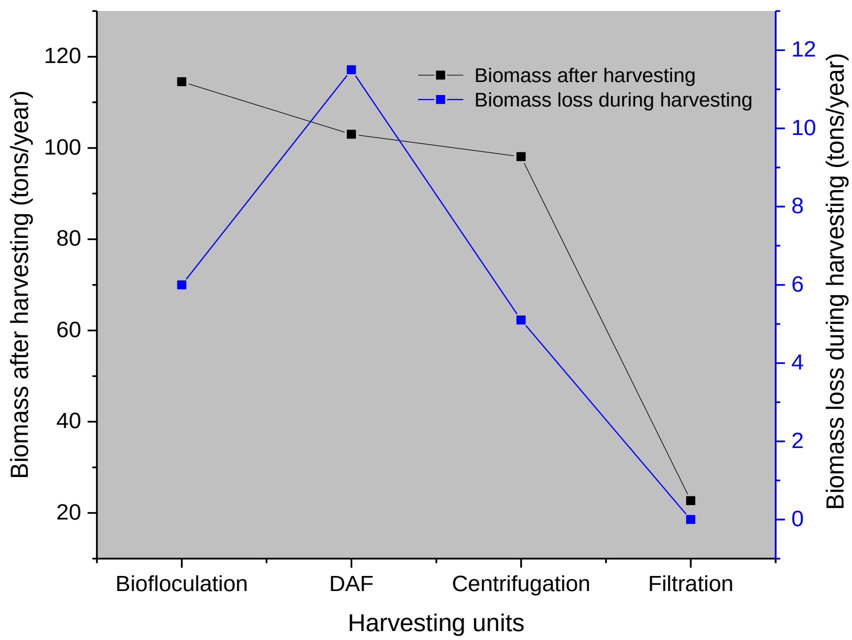

Fig. 3.16 Dry content of algae after harvesting with the corresponding loss

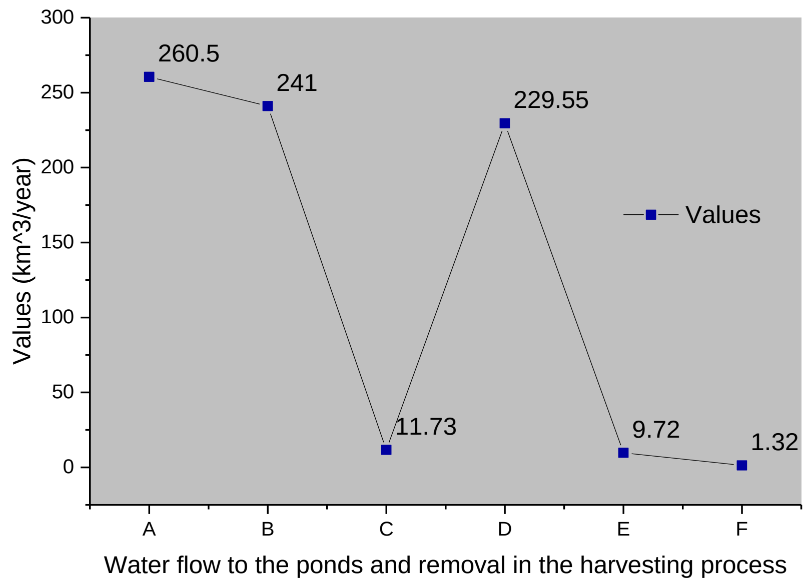

Fig. 3.17 Water removal in the harvesting process (A: total water entering to the ponds; B: total water requirement in the ponds; C: loss by evaporation from the ponds; D: removal in the flocculation; E: removal in the DAF; F: removal in the centrifugation)

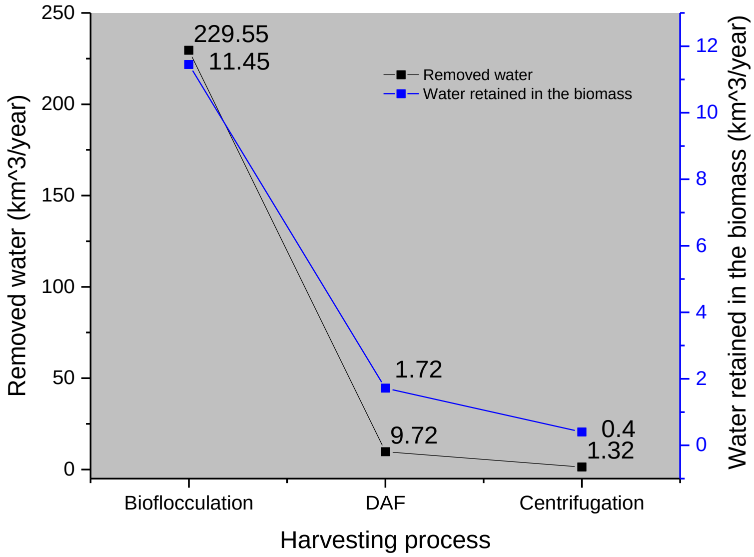

Fig. 3.18 Water removal in the harvesting process

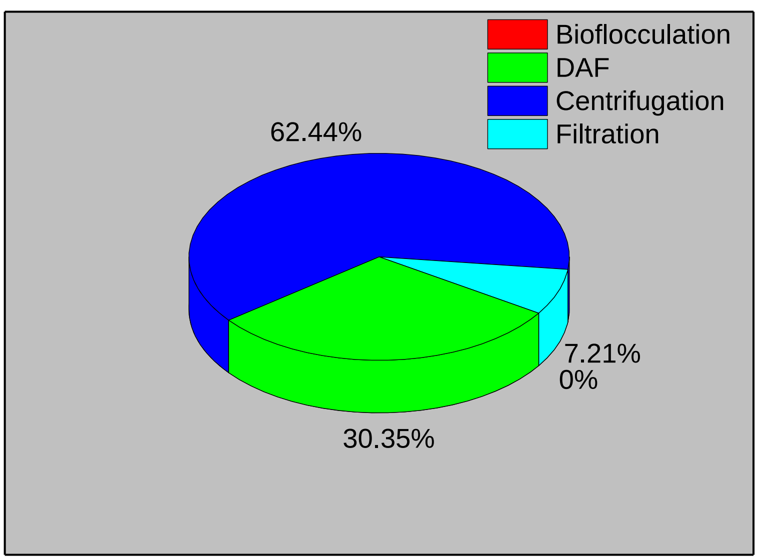

Fig. 3.19 Energy requirements for the harvesting

3.4. Conclusion

Wastewater effluents and flue gases from the sugarcane processing factory have a potential to produce algal biomass which can be used for biofuel production along with an advantage of pollution reduction, including CO_2 emission reduction and wastewater effluent reduction. It has been found that the nitrogen to phosphorus mass ratio in the wastewater is too low to be used sufficiently without supply of makeup nutrient. Consequently, in the coupling addition of appropriate nutrient to avoid nitrogen deficit would be required. The CO_2 in the flue gases from the factory has more potential to produce algal biomass than the nutrients in the wastewater effluents. Since there are several huge factories and ongoing projects for the production of sugar and ethanol in Ethiopia, microalgae cultivation by coupling with such factories would be important so as to both increase profitability and reduce environmental pollution.

The study in this chapter has investigated the importance of the process integration for both environmental foot print and cost reduction by using the inexpressive waste resources. It can also contribute a lot by opening the way for further integrated studies including improving of algal strain and finding energy and material efficient alternative processing technologies. This way the future possible bio-refinery and environmental pollution reduction approach could be well investigated to decide the provision of such activities.

CHAPTER FOUR

4. Microalgal Biofuel Production coupled with Sugar Cane Factories

4.1. Introduction

Due to the diverse characteristics regarding biodiversity and elasticity of microalgae along with their higher growth rate compared with terrestrial plants, the ability to grow on non-productive land and use of poor quality water, the ability to remove pollutants from wastewater and to sequester CO_2 from flue gases, etc., microalgae have been considered as a promising future biofuel feedstock (Bennion et al., 2015; Frank et al., 2013). There are several pathways for processing microalgae into biofuel: biodiesel production through transesterification of lipids (Chisti, 2008), bioethanol production through fermentation of the algal biomass, biogas production through anaerobic digestion, and bio-crude production through thermo-chemical conversion are among the alternatives processes (de Boer et al., 2012; Huang et al., 2010; López et al., Barreiro, 2013). Simultaneous production of biodiesel and biogas from microalgae has received interest as it enables the utilization of lipid-extracted algae for further processing and producing biogas, so that it could help to enable a maximum utilization of the algae biomass (Broberg et al., 2011; Lundquist et al., 2010)

Anaerobic digestion has also become a special focus in the utilization of microalgae for biofuel production particularly from the bio-refinery point of view. For a viable production of biofuel from microalgae, some challenges, such as managing a high energy and capital intensive harvesting/dewatering process (Grima et al., 2003), coping with the high amount of residues left after lipid extraction in the case of lipid-based biofuel production (microalgae biomass contains 30–40% lipid: and up to 70% of the residual biomass is left after the extraction process) (Pragya et al., 2013), and the need for fertilizers (Fenton & Ohuallacháin, 2012) need to be overcome. Anaerobic digestion can provide a pathway to avoid some of these problems by recovering nutrients from the extracted residual biomass and producing electricity from the methane biogas (Heaven et al., 2011).

The production of biofuel from microalgae however has not yet been realized in large-scale production. Major research gaps, such as reducing energy input, maximizing yield, and those related to an efficient material and energy usage, are waiting to be addressed. In microalgae cultivation, the nutrient supply has a significant impact on cost, sustainability, and production sitings (Farrell & Sarisky-Reed, 2010) whereas the major nutrients (nitrogen, and phosphorous) need primary focus.

It has been reported that the integration of microalgal biofuel production with industrial or power plants might help to increase the feasibility of the process (Broberg et al., 2011; Lundquist et al., 2010). The aim of this research is to conceptually couple microalgae cultivation with an existing Ethiopian sugar factory, which has an annexed ethanol factory, so that the wastewater and the flue gas from the factories are used as nutrient and CO_2 sources for the microalgae growth. The study explores a future possible microalgal cultivation integration approach with sugar and ethanol production factories by following a case study approach which uses the wastes and by-products as inexpensive CO_2 and nutrient sources for the growth of the algae. The primary goal was to produce biodiesel and biogas using this integrated process. Bio-fertilizer was is also considered as a by-product of the coupled process. The coupled process was evaluated with regard to product output, energy requirement and energy output. Likewise, the effect of several factors, such as oil content in the microalgae, and the nitrogen & phosphorus content in the wastes on the production of biodiesel were investigated.

4.2. Materials and methods

4.2.1. Process design and integration

The same factory as that of chapter 3, Metahara sugar factory, was selected. The key process parameters for the factory are shown in Table 3.2 of chapter three. The process design is based on the nitrogen and phosphorus contents in the wastewater effluent from the sugar mill (Table 3.1 of chapter three) and the vinasse from the annexed ethanol production plant of the factory (Table 4.1). Photoautotrophic cultivation of the microalgae in ponds was assumed where the wastewater and the recycled nutrients from an anaerobic digestion step could be used as nutrient sources and the flue gas from the factory as a CO_2 source. It was supposed that the amount of CO_2 required would be based on the amount of nutrients in the waste effluents (the wastewater and the vinasse). Hence, the nutrients should be considered as the limiting resources. Here apart from the study in chapter three it was proposed that the vinasse from the ethanol production plant would be used as an additional nutrient source after anaerobically digested along with other influent in the anaerobic digestion step. Furthermore the algal biomass produced in the ponds would further be processed to produce biofuels and bio-fertilizer. It was also considered that all the assumptions applied for each process step in chapter three; primary wastewater treatments, microalgae strain characteristics, cultivation and harvesting were also used here, as this study is the extension of chapter three.

Table 4.1 Assumed characteristics of molasses' vinasse from Metahara Ethanol production plant, Ethiopia (Moreira, 2002)

Parameter	Value

pH	4.1-5.0
Temperature (°C)	80-100
BOD (mg O_2/L)	25000
COD (mg O_2/L)	65000 (range 50000-150000)
Total solids (mg/L)	81500
Free solids (mg/L)	60000
Fixed solids (mg/L)	21500
Nitrogen (mg N/L)	1000 (450-1610)
Phosphorus (mg P_2O_5/L)	150 (100-290)
Potassium (mg K_2O/L)	3740-7830
Calcium (mg CaO/L)	450-5180
Magnesium (mg MgO/L)	420-1520
Sulphate (mg SO_4/L)	6400
Carbon (mg C/L)	11200-22900
C/N ratio (mass ratio)	16-16.27
Organic material (mg/L)	63400

Reducing substances (mg/L)	9500
Total vinasse flow rate (m^3/day)*	396

* Flow rate of the vinasse was estimated based on data from Table 3.2 of chapter three.

4.2.2. Biofuel production

There are different options for the production of a biofuel from algal biomass and the pathways are highlighted, as illustrated in Fig. 4.1. The criteria to choose the best pathway to utilize the biomass depends on several factors including material and energy efficiency, availability of infrastructures, CO_2 emissions and other environmental issues. In the present study, microalgae with oil content of 30% are regarded to be used as a base value (Lundquist et al., 2010) and this shows that only 30% of the total biomass will be utilized if, for example, the microalgae are only used for biodiesel production. To be more efficient in material utilization, other strategies enabling a utilization of the lipid-extracted algae (LEA) need to be employed. In this regard, biodiesel and biogas production has been found important to be both material and energy-efficient (which is highlighted in the figure in blue and orange colors) (Lundquist et al., 2010; Broberg et al., 2011). Hence, biomass is assumed to be utilized for biofuel production via a biodiesel-biogas production pathway.

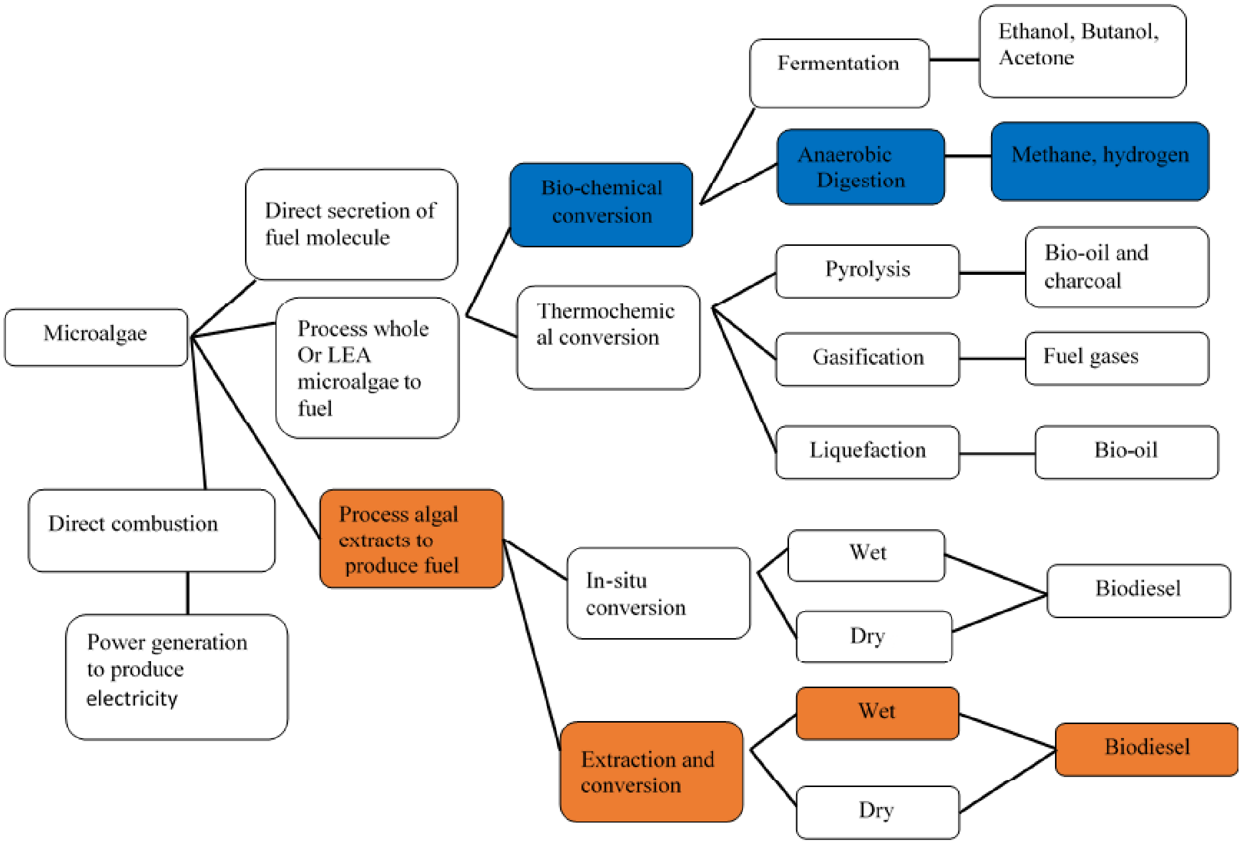

Fig. 4.1 Energy production from algae via different pathways adapted from (Wang et al., 2008; de Boer et al., 2012)

4.2.2.1. Biodiesel production

The biodiesel production should involve cell disruption, extraction and transesterification of the oil to biodiesel.

4.2.2.1.1. Cell disruption

In the cell disruption unit, the algal biomass needs to be treated using an appropriate technology to increase the recovery of intracellular products during wet extraction. For the present work, high-pressure homogenization was considered. This method was selected because it is a well-established technology both on laboratory and industrial scale, and has thus developed to one of the most commonly used methods (Prokop et al., 2015). The biomass with 25 wt.% from the harvesting processes should be treated for cell disruption and lysis using pressure homogenization before forwarding it to extraction (Davis et al., 2011). Energy consumption for pressure homogenization was assumed to be 0.20 kWh per kg of dry biomass and 90% efficiency, corresponding to a 25 wt.% input (Frank et al., 2011; ANL;NREL; PNNL;, 2012). It is thought that the undisrupted algae in the homogenizer flow through the extraction (with no lipid recovery) to the digester with the residues.

4.2.2.1.2. Lipid extraction

Lipid extraction from algae is mostly performed either from wet algal paste or dry algal cake, with or without cell disruption (Prokop et al., 2015). In the present study, lipid extraction from wet algal paste using the solvent extraction technique with pre-treatment or cell disruption is carried out. It is supposed that lipid extraction should be performed using ethanol. Ethanol should be used because of its polar nature enabling it to penetrate the polar cell membrane of the lipids so that more cell material could be made free and be extracted (Lohrey & Kochergin, 2012). Moreover, ethanol is low toxic and available in the factory (ethanol is produced from cane molasses in Metahara factory). In some other extraction studies, a ratio of solvent to dry biomass of 5:1 (w/w) was used, and the same ratio was assumed for the present study (ANL;NREL; PNNL;, 2012; Nappa et al., 2015). A lipid recovery of up to 97% was reported in the literature (Halim et al., 2011). However for the present study, an 80%-lipid recovery is considered as a base value. The lipid-rich solvent and the algae residue slurry are assumed to be separated through disk stack centrifugation (Davis et al., 2011). The algal residues should then be forwarded to the AD for the biogas production, while the algae oil-solvent solution should be forwarded to a stripping column where the ethanol would be separated from the oil and recycled, leaving a 99.50% pure lipid stream

(Lundquist et al., 2010; Broberg et al., 2011). The electricity requirement for the extraction step is assumed to be 0.28 kWh/kg per dry biomass (Frank et al., 2011; ANL;NREL; PNNL;, 2012) while a thermal energy of 1.30 kWh/kg per dry biomass was accounted (Delrue et al., 2012). Solvent loss in circulation and lipid loss in the stripper are thought to be 5.20 g ethanol/kg of oil and 5 wt.%, respectively (Frank et al., 2011; Nappa et al., 2015). Key assumptions for the extraction model are shown in Table 4.2.

Table 4.2 General assumptions for extraction unit

Parameters	Value	References
Boiling temperature of ethanol at 1 atm. (^oC)	78	
Electricity demand of extraction (kWh/kg-DWB)	0.28	(Frank et al., 2011)
Thermal energy demand of extraction (kWh/kg-DWB)	1.3	(Delrue et al., 2012)
Lipid loss in the stripper (wt. %)	5	(Broberg et al., 2011)
Ethanol loss (g/kg-oil)	5.2	Frank et al., 2011)
Lipid content of microalgae (wt. %)	30 (base value)	(Lundquist et al., 2010)

Ethanol required (kg-ethanol/kg–dry biomass)	5	(ANL;NREL; PNNL;, 2012; Nappa et al., 2015)
Un extracted biomass (wt. %)	10	(Shurtz, 2013)
Recovery of lipids (wt. %)	80	(Halim et al., 2011)

4.2.2.1.3. Transesterification

The extracted lipids would be transported and converted to biodiesel by transesterification using methanol in the presence of sodium hydroxide as a catalyst (1 wt. %) (Delrue et al., 2012). The methanol to fatty acid mass ratio in the reactor was assumed to be 1:10 (Delrue et al., 2012). An 80% (wt. %) conversion rate of oil to biodiesel was assumed in the reactor as a base value (Pokoo-Aikins et al., 2010; Zappi et al., 2003). There are some other studies which show that the free fatty acid content in the microalgae is very low, approximately 0.05% (Pokoo-Aikins et al., 2010). When taking this into account, a pretreatment step would not be necessary in the present study. The glycerol formed during transesterification was thought to be separated in a decanter with a purity of 85% glycerol and 15% methanol (wt.%) (Broberg et al., 2011), and would then be forwarded to the AD. It was assumed that 0.1 kg glycerol would be formed per kg of biodiesel (Pokoo-Aikins et al., 2010). The unreacted methanol was assumed to be recovered via distillation and recycled back to the reactor. The final purity of the fatty acid methyl ester (FAME) was regarded as 96.50% (wt. %). The contents of water, glycerol, and methanol in the FAME were expected to be 0.50, 0.24, and 0.20 wt.% respectively (Broberg et al., 2011; Lin et al., 2020).

Electrical and thermal energy requirements for the transesterification were expected to be 3.80 $\times 10^{-4}$ and 0.68 kWh per kg of converted oil, respectively (Delrue et al., 2012). The density and energy content of the biodiesel were accounted to be 900 (kg/m^3) and 42 (MJ/kg), respectively (Delrue et al., 2012). Key assumptions are shown in Table 4.3.

Table 4.3 General assumptions for transesterification process

parameter	Value	References
Methanol to fatty acid ratio (kg/kg)	1:10	(Delrue et al., 2012)
Concentration of catalyst (wt. %)	1	(Delrue et al., 2012)
Conversion rate of oil to biodiesel (wt. %)	80% (base value)	(Zappi et al., 2003; Pokoo-Aikins et al., 2010)
Amount of glycerol formed (kg glycerol per kg of biodiesel)	0.1	(Pokoo-Aikins et al., 2010)
Retention time in the decanter (h)	2	(Atadashi et al., 2011)

Final purity of FAME (Fatty Acid Methyl Ester) (wt. %)	96.5	(Atadashi et al., 2011)
Maximum allowed content of glycerol (wt. %)	0.24	(Atadashi et al., 2011)
Maximum allowed content of methanol (wt. %)	0.2	(Broberg et al., 2011; Atadashi et al., 2011)
Maximum allowed content of water (mg/kg)	500	(Atadash et al., 2011; Barreiro et al. 2013)
Electricity demand (kWh/kg-converted oil)	0.00038	(Delrue et al., 2012)
Thermal energy demand (kWh/kg-converted oil)	0.68	(Delrue et al., 2012)

Purity of glycerol sent to anaerobic digester (wt. %)	85	(Broberg et al., 2011)
Composition of crude glycerol (wt. %)	0% biodiesel, 15% methanol 85% glycerol	(Broberg et al., 2011)
Microalgae LHV (MJ/kg)	19	(Delrue et al., 2012)
Biodiesel LHV (MJ/kg)	42	(Delrue et al., 2012)
Biodiesel density (kg/m^3)	900	(Delrue et al., 2012)

4.2.2.1.4. Anaerobic digestion/Biogas production

In the biogas production model, it is assumed that the inflows to the AD derive from five process steps. These include the vinasse, a by-product in ethanol production; the primary sludge from the wastewater primary treatment stage; the algae residues (lipid extracted algae/LEA and the undisrupted algae) from the oil extraction step; the filtered algae in the harvesting section and crude glycerol, a by-product from the transesterification step in the biodiesel production.

The vinasse from the ethanol production factory is one of the components with a high mass flow rate (Table 4.1). Considering the molasses based distillery effluent, vinasse, as the main component in the anaerobic digestion, the following four reactor configurations were implemented on a commercial scale: a continuous stirred tank reactor (CSTR), an up-flow anaerobic sludge blanket (UASB) reactor, a fixed film/media digester (or Anaerobic filter – AF), and a thermophilic digester (Souza et al., 1992; Rajeshwari et al., 2000; Patil, 2013). The most successful configurations today are UASB and CSTR reactors (Patil, 2013; Rajeshwari et al., 2000). The UASB reactors are used for the treatment of a wide range of industrial wastewaters (from low to high strength wastewater) including vinasse (Souza et al., 1992; España-Gamboa et al., 2011; España-Gamboa et al., 2012). UASB reactors are being encouraged because of their several advantages including plain design, uncomplicated construction and maintenance, low construction and operating costs, low sludge production, robustness in terms of Chemical Oxygen Demand (COD) removal efficiency and wide applicability, less CO_2 emissions due to less energy requirement as well as quick biomass recovery (Daud et al., 2018).

In the present study, the vinasse is characterized by a high total solid and high COD content. Glycerol, high strength wastewater (with a high concentration of CODs), lipids and some fatty acids would be added along with the vinasse which are characterized by a high solid content. In a UASB reactor, the hydraulic retention and solids retention time are not the same and an uncoupling of the substrate from the hydraulic system is observed. Hence, operating substrates with a high total solid content in the UASB possibly damages the granular structure. As all those compounds are complex molecules they also might adversely affect the performance of the UASB reactor (Chong et al., 2012). Furthermore, the phenolic compounds in the vinasse might contribute to the color of the vinasse and make biodegradability to be difficult in the UASB (Aquino & Pires, 2016).

On the other hand, complex organic materials with high solid content can better be degraded by means of CSTR reactors. Using municipal organic waste, which is characterized by a high total solid content (171 kg/m^3) and a high COD (235 kg COD/m^3) as a substrate, allows a degradation of 68% COD to be achieved as was reported for a CSTR (Held et al., 2002). González et al. (González et al., 2017) recommended that co-digestion of vinasse with press mud using a CSTR reactor would be an excellent option for the treatment of streams of the alcohol sugar industry. Thus, a CSTR reactor was supposed to be used for the present study. In such a CSTR, a 65% COD removal and 0.29 m^3 per kg COD removed is expected for the vinasse. The solids concentration in the digester would be obtained from the total mass flow of solids transferred.

4.2.2.1.5. Inputs to the digester

In the AD, its operating conditions are shown in Table 4.4, the production of biogas was modeled based upon the volatile solids (VS), total solids (TS), methane yield per gram of volatile solids (g-VS), chemical oxygen demand (COD), and percent methane (CH_4) content in the biogas. The total amount of CH_4 produced in the AD was estimated using the CH_4 yield for each component transferred to the AD.

One of the inputs, which would directly go to the AD, was the algal residue (LEA and undisrupted algae in the disruption unit) from the extraction step. The solid concentration of algal biomass from the centrifugation step would be 25% as explained before. As the lipid content of the algae was considered to be 30% and from 24% of it would be extracted in the extraction unit, the solid concentration entering in the digester could be calculated using the mass flows of carbon, hydrogen, oxygen, nitrogen and phosphorous from the oil extraction (Broberg et al., 2011). By assuming that TN and TP would not be affected in the extraction step but the carbon, the mass of the LEA, was estimated by subtracting the total carbon extracted from the total algal biomass. It was assumed that the pretreatment step for the biomass, pressure homogenization, would help to increase the CH_4 yield by 20% in the AD (Broberg et al., 2011). The whole biomass and the LEA were characterized to contain 0.73 g-VS g-TS^{-1} and 0.63 g-VS g-TS^{-1}, respectively (Broberg et al., 2011). Likewise, a biogas yield of 0.43 L-CH_4 g per VS for the pretreated algae was supposed.

The primary sludge (Table 3.1) that contains grease, a carbon-containing component, which is removed during the primary wastewater treatment, was the second input to the AD. Broberg et al. (Broberg et al., 2011) in their modeling considered that such grease consists of oleic acid with the empirical formula $C_{18}H_{34}O_2$ and a density of 0.90 g/mL, and also a fatty acid found in sources of animals as well as vegetables. In this study, this assumption was applied. The amount of the grease could be estimated using a component concentration and the wastewater flow. It was assumed that the primary sludge contains 5.5% solid concentration (wt.%) (Davis et al., 2011; Lundquist et al., 2010), with a sludge flow rate of 32 m^3/day. For the given flow rate, an average density for water and grease (of 0.99 g/mL) was assumed. In this case, the amount of solid (grease) was obtained to be as high as 1750 kg/day, which corresponds to the amount of total solids, TS. Of the total solids, typically about 98.50% are volatile (VS content of oleic acid) (Luostarinen et al., 2009) and would be broken down in the AD (Lundquist et al., 2010). The methane yield for oleic acid was assumed to be 0.32 L-CH_4/g-VS (Agrawal et al., 1997).

The third input was the crude glycerol which was assumed to consist of 85% glycerol and 15% methanol (Broberg et al., 2011). The VS content in glycerol amounted to 0.85 g-VS/g-glycerol (Lin et al., 2020) and 99% of methanol was also assumed to be volatile (Park & Park, 2003). A methane yield of 0.43 L-CH_4/g-VS and 0.53 L-CH_4/g-VS were estimated for glycerol and methanol, respectively (Nakazawa et al., 2015). In anaerobic co-digestion of mixtures it is recommended that the amount of glycerol should not exceed 1% (v/v) (Fountoulakis et al., 2010), and thus this criteria was satisfied in the present study.

The last inflow to the digester would be the vinasse from the ethanol production factory. All the vinasse would go to the AD to be anaerobically digested together with the other inflows. The characteristics of all the inputs entering to the AD are shown in Table 4.5. It has been reported that methane production of up to 0.344 m^3/kg COD removed was possible (Elda et al., 2012). For the present study a methane yield of 0.29 m^3/kg COD removed was assumed.

The residue from the digester can be used for irrigation of either sugarcane or for the cultivation of microalgae in the pond. In this study, the supernatant was assumed to be recycled to the pond so that it would provide the microalgae with nutrients in addition to the wastewater from the sugar factory while the solid by-product would be used as a bio-fertilizer for the sugar cultivation. The COD, TN and TP reduction factors in the digester are shown in Table 4.6. The process energy per volume of CH_4 produced or nutrient recovered depends on the digestion time and digester size. Digesters for wastewater treatment (WWT) applications are typically designed for a 20-50 days solid retention time and 2-10 kg COD/m^3-day organic loading rate (OLR). For the present study, for the AD system, a power consumption of 0.22 kWh thermal/kg-TS and 0.09 kWh electrical/kg-TS with a solid retention time of 40 days corresponding to 6 kg COD/m^3-day OLR was presumed (Ehimen et al., 2011; Frank et al., 2011; ANL;NREL; PNNL;, 2012; Delrue et al., 2012). This assumption included the additional electric power, used by a disc-stack centrifuge, for concentrating solids from the digestate. Then the digestate was supposed to be dried and used as a fertilizer. The solids concentration in the digester would be obtained from the total mass flow of solids transferred. It was supposed that a biogas with a methane content of 84% and a balance CO_2 would be produced from the AD (España-Gamboa et al., 2012).

Table 4.4 Operating conditions for the reactor

Parameter	Value
Reaction temperature (ºC)	55 (thermophilic)
Volumetric loading rate (kg COD/m^3/d)	6 (5-7)
BOD removal efficiency (%)	87 (85-90)
COD removal efficiency (%)	65
Methane content in biogas (%)	84
H_2S content of biogas (%)	3.0 (2.0-4.0)
Electrical energy requirement (kWh/kg-TS)	0.085
Thermal energy requirement (kWh/kg-TS)	0.22

Table 4.5 Characteristics of the input to the AD

Parameter	Value	References
Volatile materials in the whole biomass (g-VS g- TS^{-1})	0.73	(Broberg et al. 2011)
Volatile materials LEA (g-VS g-TS^{-1})	0.63	
Methane yield for the pretreated algae (L-CH_4 g-VS^{-1})	0.43	(Yen & Brune, 2007; Ehimen et al., 2011)
Solid concentration of primary sludge (wt. %)	5.5	(Broberg et al., 2011; Davis et al., 2011)
Sludge flow rate of (m^3/day)	32	Calculated
VS content of oleic acid (%)	98.5	(Luostarinen et al., 2009)

Methane yield for oleic acid (L-CH4/g -VS)	0.32	(Agrawal et al., 1997)
Composition of crude glycerol (%)	85% glycerol, 15% methanol	(Broberg, 2011)
Methane yield of glycerol and methanol (L-CH_4/g-VS)	0.43 and 0.53 respectively	
Methane yield for the vinasse (m^3/kg COD)	0.29	(Elda et al., 2012)

Table 4.6 Reductions during anaerobic digestion of mixtures in the AD

Parameter	Reduction factor	References
BOD_5	0.6	
COD	0.65	(Elda et al., 2012)
Carbon		

Nitrogen	0.16
Phosphorus	0.21

4.2.2.1.6. Biogas upgrading

Biogas is commonly used to generate electricity and/or heat. Biogas can also be used as transportation fuel after purifying it into biomethane. Metahara sugary factory produces bioethanol to be used as transportation fuel by blending it with petro-diesel. Along with this bioethanol, in the present study, it is intended to deliver the biogas and the biodiesel, which would be produced in the coupled process, to the energy grid of the country and subsequently to be used as transportation fuel. The content of CH_4 in the gas needs to be greater than 95% (96% was assumed in the present study) for the gas to be used as a transportation fuel (Broberg et al., 2011). Thus the gas needs to be upgraded using an appropriate technology. Four types of technologies are commonly employed for the removal of CO_2, H_2S, and other impurities: membrane separation, adsorption, cryogenic distillation, and absorption. Absorption processes are suitable for large-scale processing units. Water scrubbing is common for biogas production. In this study, water scrubbing was used. The principle of a water scrubber is that CO_2 is highly soluble in water whereas CH_4 is not. The gas is fed at the bottom of the scrubber tower while the water enters the tower from the top, so that the CO_2 is dissolved in the water and the gas rich in CH_4 comes out from the top. In the reverse absorption (the stripper tower), the CO_2 desorbs from the CO_2 rich water. The CO_2 desorbs from the water as the solvent travels down the tower. It was assumed that 0.50% of the CH_4 would be lost during the upgrading process (Broberg et al., 2011). The CO_2 gas from desorption step can be used as a carbon source in the pond, depending on mass balance. It was presumed that the energy demand of the water scrubber has to be 0.17 kWh/m^3 biogas and the temperature in the scrubber process 20°C (Andersson et al., 2011). Considering that the energy density of CH_4 is 39.90 MJ/m^3 (11.20 kWh/m^3), the energy content of the biogas could be determined (Ehimen et al., 2011).

4.2.2.1.7. Nutrient recovery

It was thought that the concentration of TN and TP after digestion could be reduced by 16% and 21%, respectively (Frank et al., 2011). Of the inflows to the AD, only the vinasse and the algae residue were assumed to contain nitrogen and phosphorus and thus used to supply the pond. The output from the AD would be split into two fractions; the supernatant and the solid digestate, and it would then enter the pond to provide the microalgae with nitrogen and phosphorus, while the digester solid would be used as a bio-fertilizer after treatment. It was expected that 25% of the TN would reside in the sludge and 75% would reside in the supernatant, while the TP would be split 50/50 between the solid and liquid phases (Frank et al., 2011). It was also assumed that there would be 5% and 20% loss for TN and TP, respectively, as was also presumed before for the wastewater.

4.2.3. Stoichiometric and mass balance model for the cultivation step

A schematic of the cultivation step is shown in Fig. 4.2, and the following equations which help to calculate the unknowns are derived.

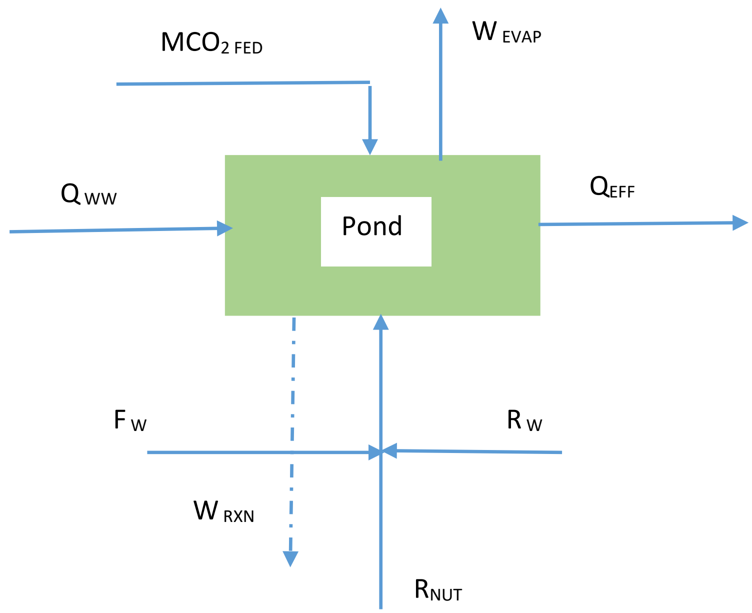

Fig. 4. 2 Schematic of the cultivation model

The total mass of the algae cultivated in the pond is assumed to go the harvesting step, thus it is equal to M_1. Then:

$$M_{N_{FED}} = X_N Q_{WW} + M_{N,R_{NUT}} \tag{4.1}$$

$$M_{P_{FED}} = X_P Q_{WW} + M_{P,R_{NUT}} \tag{4.2}$$

$$M_{N_{TOT}} = K_N N_{FED} \tag{4.3}$$

$$M_{P_{TOT}} = K_P P_{FED} \tag{4.5}$$

$$M_{CO_{2_{TOT}}} = K_{CO2} M_{CO_{2_{FED}}} \tag{4.6}$$

where:

$M_{N_{FED}}$ is total amount of nitrogen in the wastewater added to the cultivation step [kg/day]

$M_{N,R_{NUT}}$ is total amount of nitrogen in the recycle nutrient added to the cultivation step [kg/day]

$M_{P_{FED}}$ is total amount of phosphorus in the waste water added to the cultivation step [kg/day]

$M_{P,R_{NUT}}$ is total amount of phosphorus in the recycle nutrient added to the cultivation step [kg/day]

$M_{CO_{2FED}}$ is total amount of CO_2 in the flue gas added to the cultivation pond [kg/day]

$M_{N_{TOT}}$ is total amount of nitrogen to be converted to microalgae [kg/day]

$M_{P_{TOT}}$ is total amount of phosphorus to be converted to microalgae [kg/day]

$M_{CO_{2TOT}}$ is total amount of CO_2 to be converted to microalgae [kg/day]; and the other parameters used in the formulae, such as X_N, X_P, and K_N,etc., are as defined in Equations 3.3 to 3.8 of chapter three.

Based on the limiting nutrient, it may be either nitrogen of phosphorus, the mass flowrate of CO_2 can be calculated using the following equations:

when phosphorus is the limiting nutrient

$$M_{CO_{2FED}} = \left(\frac{M_{wt\,CO_2}}{M_{wt\,C}}\right)\left(\frac{\%C}{\%N}\right) M_{N_{FED}} \tag{4.7}$$

when nitrogen is the limiting nutrient:

$$M_{CO_{2FED}} = \left(\frac{M_{wt\,CO_2}}{M_{wt\,C}}\right)\left(\frac{\%C}{\%P}\right) M_{P_{FED}} \tag{4.8}$$

$$M_{Q_{EFF}} = K_N(M_{N_{TOT}})/\%N \tag{4.9}$$

OR

$$M_{Q_{EFF}} = K_P(M_{P_{TOT}})/\%P \tag{4.10}$$

$$A_{TOT} = M_{Q_{EFF}}/P_B \tag{4.11}$$

$$A_{TFF} = A_{TOT}/R \tag{4.12}$$

$$N_P = A_{TOT}/A_{SUR} \tag{4.13}$$

where:

$M_{Q_{EFF}}$ is total algal biomass production rate [kg/day]

P_B is productivity of algae in the ponds [kg/m^2.day] (Table 3.7)

A_{TOT} is total cultivation area required [m^2]

A_{TFF} is total facility foot print [m^2]

R is ratio of total area to total facility foot print [none] (Table 3.7)

A_{SUR} is surface area of a pond [m^2] (Table 3.7)

N_p is number of ponds [none]

Water balance in the cultivation step

It is assumed that the water in the recycled supernatant is insignificant, Q_{WW}, R_W, W_{M_1}, W_{EVAP}, are known and, W_{RXN} is determined from the stoichiometric model as:

$$W_{RXN} = \left(\frac{Mwt_{H2O}}{Mwt_{H2}}\right)\left(\frac{\%H}{\%N}\right)M_{N_{TOT}} \tag{4.14}$$

Then the requirement of fresh water is determined from the following relations:

$$F_W = (Q_{WW} + R_W) - (W_{M_1} + W_{EVAP} + W_{RXN}) \tag{4.15}$$

4.2.3.1. Energy Requirements for mixing paddle wheel, flue gas injection, and wastewater pumping

As mentioned in chapter three the energy requirements are determined by using Equations 3.21 to 3.24. The units of A_{TOT}, Q_{WW}, and Q_{EFF}, $CO_{2_{FED}}$ should be in ha, L/day, L/day, and kg/day respectively, and their values are known from our previous assumptions and calculations.

$$E_{PW} = C_{PW} A_{TOT} \tag{3.21}$$

$$E_{PP} = C_{PP} Q_{WW} \tag{3.22}$$

$$E_{PF} = C_{PF} Q_{EFF} \tag{3.23}$$

$$E_{FI} = C_{FI} CO_{2_{FED}} \tag{3.24}$$

Algal biomass balance in the harvesting step (Fig 4.3)

$$M_{Q_{EFF}} = M_{C_1} + M_{D_1} \tag{4.16}$$

$$M_{C_1} = M_{C_2} + M_{D_2} \tag{4.17}$$

$$M_{C_2} = M_{C_3} + M_{D_3} \tag{4.18}$$

where: M_{C_1}, M_{C_2}, M_{C_3}, M_{D_1}, M_{D_2}, and M_{D_3} are the amounts of biomass in the concentrate and dilute streams respectively [kg/day].

By assuming that all the microalgae is filtered in the filtration the amount of algae filtered is determined from the following relations:

$$M_{C_{FIL}} = M_{D_1} + M_{D_2} + M_{D_3} \tag{4.19}$$

Where: $M_{C_{FIL}}$ is the mass of the biomass filtered in the filtration unit.

Water balance around the cultivation model

The mass flowrate of water in each concentrate stream is calculated from the following relations

$$f_n = M_{C_n}/(M_{C_n} + W_{C_n}) \tag{4.20}$$

where:

f_n = mass fraction of microalgae in concentrate stream, C_n [kg/kg]; and

W_{C_n} = mass flow rate of water in concentrate stream [kg/day].

By rearranging Equation (4.20) can be written as:

$$W_{C_n} = M_{C_n} \frac{(1-f_n)}{f_n} \tag{4.21}$$

Then flow rate of water in the dilute stream can be calculated from the following relations:

$$W_{F_n} = W_{C_n} + W_{D_n} \tag{4.22}$$

Since the mass concentration of biomass in the effluent from the ponds is specified, W_{F_1} is known. Thus W_{D_1} can be calculated. It is also possible to calculate the flow rate of every concentrate and dilute stream by using the following equation:

$$W_{F_{n+1}} = W_{C_n} \tag{4.23}$$

Then the mass flowrates are converted to volumetric flowrates using the following relations:

$$Q_{D_n} = \frac{W_{D_n}}{\rho} \tag{4.24}$$

where:

Q_{D_n} = volumetric flowrate of dilute stream D_n [m^3/day]

ρ = is the density of the water [kg/m^3]

Energy requirements in the harvesting step

$$E_{DAF} = C_{DAF}Q_{wDAF} \tag{3.33}$$

$$E_{cent} = C_{cent}Q_{wcent} \tag{3.34}$$

$$E_{filt} = C_{filt}M_{algfilt} \tag{3.35}$$

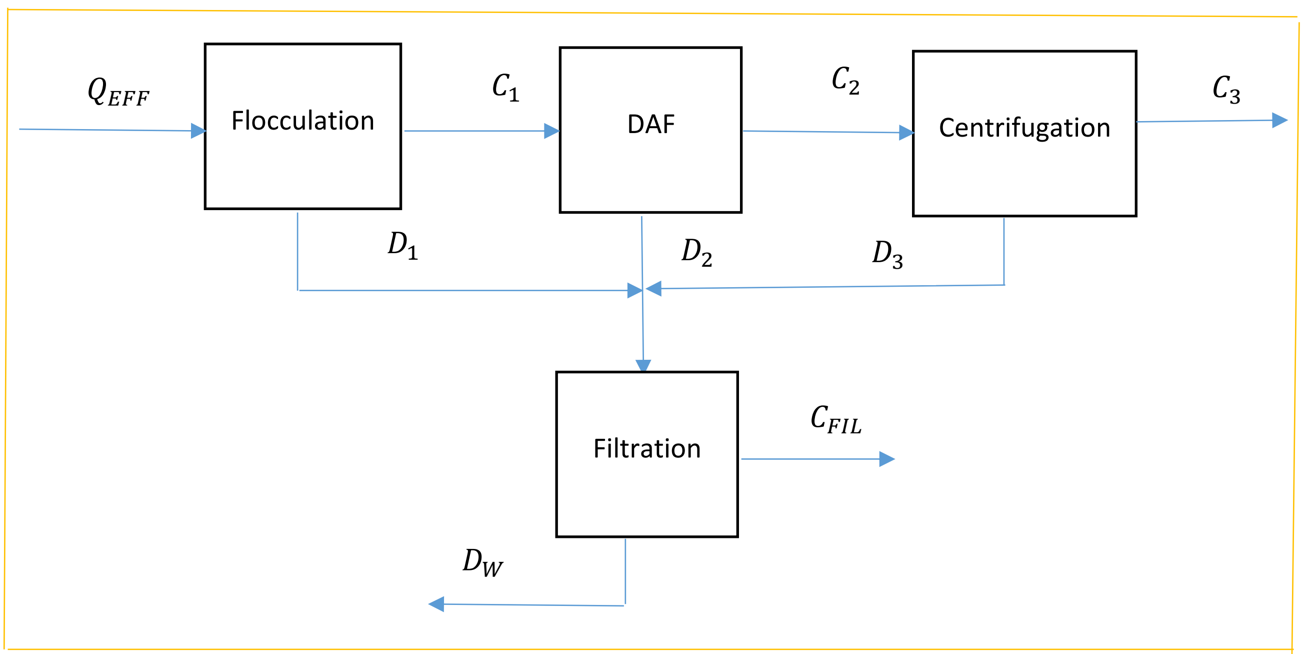

Fig. 4. 3 Schematic of the harvesting model

Mass balance in the extraction step (Fig 4.4)

Around the disruption unit

$$M_{C_3} = M_{F_{CD}} \tag{4.25}$$

$$M_{D,F_{CD}} = C_{CD}M_{F_{CD}} \tag{4.26}$$

where:

$M_{F_{CD}}$ is the algae biomass in the feed stream to the extraction unit [kg/day]

C_{CD} is the disruption factor [none] (Table 4.2)

$M_{D,F_{CD}}$ is the disrupted biomass in the feed stream to the extraction unit [kg/day]

Algal biomass balance around the extraction unit gives:

$$M_{F_{CD}} = M_{F_{PS}} \tag{4.27}$$

$$M_R = M_{F_{PS}} - M_{LIP} \tag{4.28}$$

$$M_{LEA} = M_R - M_L - (1 - C_{CD})M_{F_{CD}}) \tag{4.29}$$

$$M_L = C_{EXT}M_{D,F_{CD}} \tag{4.30}$$

where:

$M_{F_{PS}}$ is the biomass feed to the phase separation unit [kg/day]

M_R is the mass of biomass in the residue [kg/day]

M_{LEA} is the mass of lipid extracted algae in the residue [kg/day]

Because the ratio of solvent to dry algal biomass is known (Table 4.2), the mass of the solvent, M_S is known.

Energy requirements in the extraction step

$$E_{E,ext} = C_{E,ext}M_L \tag{4.31}$$

$$E_{TH,ext} = C_{TH,ext}M_L \tag{4.32}$$

where:

$E_{E,ext}$ is total electricity requirement for the extraction [kwh/day]

$C_{E,ext}$ is electricity requirement for the extraction [kwh/kg-oil] (See Table 4.2)

$E_{TH,ext}$ is total thermal energy requirements for the extraction [kwh/day]

$C_{TH,ext}$ is thermal energy requirements for the extraction [kwh/kg-oil]

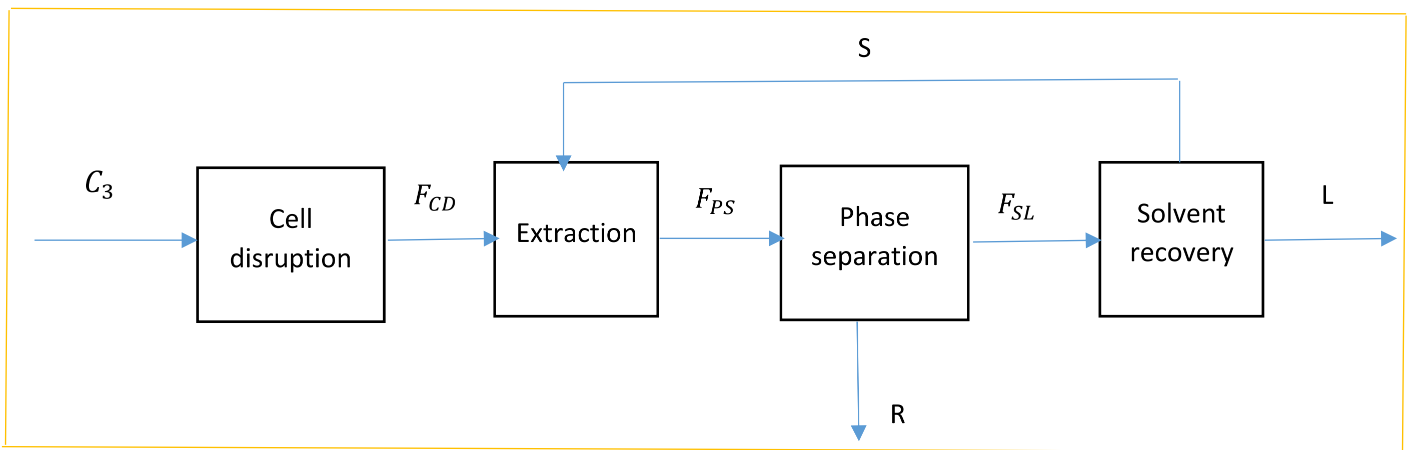

Fig. 4. 4 Schematic of the extraction model

Schematic of the transesterification model is shown in Fig 4.5. Because the transesterification efficiency, and the composition of glycerol is known **(**Table 4.3), the mass of the biodiesel and glycerol can be determined:

$$M_{BD} + M_{GLY} = C_{TR} M_{LIP} \tag{4.33}$$

$$R_{GB} = M_{GLY}/M_{BD} \tag{4.34}$$

where:

M_{BD} is the mass of the biodiesel [kg/day]

M_{GLY} is the mass of the glycerol [kg/day]

C_{TR} is the transesterification efficiency [none]

R_{GB} is the ratio of glycerol to biodiesel in the product mixture [kg/kg]

Energy requirements in the transesterification step

$$E_{E,tr} = C_{E,tr} M_{BD} \quad (4.35)$$

$$E_{TH,Tr} = C_{TH,tr} M_{BD} \quad (4.36)$$

where:

$E_{E,tr}$ is total electricity requirement for the transesterification [kwh/day]

$C_{E,tr}$ is electricity requirement for the transesterification [kwh/kg-biodiesel] (See Table 4.3)

$E_{TH,tr}$ is total thermal energy requirements for the transesterification [kwh/day]

$C_{TH,t}$ is thermal energy requirements for the extraction [kwh/kg-biodiesel]

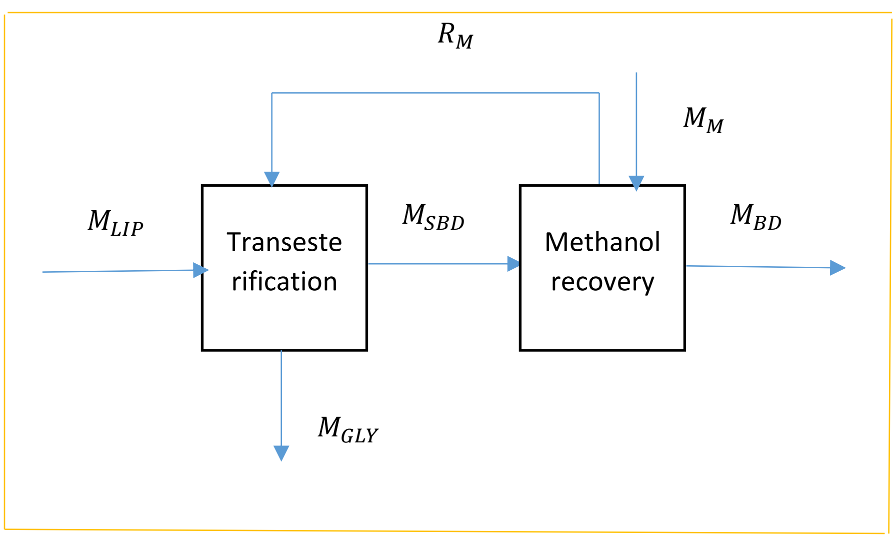

Fig. 4. 5 Schematic of the biodiesel production model

Calculation of the methane yield in the biogas production unit (Fig 4.6)

$$V_A = \upsilon_A Y_A (1 - C_{CD}) M_{F_{CD}} \tag{4.37}$$

$$V_{LEA} = \upsilon_{LEA} Y_{LEA} M_{LEA} \tag{4.38}$$

$$V_{GLY} = \upsilon_{GLY} Y_{GLY} M_{GLY} \tag{4.39}$$

$$V_{SLD} = \upsilon_{SLD} Y_{SLD} M_{SLD} \tag{4.40}$$

$$V_M = \upsilon_M Y_M M_M \tag{4.41}$$

$$V_{VIN} = \mathrm{r}_{COD} Y_{COD} M_{COD} \tag{4.42}$$

where:

V_A, V_{LEA}, V_{GLy}, etc., the methane production from the whole algae, the lipid extracted algae (LEA), the glycerol etc., [m^3-CH_4/day]

υ_A, υ_{LEA}, υ_{GLy}, etc., are volatilities of the whole algae, the lipid extracted algae (LEA), the glycerol etc., [kg-VS/Kg-TS]

Y_A, Y_{LEA}, Y_{GLY}, etc., are the methane yields of the volatile solids (VS) for the whole algae, the lipid extracted algae (LEA), the glycerol etc., [m^3-CH_4/Kg-VS]; and

M_A, M_{LEA}, M_{GLY}, etc., are the mass flow rates of the total solids (TS) for the whole algae, the lipid extracted algae (LEA), the glycerol etc., [kg/day].

Since the percentage of purity is known in the upgrading unit of the amount of upgraded biogas is determined. The production rate of bio-fertilizer, BF determined from the following equations:

$$M_{BF} = \%\mathrm{N}_{ris} (1 - r_{N,AD}) N_{TOT,AD} \tag{4.43}$$

Where

M_{BF} is the production rate of bio-fertilizer [kg/day]

$\%N_{ris}$ is the % of nitrogen in the residue from the AD after separation

$r_{N,AD}$ is the reduction rate of nitrogen in the AD [none]

$N_{TOT,AD}$ is the total nitrogen feed to the AD [kg/day]

Energy requirements for the biogas production step

$$E_{E,AD} = C_{E,AD} M_{TS} \tag{4.44}$$

$$E_{TH,AD} = C_{TH,AD} M_{TS} \tag{4.45}$$

$$E_{E,upg} = C_{E,upg} Q_{BG} \tag{4.46}$$

where:

$E_{E,AD}$ is total electricity requirement for the AD [kwh/day]

$C_{E,AD}$ is electricity requirement for the extraction [kwh/kg-TS] (See Table 4.2)

$E_{TH,AD}$ is total thermal energy requirements for the extraction [kwh/day]

$C_{TH,AD}$ is thermal energy requirements for the extraction [kwh/kg-TS]

$E_{E,upg}$ is total electricity requirement for the upgrading [kwh/day]

$C_{E,upg}$ is electricity requirement for the upgrading [kwh/m^3] (See Table 4.4)

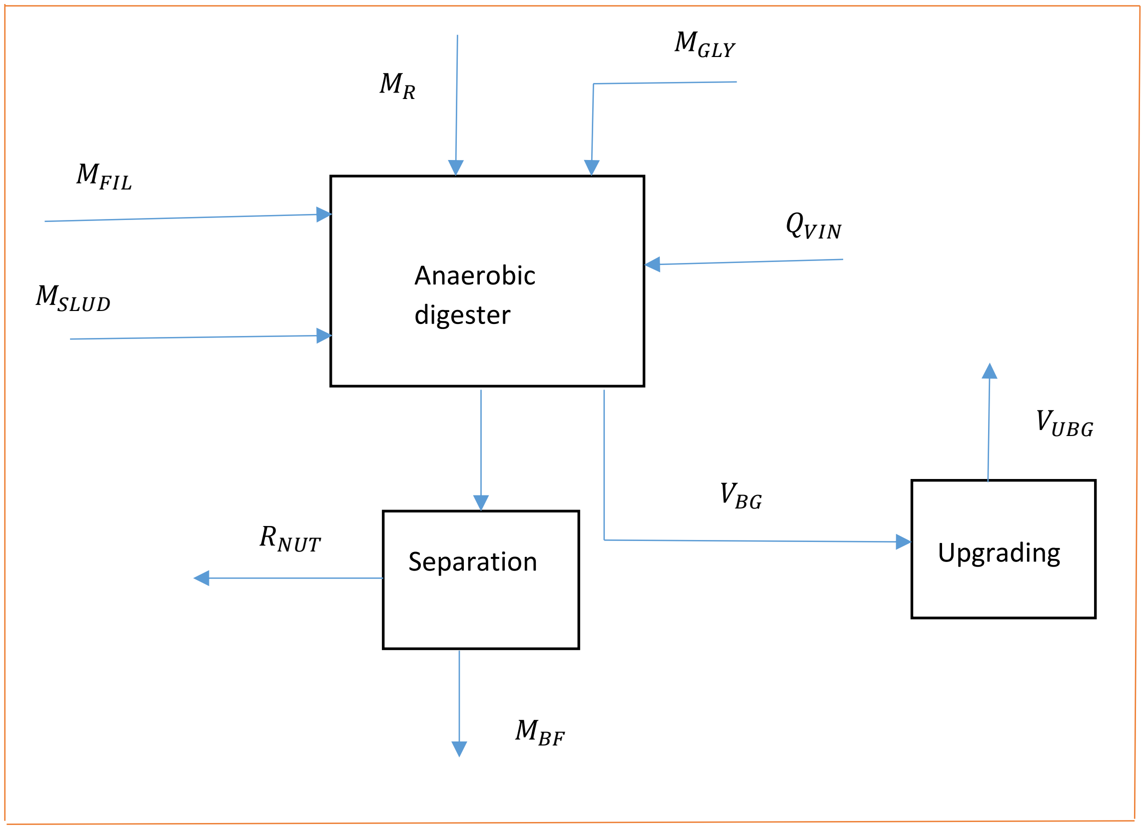

Fig. 4. 6 Schematic of the biogas production model

4.2.4. Product evaluation for the coupled process

The coupled process was evaluated with regard to product outputs, energy inputs and outputs using a spread sheet.

4.2.5. Sensitivity analysis

Sensitivity analysis is important to determine which parameters potentially affect the response variable (Davis et al., 2011). In the present study, four parameters viz. oil content of the microalgae, the nitrogen content in the vinasse from the ethanol factory, the extraction efficiency, and the transesterification efficiency of the crude oil to biodiesel were selected, and it was studied how their change in value affects the biodiesel yield in the integrated process. Low, base, and high values were assigned for each parameter and the sensitivity of the biodiesel yield to the change of the parameters from the base value was investigated.

The oil content of microalgae may vary depending on the type of the microalgae strain and its cultivation conditions (Mandal & Mallick, 2009). The oil content in microalgae can reach 80% and even more while a 20-50% oil content is common (Chisti, 2008), which indicates that the microalgae have great potential to be a future renewable biofuel resource. As mentioned in Section 4.2.2, for the present study a realistic value of 30% was considered as a base value. For the sensitivity study an oil content of 20% and 40% was used as the low and high value, respectively.

The biomass production in the ponds depends of their nutrient supply and the biomass production in turn affects the biodiesel production. Nitrogen is an essential constituent of all structural and functional proteins in algal cells accounting for about 7–10 % of the cell dry weight (DCW) (Richmond & Hu, 2013). Likewise, the sensitivity of the biodiesel to a change in the nitrogen content of vinasse, the main nitrogen source, was studied. From the mass balance equations, the anaerobically digested vinasse can provide the microalgae with 99 tons/year of nitrogen. Base values of 70 and 120 tons/year were used as low and high values, respectively.

The extraction efficiency of the oil from the microalgae is also an important factor, which affects the production of biodiesel. Depending on the type of the solvent used and the microalgae species, a recovery of > 95% of the lipids is possible. An extraction efficiency of up to 98% was reported in the literature (Halim et al., 2011). For the present study, 60, 80, and 97% extraction efficiencies were assumed as low, base and high values, respectively, for the sensitivity analysis.

The biodiesel production is also directly proportional to the transesterification efficiency of the crude oil. For the sensitivity analysis of the present study, a 70, 80, and 90% transesterification efficiency of the oil in the reactor was considered (Zappi et al., 2003; Pokoo-Aikins et al., 2010).

4.3. Results and discussions

4.3.1. Process design and integration

The integrated process is shown in Fig. 4.7. The wastewater (after primary treatment) and the flue gas from the sugar factory are used as nutrients and CO_2 sources for the growth of the microalgae in the ponds. The selected microalgae are grown in the PBRs before they have been transferred to the ponds. The algae biomass cultivated in the ponds is harvested in a series of harvesting units (settling, DAF and centrifugation). The unrecovered biomass formed due to the inefficiencies of the harvesting units is recovered using the filtration unit. The algal biomass from the last harvesting unit, the centrifugation, is transferred to the oil extraction unit after a pre-treatment in the cell disruption unit. The oil produced in the extraction unit is transferred to the transesterification unit where the biodiesel is produced. The glycerol, the by-product in the transesterification unit; the LEA and the undisrupted algae from the extraction unit; the filtered algae from the filtration unit; the sludge from the primary treatment plant; and the vinasse, the byproduct, from the ethanol production plant, are digested in the AD to produce the biogas. The bottom product from the AD is separated into two products; the supernatant, and the bottom product using centrifugation. The supernatant is recycled and fed to the ponds where it is used as a source of nutrient along with the wastewater from the sugar factory. The bottom product is used for the production of the bio-fertilizer. The biodiesel (BD), biogas (BG), and bio-fertilizer (BF) are the three main products of the integrated process.

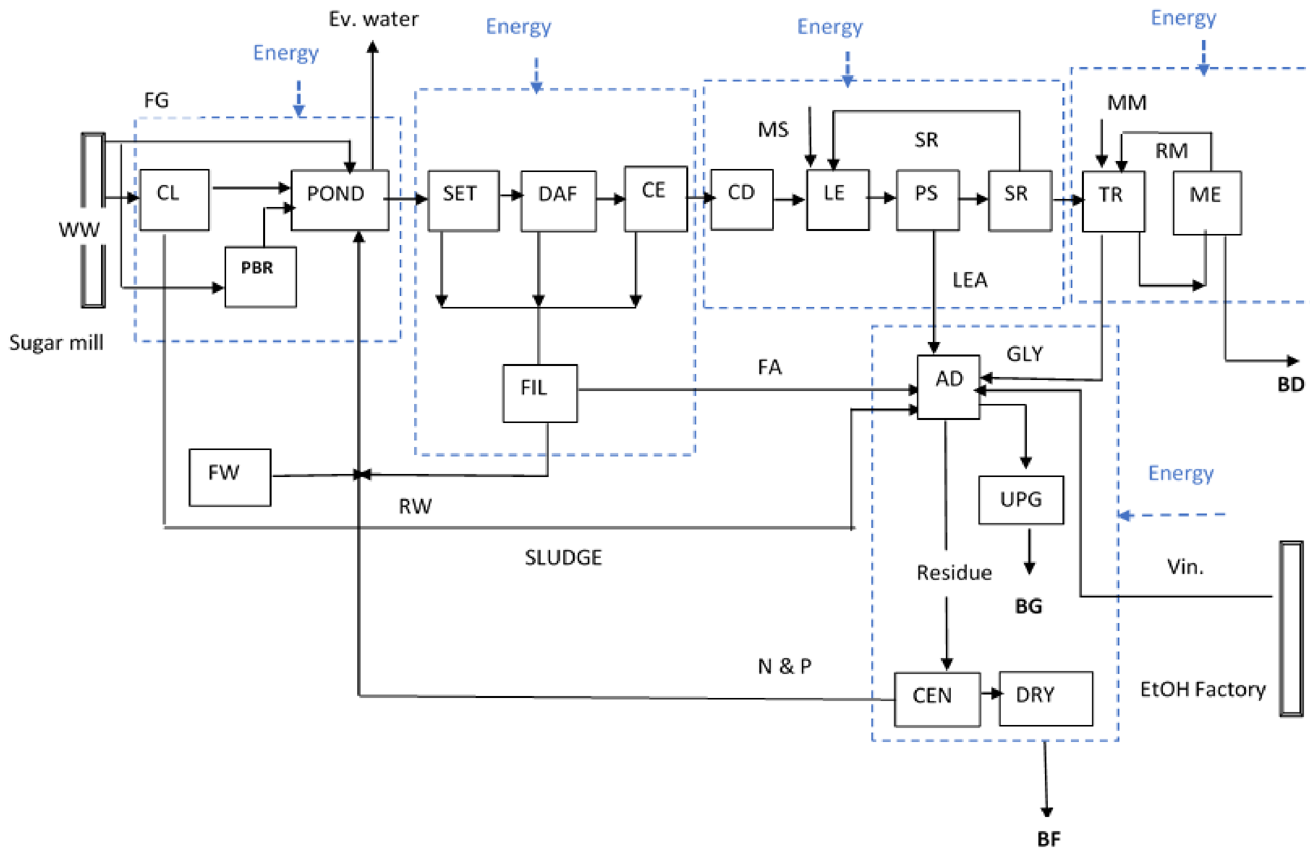

Fig. 4. 7 Process flow diagram for the integrated process (*WW-* ***Waste water,*** *FG-* ***Flue gas,*** *Ev.W-* ***Evaporated water***, *CL-* ***Clarification,*** *PBR-****photo-bioreactor,*** *SET-****settling,*** *DAF-****dissolved air floatation,*** *CEN-* ***Centrifugation,*** *CD-* ***cell disruption,*** *LE-****lipid extraction,*** *LEA-lipid extracted algae, PS-****phase separation,*** *SR-****solvent recovery/recycle,*** *TR-****transesterification,*** *ME-****methanol evaporation,*** *RM-****recycle methanol,*** *MM-* ***makeup-methanol,*** *MS-* ***make up solvent,*** *FIL-****filtration,*** *FA-****filtered algae,*** *FW-****fresh water,*** *RW-****recycle water,*** *AD-****anaerobic digester,*** *GLY-****glycerol,*** *Vin-* ***vinasse*** *UPG-****upgrading,*** *BD-****biodiesel,*** *BG-****biogas,*** *N-****nitrogen,*** *P-****phosphorus,*** *DRY-* ***drying,*** *BF-* ***bio-fertilizer,*** *EtOH-****Ethanol,*** *Sug. Fac-* ***sugar factory)***

4.3.2. Cultivation and harvesting of the microalgae

The results from the cultivation and harvesting models are shown in Table 4.7. The wastewater effluent from the sugar factory would be reduced in the primary treatment plant. This operation could help to treat the wastewater before going to the ponds; the solids, the COD and the BOD are reduced in the wastewater and this possibly could increase the photosynthesis efficiency of the algae in the ponds which in turn increases the algal biomass production in the ponds.

The total algal biomass production in the ponds was found to be 1412 tons/year. As per the assumptions considered in the present study, this biomass would be obtained by using photoautotrophic cultivation in an open system, by assuming a real value of 25 g/m^2/day for open pond productivity. Literature review reveals that due to their higher surface-to-volume (S/V) ratio, PBRs can help to achieve higher volumetric productivities and cell concentrations (Stephenson et al., 2010; Lee, 2012). Hence, if PBRs have been assumed for the cultivation of algae instead of using open ponds, the productivity would have increased and more biomass could have been obtained. In addition to this, closed systems are preferred to open ponds because the contamination of algae is reduced (Singh et al., 2015). However, due to their low investment and maintenance costs, which also results in lower production costs (Ugwu et al., 2008; Pires et al., 2012), open pond systems are the most used systems in microalgae cultivation. These ponds can be constructed on the degraded lands (Chisti, 2008) without competing with fertile land used for the cultivation of the sugar cane in the case of the present study. These advantages along with their simple design, scalability and low energy input, makes the open systems suitable to be possibly implemented for the realization of the proposed idea. Due to the inefficiencies of the harvesting operations, 265.10 tons/year of algal biomass remains unrecovered and overflown with the water. The filtration unit would be used to solve this problem. The algal concentration in the pond is low (0.50 g/L).

Harvesting such dilute microalgal suspension to achieve the final concentration of 250 g/L is highly energy and capital intensive. In another study, it has been reported that harvesting accounts for 20%-30% of the overall production costs of microalgal biofuels (Rawat et al., 2013). The lack of energy-efficient and cost-effective harvesting methodologies has been considered as the major problem for the economic production of algal biofuels (Cooney et al., 2009). In this regard, auto flocculation using gravity can decrease the energy consumption in the next harvesting operations. Taking this into account in the present study, the DAF and the centrifugation steps would be preceded by an auto flocculation step. In this auto flocculation step, the separation would be carried out by gravity; and no energy is required. As it is presented in Table 4.7, 95% of the water in the algal broth would be removed during the auto flocculation step; which would decrease the energy consumption tremendously in the next harvesting units. Thus it is considered that flocculation is an important step to decrease the biofuel production cost.

The N:P ratio in the wastewater was found to be 1.62 (Table 3.9 of chapter 3). Literature review shows that for microalgae grown by utilizing all the nitrogen and phosphorus, the N:P ratio should be greater than 4:1 and less than 40:1 (Lardon et al., 2009; Andersson et al., 2011). In this regard, the primarily treated sugar factory wastewater is considered as nitrogen deficient. This deficiency can be compensated by makeup nutrients such as nitrates and ammonia. To avoid the use of such a makeup nutrient it was assumed that the supernatant, the top product obtained by separating the sludge from the anaerobic digester into top and bottom product, would be recycled to the ponds and used as a source of nutrients. If no recycling is assumed it would require to supply 7.91 ton/year of nitrogen as a makeup nutrient based on the amount of the total phosphorus contained in the wastewater. In this way, it would be possible to produce 121 ton/year of biomass in the ponds. However, as can be seen from Table 4.7 it was possible to produce a substantially increased (1412 ton/year) algal biomass by recycling of the nutrients recovered in the anaerobic digestion unit without the use of any makeup nutrient. The increased biomass production in turn could increase the oil and biofuel production capacity (Table 4.8 - Table 4.10). This shows that the usage of all the wastes in the process, in a zero waste approach, could possibly increase the feasibility of the integrated process. Likewise, the results of the cultivation and the harvesting model demonstrate that, when there is recycling, there will also be an increase of the cultivation area from 2 ha to 23 ha (Fig. 3.15), and an increase in energy requirements in parallel with the increase of biomass production. The two cases, when sugar mill is used as the only nutrient source with the makeup nitrogen, chapter 3, and when there is nutrient recovery from the AD, chapter 4, are shown in Fig. 4.8 and Fig 4.9. Equally the energy requirements by percentage for each process step of the whole process is shown in Fig. 4.10.

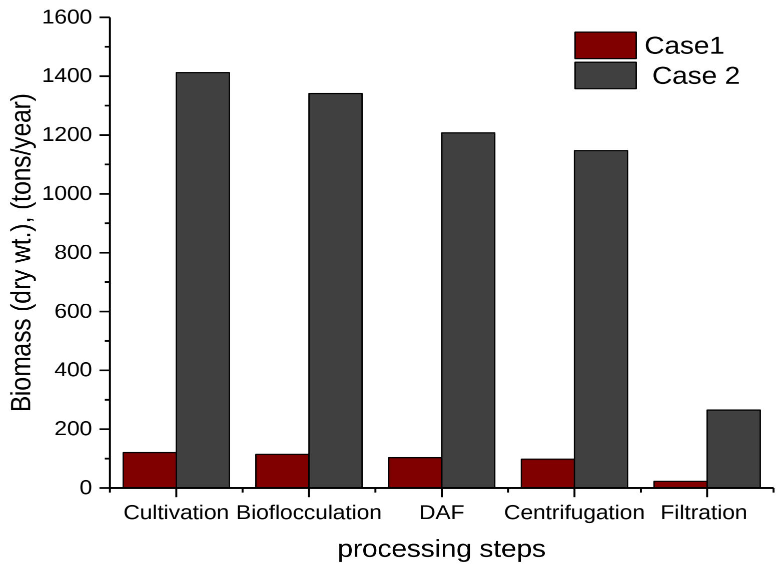

Fig. 4. 8 Dry content of algae in the cultivation (ponds) and after harvesting (Case1: when sugar mill WW is used as the only nutrient source with makeup nutrient, chapter 3, and Case 2: when there is nutrient recovery from the AD, chapter 4)

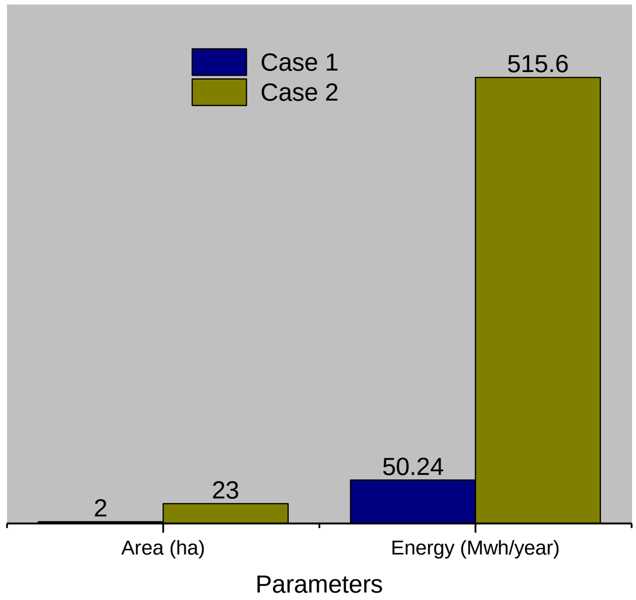

Fig. 4. 9 Comparison of Chapter 3, Case 1, and chapter 4, Case 2 for area and energy requirements

(Note: energy requirement for cultivation and harvesting processes is considered for both cases)

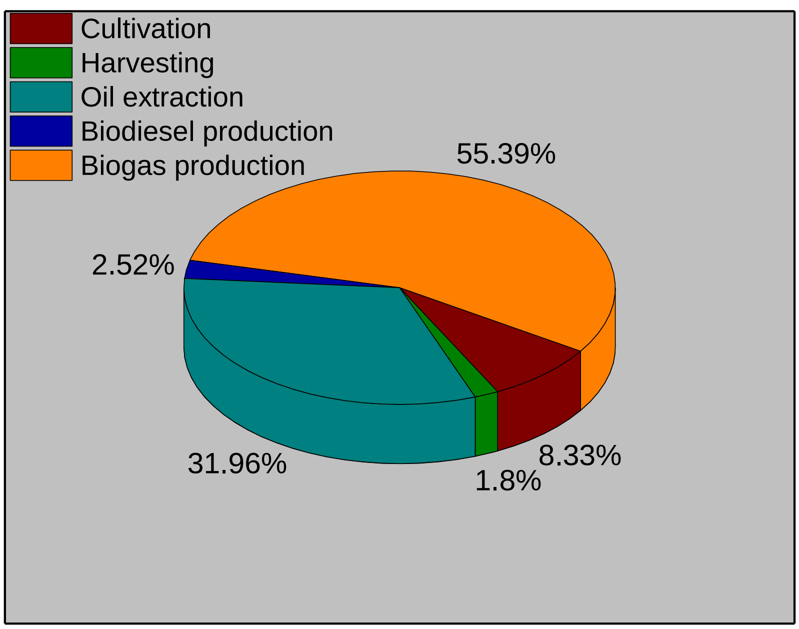

Fig. 4. 10 Energy requirements of each process step by %

Table 4. 7 Outputs from the cultivation and harvesting models

Parameters	Cultivation	Harvesting		
	Pond	Bio-flocculation	DAF	Centrifugation
Biomass concentration (g/L)	0.50	10	60	250
Dry content of algae (tons/year)	1412	1,341.40	1,207.30	1,146.90
Area for cultivation land (ha)	~23	-	-	-
Algae over flow (tons/year)	-	70.60	134.10	60.40
Filtered algae (tons/year)	-	70.60	134.10	60.40
Total water required in the pond (m^3/year)	2,824,000	-	-	-
Total water feed to the ponds from the	260,500	-	-	-

sugar factory (m^3/year)				
water loss from pond by evaporation (m^3/year)	390,841.60	-	-	-
Treated water (m^3/year)	-	2,689,860	114,019	15,533
Water recycle to the pond (m^3/year)	2,819,412	-	-	-
Additional fresh water to pond (m^3/year)	134,930	-	-	-
water with the microalgae (m^3/year)	2,824,000	134,140	20,121	4587.60
Electricity demand (MWh/year)	423.90	-	11.40	77.70
Electricity demand for filtration (MWh/year)	-	0.70	1.30	0.60

4.3.3. Oil extraction

Based on the assumption considered in Section 4.2.2.1.2, the main results from the extraction model are given in Table 4.8. The model shows that the total recovered extracted oil entering the esterification reactor was found to be 235.36 tons/year with a thermal energy and electricity demand of 1341.95 and 284.91 MWh/year, respectively. A high thermal energy demand was required as wet extraction was assumed. In wet extraction, the presence of water could be a problem because it can either promote the formation of emulsions in the presence of ruptured cells or participate in side reactions when present in the bulk solution. At the cellular level, intracellular water can be a barrier between the solvent and the solute. Increasing the temperature and the pressure can reduce the problem but at the expense of a high energy input. To reduce the temperature and pressure requirements during extraction, cell disruption can be applied (Lee, 2012). Thus in the modeling, the pressure homogenization step was assumed to help reduce the high temperature and pressure demand. As it is evident in Table 4.8, the cell disruption and extraction steps shares 35% of the total energy demand in the whole integrated process; this accounts the larger energy share next to the biogas production section which accounts for 53% of the total energy in the process.

Table 4. 8 Outputs from the extraction model

Parameter	Value
Disrupted algae flow to extraction step (tons/year)	1032.27
Undisrupted flow to extraction step (tons/year)	114.70

Total recovered extracted oil entering to esterification reactor (tons/year)	247.75
Lipid lost (tons/year)	12.39
Oil going to transesterification (tons/year)	235.36
Ethanol required (tons/year)	5161.35
Make-up flow of ethanol (tons/year)	1.29
LEA to AD (tons/year)	784.53
Undisrupted algae to AD (tons/year)	114.70
Electricity demand for homogenizer (cell disruption) (MWh/year)	206.45
Electricity demand for extraction (MWh/year)	284.91
Thermal energy for extraction (MWh/year)	1341.95

4.3.4. Biodiesel production/Transesterification

Based on the assumption shown in Section 4.2.2.1, the main results from the transesterification model are given in Table 4.9. The model result indicates that it is possible to produce 188.29 tons/year of biodiesel from 1412 tons/year of algal biomass produced in the ponds. The thermal energy demand for the biodiesel production was found to be 128.04 MWh/year, which is much lower than the thermal energy (1341.95 MWh/year) required in the extraction step. The energy content of the biodiesel was estimated to be 2197.34 MWh/year. The energy required for the biomass conversion to biodiesel is the total sum of the energy required in the cell disruption, cell extraction and transesterification sections, which sums up to 1954.40 tons/year. A negative energy balance has been considered as the major bottleneck in the microalgae biomass extraction/conversion process (Hirano et al., 1998). As it was estimated in the present study, the energy content of the biodiesel (output energy) was greater than the input energy implying that the extraction/conversion process resulted in a positive energy balance. This might be attributed to both the homogenization step assumed to help reduce the high temperature and pressure demand, and the high extraction and transesterification efficiencies assumed in the process.

Table 4. 9 Outputs from the esterification model

Parameter	Value
Lipid flow (tons/year)	235.36
Methanol flow (tons/year)	23.54
Make-up flow methanol (tons/year)	3.71
Catalyst used (tons/year)	1.88
Biodiesel output (tons/year)	188.29
Energy of biodiesel (MWh/year)	2197.34

Purity of biodiesel (wt. %)	97% of 0.24% glycerol, 0.2% methanol, 0.0005% water, and the balance of other impurities
Glycerol output (tons/year)	18.83
Glycerol lost (tons/year)	0.05
Glycerol going to AD (tons/year)	18.78
Glycerol purity (wt. %)	85% glycerol, 15% methanol
Electricity requirement (MWh/year)	0.07
Thermal energy requirement (MWh/year)	128.04

4.3.5. Biogas production

In the biogas production model it was assumed that five different flows of substrates would be transferred into the anaerobic digester; primary sludge from the primary wastewater treatment, algae residues from the oil extraction, unrecovered algae from the harvesting section, glycerol from the transesterification step and vinasse from the ethanol factory. It was also assumed that the biogas from the AD would need to be upgraded so as to meet the requirements for transportation fuel.

Based on the assumptions shown in Section 4.2.2.1, the main outputs from the biogas production and upgrading model are presented in Table 4.10, Table 4.11 and Table 4.12. The results in Table 9 demonstrate that for 250 working days in a year, a total methane yield of 1905414 m^3/year (1974882 m^3/year upgraded biogas) would be obtained. The total energy requirement for the biogas production and the upgrading units operations was estimated to be 2796.55 MWh/year. The total liquid influent to the AD is 432.52 m^3/day (which is the sum of vinasse, 396 m^3/day; sludge, 32 m^3/day; algae residue, 4.5 m^3/day; and crude glycerol, 0.019 m^3/day).

Total nitrogen content of the bio-fertilizer was estimated using the assumption that the digestate solid would contain 25% of the nitrogen contained in the solid digested algae residue and digested vinasse. Thus it was found that the bio-fertilizer obtained by drying the digestate solid would contain 42.06 ton TN/year. Table 4.11 and Table 4.12 show the output and input energies in the biogas production section.

Assuming 65% COD reduction in the AD the total COD reduction would be 421.6 ton/year. Thus for COD loading rate of 6 kg COD/day-m^3 and HRT of 40 days the total volume of the digester could be calculated. This value was estimated by assuming that the COD content of the primary sludge was 5.3 tons/year of which 65% was supposed to be reduced in the digester.

As it is shown in Table 4.11 and Table 4.12 the output and input energies in the biogas production section were 22118.68 and 2796.55 Mwh/year respectively resulting a positive energy balance (Fig. 4.11). This might possibly be due to the use of the vinasse from the ethanol factory to produce a huge amount of biogas.

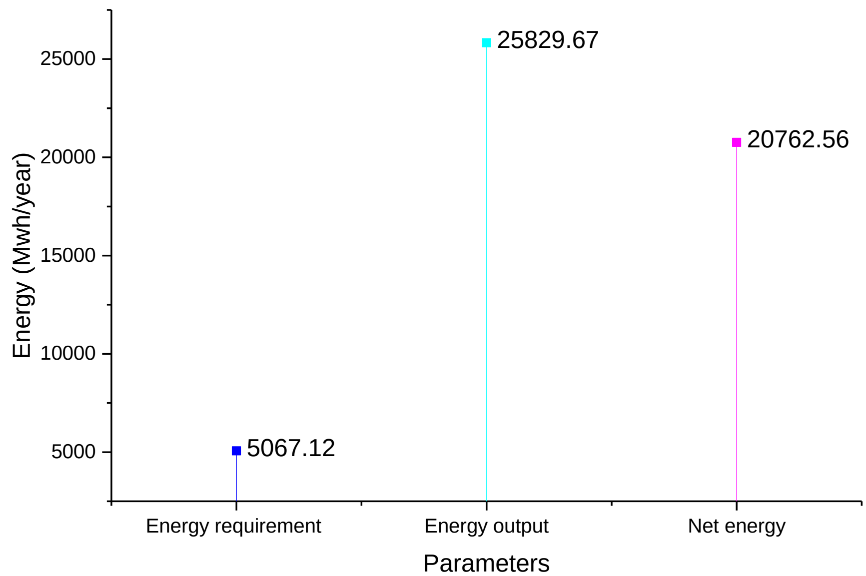

Fig. 4. 11 Energy requirements and outputs for the whole process

Table 4. 10 Mass flow into the AD and methane output

Inputs	TS (tons/year) or COD reduced (tons-O_2-year^{-1})	VS to TS ratio (Kg-VS-kg-TS^{-1})	Methane yield (m^3-CH_4-kg-VS^{-1}) OR (m^3-CH4/kg-COD utilized)	Total methane yield (km^3 -year^{-1})
LEA	784.53	0.63	0.43	212.53
Un extracted algae	114.70	0.73	0.43	36.00

Primary sludge	437.5	0.985	1.01	435.25
Glycerol	18.83	0.85	0.43	6.88
Methanol	3.32	0.99	0.53	1.74
Vinasse	4,182.8	-	0.344	1,438.88
Total				2131.28

Table 4. 11 Outputs from the water scrubber

Parameter	Value
Upgraded biogas (m^3/year)	1974882
CO_2 outflow/removed (m^3/year)	270420
Energy content of produced upgraded biogas (MWh/year)[d]	22118.68

[d] Value is calculated for energy content of methane =11.2 kWh/m^3

Table 4. 12 Energy demand for biogas production and upgrading

Process step	**Value**

Electricity demand of anaerobic digester for mixing (MWh/year)	685.82
Thermal energy of anaerobic digester for heating (MWh/year)	1775
Electricity demand for water scrubber (MWh/year)	335.73
Total (MWh/year)	2796.55

4.3.6. Sensitivity analysis

How the biodiesel production responds to the change of oil content of the algae, extraction efficiency, transesterification efficiency and nitrogen content of the vinasse was studied and shown in Figure 4.12.

Figure 4.12 shows that the change in value of the biodiesel production from 188 tons/year, the value obtained at the base values of the parameters considered in the present study. The result shows that the production of the biodiesel was most sensitive with change in the oil content of the algae. Its value was reduced from 188 to 135 tons/year (by 28%) when the oil content in the microalgae lowers from 30% (base value) to 20%. While it was increased by 25% when the oil content in the microalgae raised to a value of 40%. The result also shows the biodiesel production was reduced by 28, 25, and 20% when the values of the nitrogen content in the vinasse, the esterification efficiency, and the extraction efficiency were reduced by 29, 25, and 25 % from their base values respectively. On the other hand the biodiesel production was increased by 20, 23 and 16% when the nitrogen content in the vinasse, the esterification efficiency, and the extraction efficiency were increased by 21, 23, and 21% from their base values respectively. Generally the results show that all the investigated parameters are important and need to be considered in the production of biodiesel from microalgae. The nitrogen content in the vinasse depends on the composition of the vinasse which in turn may depend on several factors including the ethanol production process. The oil content of microalgae can possibly be improved by applying algal strain modification strategy. The extraction efficiency could be improved by selecting a solvent with higher extraction efficiency and optimizing the operating parameters while the esterification efficiency could be improved by decreasing the impurities in the crude oil.

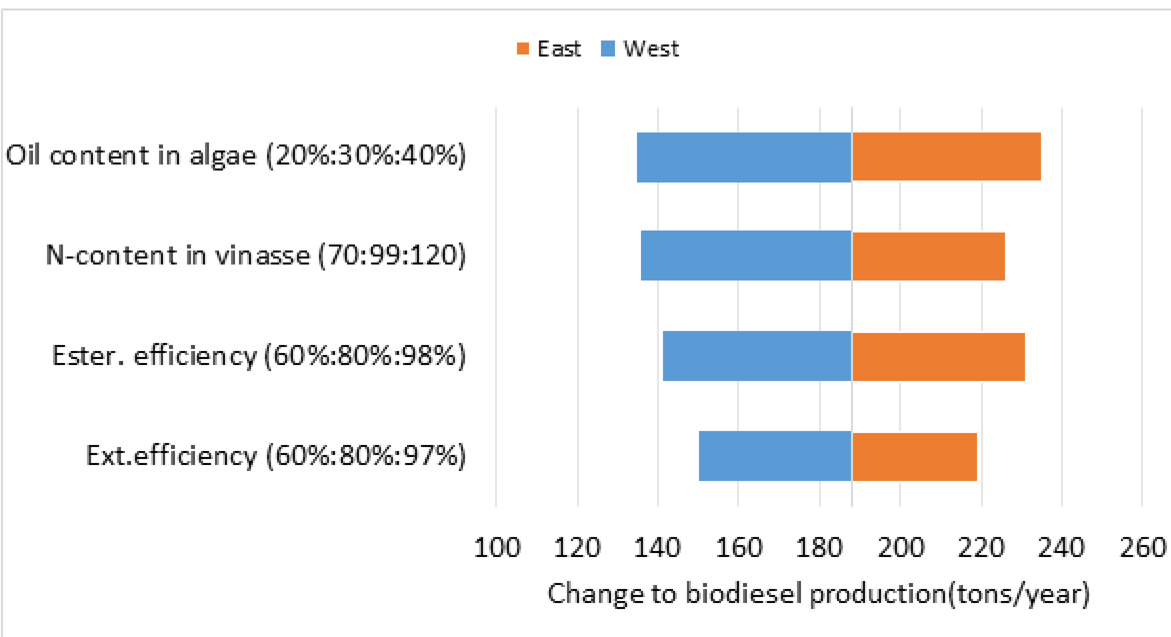

Fig. 4. 12 Biodiesel production sensitivity analysis

4.4. Conclusion

The wastes from the factory have a high potential for production of microalgal biomass and microalgal biofuel; biodiesel and biogas. Moreover, the process integration shows that another important product, bio-fertilizer, can be produced which can possibly make the synergy of the processes feasible. The result shows that the vinasse from the ethanol factory is the major nutrient source for the microalgae cultivation, as most of the nitrogen and phosphorus utilized by the algae in the pond is obtained from the vinasse after it is anaerobically digested in the AD. It was also found that the oil content of the algae, the nitrogen content of the wastes, and the extraction and transesterification efficiencies significantly affect the biodiesel production in the integrated process implying that improving these parameters is significant in increasing the feasibility of the integrated process. The ratio of the output energy to the input energy is about 4.8 showing that the energy balance in the integrated process is positive. This in turn indicates that the process is energy efficient. The use of the vinasse as an input in the AD played a great role for the energy efficiency of the coupled process to be convincing. Since there are several factories and ongoing mega projects for processing of sugarcane in Ethiopia, such economic activities are necessary in order to improve the value of the process and reduce the environmental pollution. However, its provision requires further research work in different areas including the biology of microalgae, the technology for processing of microalgae, and the economic feasibility of the integrated process. This study can play an important role in opening the way for such activities.

CHAPTER FIVE

5. Techno Economic Analysis of Microalgal Biofuel Production Process

5.1. Introduction

Depletion of fossil fuel (Abdullah et al., 2007; Energy outlook, 2016), global warming due to GHG emissions (Greenwell et al., 2010), food security issues due to using food crops for biofuel production (Harun et al., 2010), and the instability of price of petroleum based fuels are major global concerns urging the research and industrial community to look for renewable and sustainable alternative energy sources to secure the future energy demand (Rajkumar et al., 2014). In this regard microalgae have been found promising future biofuel sources (Alam et al., 2015). There are several alternative pathways for production of biofuel from microalgae such as thermochemical conversion, biochemical conversion, in situ conversion, and conversion after extraction (Wang et al., 2008; de Boer et al., 2012). Some research findings show that production of biodiesel via transesterification of extracted oil and biogas production via anaerobic digestion simultaneously is both material and energy efficient (Lundquist et al., 2010; Broberg et al., 2011). The main interesting features of using microalgal oil for biodiesel and biogas production include: the possibility of using CO_2 gas from industrial plants such as ethanol, sugar and other coal-burning plants with up to 90% conversion efficiency and possibility of utilization of nutrients (Broberg et al., 2011) from wastewater for the microalgae growth while environmental pollution is reduced simultaneously (Lundquist et al., 2010).

Though there are efforts for commercialization of algal biodiesel production the economic viability of producing algal biomass for low-value products such as biofuels still remains challenging (Lyon et al., 2015). The cost for microalgal biomass production is currently much higher than from other energy crops (Laurens, 2017). There are several approaches towards the viable production of biofuel from microalgae including genetic engineering to improve microalgal strain, bio-refinery approach for efficient material and energy utilization (Laurens, 2017), and integration of biofuel production with other processes (Lundquist et al., 2010). Coupling of microalgal cultivation for biofuel production with wastewater treatment for both low cost nutrient and environmental pollution reduction has found important to reduce the biofuel production cost (Broberg et al., 2011; Lundquist et al., 2010). Moreover use of flue gases from power plants and industrial plants is vital for CO_2 cost reduction and pollution reduction (Lundquist et al., 2010;; Broberg et al., 2011; Savage, 2011). In this regard sugar and ethanol factories could be important potential sites to be coupled with microalgal biofuel production so that the wastes and the byproducts could be used as nutrient sources for the microalgae while pollution is reduced simultaneously (Lundquist et al., 2010).

The focus of the present study was to perform an economic estimation of simultaneous biodiesel, biogas, and bio-fertilizer production from microalgae coupled with a sugar factory. Moreover the sensitivity of the process for change of some selected parameters was investigated using sensitivity analysis approach in Microsoft excel. For this purpose a case study approach was followed; conceptually coupled microalgal biofuel production process with a sugar factory, Metahara factory located to the Southeast of Addis Ababa, capital city of Ethiopia, was selected. The detail of the modelling for the coupled process was done using material and energy balance approach in the previous chapters, and this chapter focuses on the economic estimation of the coupled process based on the outputs from the process.

5.2. Materials and methods

5.2.1. System

This study focuses on economic estimation of a conceptual microalgal biofuel production coupled with an actual sugar factory, Metahara sugar factory. The estimation is based on the product outputs and energy requirements which were evaluated in chapter three and chapter four.

In the coupled system a photobioreactor (PBR) is used to grow a selected microalgae strain before being transferred to an open pond so that the risk of contamination would be reduced. The PBR is used to establish a monoculture of microalgae within the ponds and ensure the dominance of the desired species. The flue gas from the sugar mill and the ethanol plant would be pressurized and injected along the ponds through PVC pipes to be used as source of CO_2. The wastewater from sugar mill and the supernatant of recycle after anaerobic digestion are used as nutrient sources for the growth of the microalgae in the cultivation pond. The by-product glycerol from the transesterification unit is fed into the anaerobic digester (AD) together with sludge from the primary wastewater treatment, algae residues from the harvesting unit and the vinasse from the ethanol plant. The detail about the modelling of the process is found in chapter three and chapter four. The whole system/process considered is shown in Fig. 4.7 of chapter four.

5.2.2. Economic estimation

The economic feasibility of the process was studied using the discounted cash flow analysis method. The minimum biodiesel selling price (MBSP) was determined at a zero net present value (NPV) with a finite internal rate of return (10% IRR) while the prices of the biogas and the bio-fertilizer were assigned to the current market prices. The IRR was determined by assuming that the process would have 20 years life time.

5.2.2.1. Total capital cost estimation

All capital and operating costs of the process were estimated based on the mass and energy balance outputs obtained in chapter four. The capital cost estimate was mainly based on the works carried out by Benemann and Oswald (1996); Davis et al. (2016); ANL, NREL, PNNL (2012) report; Delrue et al. (2012), Lundquist et al. (2011); Broberg et al. (2011); and Nappa et al. (2015) (Benemann & Oswald, 1996; Lundquist et al., 2010; Brogberg et al., 2011; ANL;NREL; PNNL;, 2012; Delrue et al., 2012; Nappa et al., 2015; Davis et al., 2016). All the equipment costs taken from literatures were installed equipment costs. Order-of- magnitude (ratio) estimate approach was also used to evaluate capital costs (Peters and Timmerhaus, 1991). Here the cost information from previous works is used and the new equipment cost is estimated considering the ratio of the capacities of the equipments.

The cost of the primary wastewater treatment was estimated based on Lundquist et al. (2010) using a scaling factor of 0.6. Lundquist et al. (2011) estimated the capital cost of a primary clarifier with a capacity of 22,740 ML/yr. to be $420,000.

According to Davis et al. (2016) pond cost estimate included five categories; civil work cost, liner costs, piping, electrical and “other pond costs’’ (which is basically associated to cost of paddle wheel and motor). Liner costs are associated to costs of full lining or partial lining either on the pond berm or pond turns. Pond lining costs significantly affect the capital cost. For example in the report by Davis et al. (2016) for a 10 acre pond design liner costs of 40, 000-430,000 per 100-acre were shown depending on whether the pond was partially or fully lined. To avoid such high liner cost unlined/partially lined ponds were assumed for this study. Benemann and Oswald (1996) estimated pond cost considering only the civil work and the mixing costs (“other costs’’). The capital cost of the ponds in this study was based on the works of Benemann and Oswald (1996).

The cost of the inoculum ponds was based on the report by Davis et al. (2016) which estimated \$16.12 million for 144.8 acre (58.64 ha) areal coverage of the inoculum stage. In this study the inoculum stage was assumed to cover 1% of the total cultivation area (Lundquist et al., 2010) of which the PBR, the covered pond, and the open lined pond would share 4%, 16%, and 80% respectively (Davis et al., 2016).

The cost of primary harvesting (autoflocculation) and the dissolved air floatation (DAF) were estimated based on the works of Benemann and Oswald (1966) who estimated \$2,800,000 and \$800,000 per 400 ha total cultivation area for the flocculation and DAF (a flocculant was used in the DAF) respectively. In the report by Davis et al. (2016) the cost of a centrifuge was estimated to be \$2,890,152 per a capacity of 252 m^3/hr. In this study the cost of the centrifuge was estimated using this value by scaling down approach. Capital costs for the cell disruption, extraction, and anaerobic digestion were based on the work of the report ANL, NREL, PNN (2012) while the remaining capital costs of the unit operations not included in the above works were based on Broberg et al. (2011), Derlue et al. (2012), and Nappa and Karinen (2015).

Benemann and Oswald (1996) have estimated the cost of water and nutrient delivery capital cost to be \$5200 where the nutrient supply contributes \$1000/ha. Since in the present study it was supposed that the wastewater would be used as nutrient source, and no other nutrient would be delivered, the cost of water supply was considered. However Benemann and Oswald (1996) estimated the cost based on deep brackish water that needs to be pumped to the surface which does not apply in the present study. Thus one fifth of this value was considered in the present study (Benemann and Oswald, 1996). The capital cost of the primary wastewater treatment was estimated to be equal to the cost of settling in the harvesting section. Once the total installed equipment cost had been determined the total direct and indirect costs were estimated to determine the total capital investment (TCI). To do so the approaches followed in the works of Davis et al (2016) and the report ANL, NREL, PNN (2012) was applied (Table 5.1).

Total direct costs (TDC) are basically associated to warehouse and site development costs while the indirect costs include pro-rateable costs, field expenses, home office and construction, contingency and other costs (ANL;NREL; PNNL, 2012; Davis et al., 2016). According to standard ANL; NREL; PNNL (2012) assumption direct and indirect factors are estimated based on separating out costs attributed to process areas falling inside battery limits (ISBL) and outside battery limits (OSBL). The same approach was used for the present study (Table 5.2). In this regard the cultivation, dewatering, cell disruption, extraction, transesterification, anaerobic digestion, biogas and upgrading processes were categorized as process areas falling inside battery limits (ISBL) while the rest falling out side battery limits (OSBL).

The total installed equipment cost combined with the total direct and indirect costs was assumed to give the total fixed capital investment (FCI). Then the FCI combined with the working capital and the land cost gave the total capital investment (TCI). To estimate capital costs the scaling expression shown in Equation 5.1 was applied (Davis et al., 2016).

$$\frac{Cost_A}{Cost_B} = \left(\frac{Capacity_A}{Capacity_B}\right)^n \qquad (5.1)$$

Where n varies between 0 and 1 and possibly greater than 1 depending on the type of equipment to reflect economy scale dependencies. In this study the majority of the equipments were scaled linearly while the primary water treatment, centrifuge and the biogas upgrading equipments follow a scaling factor of an average value, 0.6. All costs were estimated in 2017 US dollar and the Chemical Engineering Plant Cost Index (CECPI) was used to recalculate the capital costs obtained for another year than 2017 to the capital cost in year 2017 (Equation 5.2).

$$Cost_{2017} = Cost_{YearX} \left(\frac{CECPI_{2017}}{CECPI_{YearX}} \right) \quad (5.2)$$

Table 5. 1 Estimation of additional direct costs and indirect costs

Parameters	Assumptions	References
Site Development*	9% of ISBL	(ANL;NREL; PNNL, 2012; Davis et al., 2016)
Warehouse	4% of ISBL	(ANL;NREL; PNNL, 2012)
Pro-rateable Costs	10% of TDC	
Field Expenses	10% of TDC	
Home Office and Construction	20% of TDC	
Contingency	10% of TDC	(Davis et al., 2016)
Other Costs	10% of TDC	(ANL;NREL; PNNL, 2012)
Working Capital	5% of FCI	(ANL;NREL; PNNL, 2012; Davis et al., 2016)
Land cost	$3000/ha	Based on Ethiopian land cost estimation approach for rural area

**In the ISBL cultivation was not added since site development cost for this facility has been included in the pond cost.*

Table 5. 2 Installed capital cost estimates $/ha for 22.6 ha (~23ha) cultivation area in 2017 $

Type	Value	References
Water treatment (OSBL) ($/m^3/yr waste water influent)	0.10565	(Lundquist et al., 2010)
Pond ($/ha)	16354	(Benemann and Oswald, 1996)
Inoculation pond ($/ha)	266,857	(Davis et al., 2016)
Water supply ($/ha)	1249	(Benemann and Oswald, 1996)
Waste water treatment blow down(OSBL) ($/ha)	1487	(Benemann and Oswald, 1996)
Electrical supply & distribution (OSBL) ($/ha)	2974	(Benemann and Oswald, 1996)
Flue gas delivery ($/ha)	14869	(Benemann and Oswald, 1996)
Settling/bioflocculation ($/ha)	10407	(Benemann and Oswald, 1996)

DAF aided by flocculant ($/ha)	2974	(Benemann and Oswald, 1996)
Centrifugation ($/(m^3/hr.))	69,374	Davis et al., 2016)
Cell disruption ($/ha)	8673	(ANL;NREL; PNNL, 2012)
Extraction ($/ha)	2583	(ANL;NREL; PNNL, 2012)
Transesterification ($/ton of convertible lipids per year)	211	(Delrue et al., 2012)
Anaerobic digestion ($/ha)	8612	(ANL;NREL; PNNL, 2012)
Biogas upgrading ($/Mm^3 upgraded biogas per year)	10697	(Broberg et al., 2011)

5.2.2.2. Variable operation costs

The items in variable operation costs include power, and raw materials. The important item is the power. In the present study the power consumption of different unit operation in the process and quantities of raw materials used were determined using the assumptions considered and the outputs from the material and energy balances. It was assumed that the thermal energy requirements would be provided by the steam from the process. The Consumer Price Index (CPI) was used to recalculate costs obtained in another year than 2017 to costs in 2017 (Equation 5.3).

$$Cost_{2017} = Cost_{YearX}\left(\frac{CPI_{2017}}{CPI_{YearX}}\right) \quad (5.3)$$

Cost of electricity in 2015 in Ethiopia was estimated to be \$0.09/kwh (Think Geoenergy-2015). However the current Growth and Transformation Plan II (GTP II) in Ethiopia has a new target to increase generation capacity to over 17,000 MW by 2020, with an overall potential of 35,000 MW by 2037 (Ethiopia power sector Market, 2016) showing that the electricity cost would be expected to decrease. Thus a cost estimate of \$0.05/kwh was used for the present study. Also other unit prices including chitosan (flocculant), ethanol (solvent), and methanol are shown in Table 5.3.

Table 5. 3 Unit prices in 2017 \$

Item	Price	Reference
Power	\$0.05/kwh	Estimated
Chitosan	\$6644.78/ton	(Nappa et al., 2015)
Ethanol	\$751/ton	Selling price of ethanol from factory
Methanol	\$500/ton	(Methanol bussinus online, 2018)
Steam	\$40/Mwh	Estimated

5.2.2.3. Fixed operating costs

The facility is assumed to be staffed by a plant engineer, lab manager, supervisors, technicians, operators, and secretaries (Table 5.4). Salaries for employees was based on Ethiopian salary scale. The overhead and maintenance cost were estimated at 60% of labor cost and 2% of equipment costs respectively (ISBL) (Davis et al., 2011). Estimates of 15% of total capital investment and 1% total capital investment were taken for the capital charge and miscellaneous costs respectively (Davis et al., 2011). The minimum selling prices of the two products other than biodiesel are shown in Table 5.5

Table 5. 4 Estimation of fixed operating costs

Position	Salary/yr (in $2017)	# required
Plant engineer	10344	1
Maintenance supervisor	6206	1
Maintenance technician	4138	4
Lab manager	6206	1
Lab technician	4138	4
Shift supervisor	6206	4
Shift operators	3310	10
Clerks and secretaries	3000	2
Benefit	@ 30%	
Overhead	60% labour	
Maintenance	2% of installed equipment cost(excluding OSBL)	

Capital charge	15% TCI
Miscellaneous	1% TCI

Table 5. 5 The Minimum selling prices of products

Type	Value	Unit	Reference
Biogas selling price	29	\$/MWh	(Nappa et al., 2015)
Bio-fertilizer	1107	\$/ton	(Delrue et al., 2012)

Note: prices were estimated for the year 2017

5.3. Results and discussions

5.3.1. Total capital costs

The total capital cost of the integrated process is shown in Table 5.6 while the installed equipment cost allocation is shown in Figure 5.6 and Figure 5.7. The result for the total capital cost shows that the proposed coupled process requires a total capital investment of more than 2.3 million dollar (in \$2017). It is found that the pond system, including the inoculum system, is the most capital intensive system followed by the flue gas delivery system. They cover 21% and 16% of the total capital cost.

Table 5. 6 Total capital cost of the process

Type	**Value (in \$2017)**	**Remark**

Water treatment (OSBL)	28,367	
Cultivation pond	369,600	
Inoculation pond	6631	
Water supply	28,227	
Waste water treatment blow down (OSBL)	0	No blow down water
Electrical supply and distribution (OSBL)	67,212	
Flue gas delivery	290,817	
Settling/ bioflocculation	235,198	
DAF aided by flocculant	67,212	
Centrifugation	180,373	
Cell disruption	196,010	
Extraction	64,575	
Transesterification	58,376	
Anaerobic digestion	194,631	

Biogas upgrading	22,571
Total	**1,809,800**
Additional direct cost	222,849
Indirect cost	133,709
Fixed capital investment (FCI)	2,166,358
Working capital	108,318
Land cost	67,800
Total capital investment (TCI)	**2,342,476**

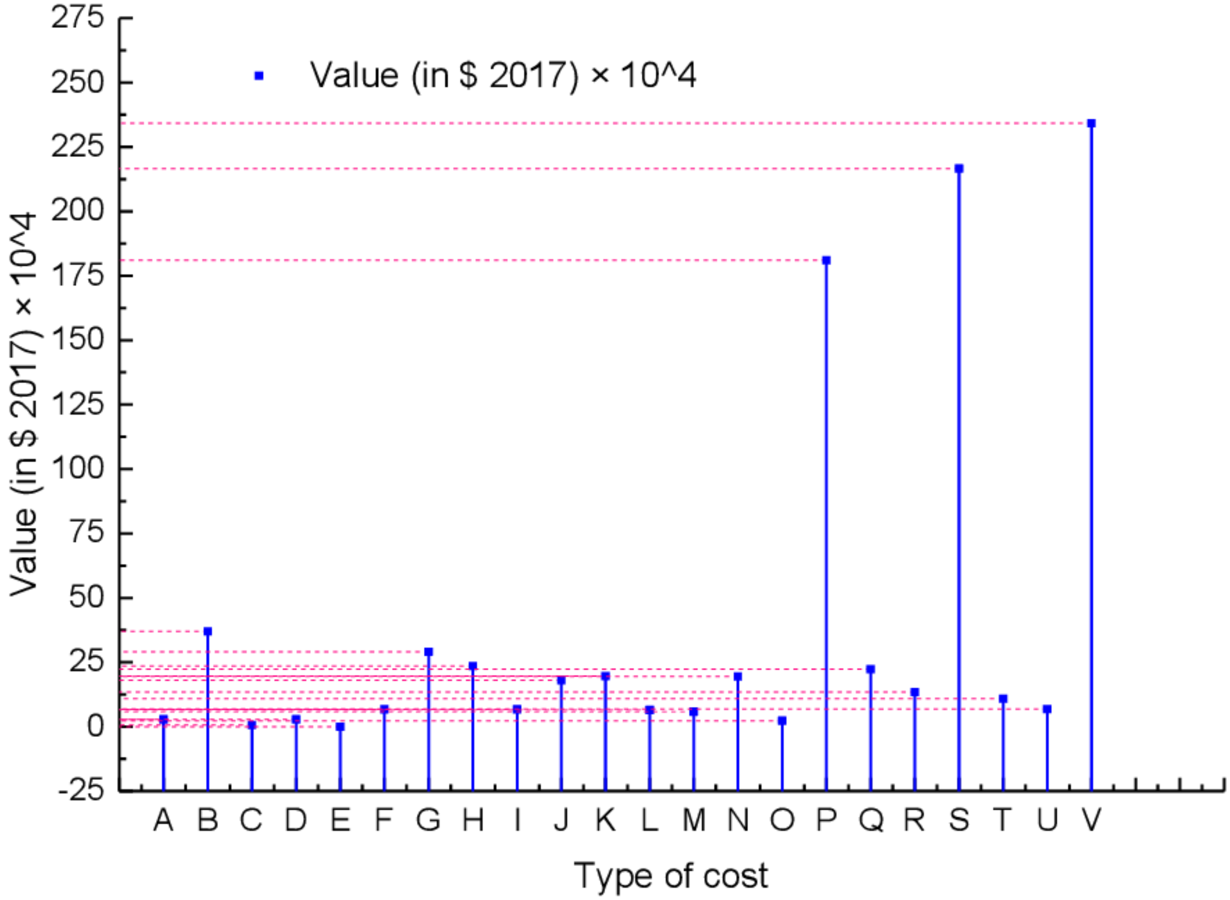

Fig. 5. 1 Capital cost of the whole process (A: WW treatment (OSBL), B: Cultivation pond, C: Inoculation pond, D: Water supply, E: WW treatment blow down (OSBL), F: Electrical supply and distribution (OSBL), G: Flue gas delivery, H: bioflocculation, I: DAF aided by flocculant, J: Centrifugation, K: cell disruption, L: Extraction, M: Transesterification, N: anaerobic digestion, O: Biogas upgrading, P: total of A-O, Q: Additional direct cost, R: indirect cost, S: FCI, T: WC, U:land cost, V: TCI)

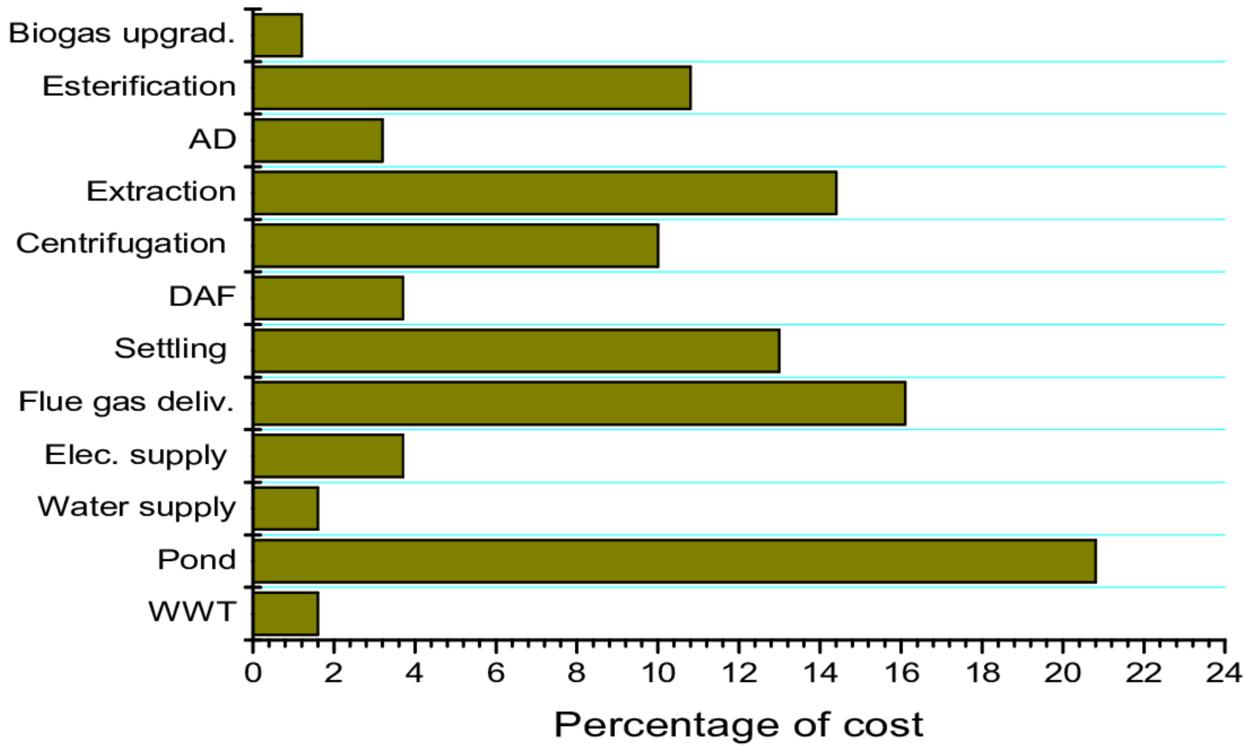

Fig. 5. 2 Installed equipment cost allocation (%)

5.3.2. Operating costs

The variable and fixed operating costs are shown in Table 5.7. The result for the operating costs shows the process costs approximately $900, 000 annually of which the variable and fixed operating costs share 20% and 80% respectively.

Table 5. 7 Operating costs of the process

Parameter	Value ($/yr)
Power for cultivation	21,195
Power for harvesting processing	4,585

Power for cell disruption and extraction	24,568
Power for transesterification	4
Power for biogas production and upgrading	52,227
Chemicals	2967
Steam	100,000
Total variable operating costs	**205,546**
Plant engineer	103,344
Maintenance supervisor	6,206
Maintenance technician	16,552
Lab manager	6206
Lab technician	16,552
Shift supervisor	24,824
Shift operators	33,100
Clerks and secretaries	6000
Total salaries	**212,784**

Overhead	127,670
Maintenance	34,284
Capital charge	351,371
Miscellaneous	23,425
Total	**749,534**
Benefits (@ 20%)	149,907
Total fixed operating cost	724,521
Total annual cost	**899,441**

**Ethanol and methanol are recycled with in the process and only make up ethanol and methanol would be required* yearly

5.3.3. Revenues

Total annual revenue of the process is shown in Table 5.8. The revenues of the process was determined by the selling price of the three products: the biogas, the bio-fertilizer, and the biodiesel. The minimum selling price of the biogas and the bio-fertilizer were specified at the current market price while the minimum selling price of the biodiesel was assigned to a variable. The minimum biodiesel selling price (MBSP) per liter was determined using discount cash flow analysis method with IRR 10% and 20 years' service life of the process. To determine the MSBP the NPV was set to a value of zero. The MBSP of the biodiesel was estimated to be $2.17per liter, which agrees with the result obtained by Davis et al., (2011) (Davis et al., 2011) who reported MBSP of $2.16/L(in $2007). In order to be competitive, the microalgal biodiesel should be sold with a price less or equal to the petroleum diesel price; the world five year (2010, 2012, 2014, 2016, 2018) average petroleum diesel price is \$1.09/L (Valev, 2016). Thus in order for the microalgal biodiesel to be competitive with the current petro-diesel, this price needs to be reduced by at least half.

On the other hand for two potential pathways, algal biomass to biofuel via lipid extraction and via hydrothermal liquefaction, by extrapolating 2014 result to 2022, minimum biofuel selling price of \$4-4.5/gasoline gallon equivalent (\$1-\$1.19/L) has been reported (Laurens, 2017). This value is almost half of the result obtained in the present study, and this variation might happened due to the extrapolated time and the difference in the assumptions considered. Likewise in another study using a thermodynamic model comparing the total cost (biomass production and harvest, oil extraction, capital, labor, to operational costs) of photoautotrophic cultivation using raceway was estimated to be \$14.44/L while the cost of \$0.88/L for canola oil was used as reference (Alabi et al., 2009). Compared to this result there is a significant production cost reduction in the present study. This may be attributed to the coupling of the process to the sugar factory as the cost of nutrients and CO_2 is avoided. Chisti (Chisti, 2008) compared the production cost of oil from microalgae with petro-diesel. During his estimation including cost of crude oil (52%), tax (20%), distribution and marketing (9%), and refining (19%) the price of petro-diesel was within the range of \$0.66 and 0.79/L. Excluding the tax, marketing and distribution costs it was estimated \$0.49/L. While estimated cost of recovered oil from microalgae using PBR in a typical facility was \$2.8/L which was approximately six fold of the selling price of petro-diesel. Chisti's (Chisti, 2008) result on the selling price of microalgal oil, which was \$2.8/L, was greater than the present result, which is \$2.17/L, almost by 29%. However the two results are not directly comparable as the oil would need further processing to produce the biodiesel, and thus it would increase the production cost. Moreover Chisti used a photo-bioreactor to produce the biomass while in the present study open pond cultivation was assumed. With regard to product cost, taking both the capital cost and the annual operating cost to produce a unit product of biodiesel, in his study for the modeling of the production of microalgal biodiesel, Mark Henson (2013) (Henson, 2013) has estimated a cost of \$12.16 per liter of biodiesel produced. In present study it was estimated to be about \$9.30 per liter of biodiesel produced. This variation might happened due to the difference in the two paths for microalgal to biofuel conversation as biogas production is involved in the present study while it is absent in Mark Henson's. In general estimation of microalgal biofuel production cost depends on projections of different scenarios that allow the effects of key variables to be compared. So in order to study different scenarios performing sensitivity analyses is useful.

Table 5. 8 Revenues from selling of the products (\$/yr)

Type	Value
Biogas	685,338
Bio-fertilizer	46,560
Biodiesel	209222 × MBSP
Total annual revenue	731,898 + 209222 × MBSP

5.3.4. Sensitivity analysis

The sensitivity of the minimum biodiesel selling price (MBSP) to change of different parameters was investigated (Figure 5.8). The MBSP is most affected by change in oil content of the algae followed by change in nitrogen-content in the vinasse. As the oil content varies from 30% (the base value) to higher value of 40%, the MBSP reduces almost by 20%, i.e. from \$2.17/L to \$1.74/L. While as the oil content decreases from 30% (by wt.) to 20% the MBSP increases by almost 40%, i.e. from 2.17 to \$3.03/L. Similarly the MBSP increases by 39% when the N-content in the vinasse lowers by 29 ton/yr from the base value and reduces by 17% when the –nitrogen content in the vinasse increases by 21 ton/year.

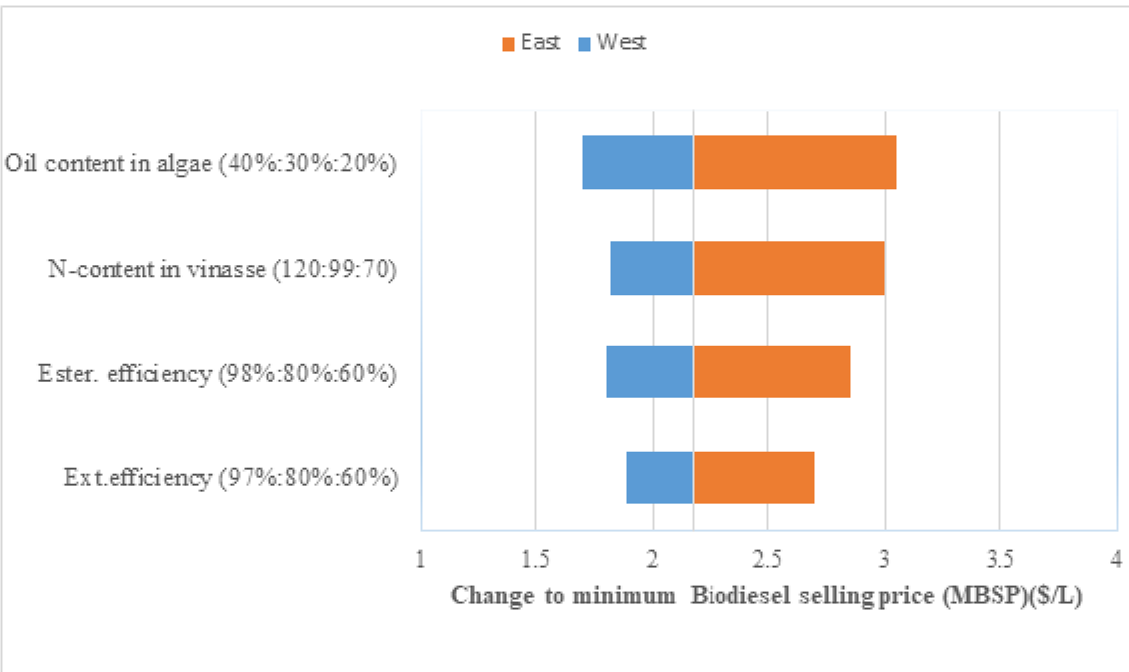

Fig. 5. 3 Effects of different parameters on the MBSP

5.4. Conclusion

The focus of this study was on the techno-economic feasibility of microalgal biomass and biofuel production which is conceptually coupled with a sugar factory in order for utilization of wastes and byproducts as source of nutrients and CO_2 for microalgal growth. For this purpose a case study approach was followed where an Ethiopian sugar cane processing factory, Metahara sugar factory, is used for the integration of the conceptual biofuel production from the microalgae. It was found that the proposed coupled process requires a total capital investment of more than 2.3 million dollar (in \$2017), and the pond system, including the inoculum system, is the most capital intensive system followed by the flue gas delivery system. Likewise, it was found that the MBSP was double as compared with the petro-diesel price. Thus in order for the biodiesel to be competitive the calculated MBSP needs to be reduced at least by half. However the MBSP was found lower when compared with some other previous works and this may be attributed to the production of the microalgal biofuel using the low cost CO_2 and nutrient supply from the factory. The result from the sensitivity analysis implies that in order for the microalgal biofuel production process to be more viable and get provision it needs further research works on the biology of the algae to get a strain with high oil content and also on developing more energy and material efficient processes. Moreover, it is worthy of considering that the decline in the price of petroleum, coupled with ongoing low prices for natural gas and absence of consistent policies on carbon pricing is a challenge in the development of cost-competitive production algae based bioenergy products like gaseous and liquid fuels (Laurens, 2017). Finally the study is important to show the significance of use of inexpensive nutrient and CO_2 sources for algal biofuel production and as well as to decide the provision of industrial scale microalgal biofuel production.

CHAPTER SIX

6. Conclusions and Recommendations

6.1. Conclusions

Global energy crises due to high population and industrialization is urging the world to find alternative renewable energy sources. Production of biodiesel from microalgae is considered to be among the most viable biofuel production pathways due to high content of lipids in microalgae. Furthermore, the low content of lignin and hemicellulose in microalgae is a key advantage that makes microalgae to be favorable feed stock for biofuel production.

There are several important challenges associated to microalgal cultivation. These challenges include: system stability when scaling up for commercial production, improving productivity at minimized cost, sustainable availability of resources such as land, water, CO_2, and nutrients.

To date attempts have been made to use microalgae as substitutes to conventional biofuel feedstocks by overcoming the above challenges. Possibility to couple microalgae cultivation with other processes to both reduce environmental foot print and cost of production by considering different location options has been found important. For example cultivation of microalgae could potentially be coupled with wastewater treatment, aquaculture, point CO_2 sources, and industrial waste streams (e.g. streams from pulp and paper, sugar and ethanol factories).

Coupling of microalgal cultivation with sugar factories for the inexpensive nutrient and CO_2 utilization is one of the identified approach towards increasing the feasibility of microalgal biofuel production. This study was devoted for coupling of cultivation of microalgae with CO_2 and nutrient sources generated from sugar factories.

Basically two scenarios (chapter 3 and chapter 4) were considered to study the potential of the sugar factory wastes and byproducts to support the growth of microalgae in open ponds. In the first scenario (chapter 3) only the wastewater from the sugar mill was used as the only nutrient source for the growth of the microalgae. Based on the key assumptions considered the coupled process established was shown in Fig. 3.10. The mass and energy outputs are also shown in Table 3.9 and Fig. 15 respectively.

In this first scenario the N:P ratio in the wastewater from the sugar mill was less than the minimum value necessary for algal growth. Literature review shows that for microalgae grown by utilizing all the nitrogen and phosphorus, the N:P ratio should be greater than 4:1 and less than 40:1. In this regard, the primarily treated sugar mill wastewater was found as nitrogen deficient. This deficiency can be compensated by makeup nutrients such as nitrates and ammonia. On the other hand the nitrogen deficiency could be solved by utilizing the vinasse from the ethanol production plant (scenario two). Most of the nitrogen and phosphorus utilized by the algae in the pond could be obtained from the vinasse after it is anaerobically digested in the AD. It was assumed that the supernatant, the top product obtained by separating the sludge from the anaerobic digester into top and bottom product, would be recycled to the ponds and used as a source of nutrients. If recycling was not considered (scenario one) a supply 7.91 ton/year of nitrogen as a makeup nutrient based on the amount of the total phosphorus contained in the wastewater would have been needed. In this way, it would be possible to produce 121 ton/year of biomass in the ponds. In addition to filling the nutrient deficit, the use of the vinasse as an input in the AD would play a great role in increasing the energy efficiency of the coupled process. Likewise, the results of the cultivation and the harvesting model demonstrate that, when there is recycling, there would be an increase of the cultivation area from 2 ha to 23 ha, and an increase in energy requirements in parallel with the increase of biomass production.

Moreover the results of this study show that the wastewater after algae treatment meets the requirements for discharge in land for TN and TP but not for COD, BOD_5, TSS and oil and grease. This suggests that the algae treatment should be employed after biological treatment step, where activated sludge oxidizes the organic matter.

The overall energy balance in the coupled process (Fig 4.7) shows a positive energy balance. In the transesterification process this attributes to both the homogenization step assumed to help reduce the high temperature and pressure demand, and the high extraction and transesterification efficiencies supposed in the process. Likewise in the biogas production process this might possibly be due to the use of the vinasse from the ethanol factory to produce a huge amount of biogas.

Excess CO_2 could be obtained from the flue gases of the sugar factory. If the whole flue gas had been utilized it would have a potential of producing biomass of 136850 tons/year, which is much greater than 1412 ton/year, the value obtained by considering the nutrients in the wastewater as the limiting factor. However the total CO_2 used in the process would be equal to the amount required by the algae in the ponds as per the limiting nutrients, and this value is the same as the net CO_2 reduced in the coupled process.

Chapter five of this dissertation was concerned with the economic analysis of the coupled process (Fig. 4.7). In the economic analysis, it was found that in the proposed integrated process, the pond system, including the inoculum system, is the most capital intensive system followed by the flue gas delivery system. Likewise, it was found that the MBSP was much higher (double) as compared with the petro-diesel price. Sensitivity analysis shows that oil content of the microalgae is the most important parameter which affects the feasibility of the process. The second important factor is the nutrient content in the waste streams followed by the processing technologies.

Overall integration of microalgal biofuel production with sugar factories is an important approach towards increasing the feasibility of production a high volume-low value product, biofuel.

Moreover another important product, bio-fertilizer, can be produced which may make the synergy of the processes more feasible.

Since there are several factories and ongoing mega projects for processing of sugarcane in Ethiopia, such economic activities are encouraged so as to increase the process value and reduce environmental pollution.

Finally the study is important to show the significance of use of inexpensive nutrient and CO_2 sources for algal biofuel production and as well as to decide the provision of industrial scale microalgal biofuel production.

6.2. Recommendations

This study has investigated coupling of microalgae cultivation with CO_2 and nutrient sources from sugar factories. Some important new findings have been demonstrated. Further research works can focus on the following areas:

- System stability is one of the important challenges during microalgal cultivation. Selecting on the biology of the microalgae to get a robust strain with high oil content is important.
- Study on the development of selective, sensitive and inexpensive control methods on some important parameters, such as pH, temperature, and photo inhibition.

- Harvesting is an energy intensive step in microalgae cultivation, and thus, it is a major challenge in the commercialization of biofuels from microalgae. So study on the development of energy-efficient and cost-effective harvesting technology is important.
- Thorough techno economic feasibility study with life cycle analysis on the production of biofuel from microalgae coupled with point CO_2 sources and industrial waste streams is imperative.
- Finally, it is worthy of considering that fluctuation in the price of petroleum and natural gas, and absence of consistent policies on carbon pricing is a challenge in the development of cost-competitive production algae based bioenergy products.

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Appendices

Appendix A: List of publications

Zewdie, D. T., & Ali, A. Y. (2020). Cultivation of microalgae for biofuel production: coupling with sugarcane-processing factories. *Energy, Sustainability and Society*, *10*(1), 1-16.

Zewdie, D. T., & Ali, A. Y. (2021).Utilization of sugarcane factories' wastes as inexpensive source of nutrients and CO_2 for microalgal biomass production: process coupling and potential evaluation. *SN Applied Sciences*, (2021), 3:297

Submitted Manuscript

Techno-economic Analysis of Microalgal Biofuel Production Coupled with Sugarcane Processing Factories. South African Journal of Chemical Engineering Manuscript Number: SAJCE-D-20-00114 (under review)

Appendix B: Composition of some selected media

Table B.1 Composition of some selected media (Borowitzka, 2013)

Substances	BBM	BG-11	Modified Allen's	Sorokin/Krauss
$NaNO_3$	0.25	1.5	1.5	
KNO_3	-	-	-	1.25
$K_2HPO_4 \cdot 3H_2O$	0.075	0.04	0.039	
KH_2PO_4	0.175	-	-	1.25
$MgSO_4 \cdot 7H_2O$	0.075	0.075	0.075	1.0

$CaCl_2 \cdot 2H_2O$	0.084	0.036	0.025	0.04
$Ca(NO_3)_2 \cdot 4H_2O$	-	-	0.02	-
$Na_2SiO_3 \cdot 9H_2O$	-	-	0.058	-
Citric acid	-	0.006	0.006	-
Fe-ammonium citrate	-	0.006	-	-
$FeCl_3$	-	-	0.002	-
$FeSO_4 \cdot 7H_2O$	0.00489	-	-	0.05
EDTA, 2Na-Mg salt	0.05	0.001	0.001	0.5
Na_2CO_3.	0.02	0.02	-	-
NaCl	0.025	-	-	-
KOH	0.031	-	-	-
H_3BO_4 (μgL^{-1})	11.42	2.86	2.86	114
$MnCl_2 \cdot 4H_2O$ (μgL^{-1})	1.44	1.81	1.81	14
$ZnSO_4 \cdot 7H_2O$ (μgL^{-1})	8.82	0.222	0.22	88
$NaMoO_4 \cdot 2H_2O$ (μgL^{-1})	-	0.391	0.391	-
$CuSO_4 \cdot 5H_2O$(μgL^{-1})	1.57	0.079	0.079	16
$Co(NO_3)_2 \cdot 6H_2O$ (μgL^{-1})	0.49	0.0494	0.0494	5
MoO_3 (μgL^{-1})	0.71	-	-	7
Adjusted final pH	-	7.4	7.8	6.8

Note: all concentrations are in g L^{-1}, unless indicated otherwise and the quantities are for 1L of culture solution

Appendix C: Material flow in the process

Appendix C. Material flow in the process

Material balance

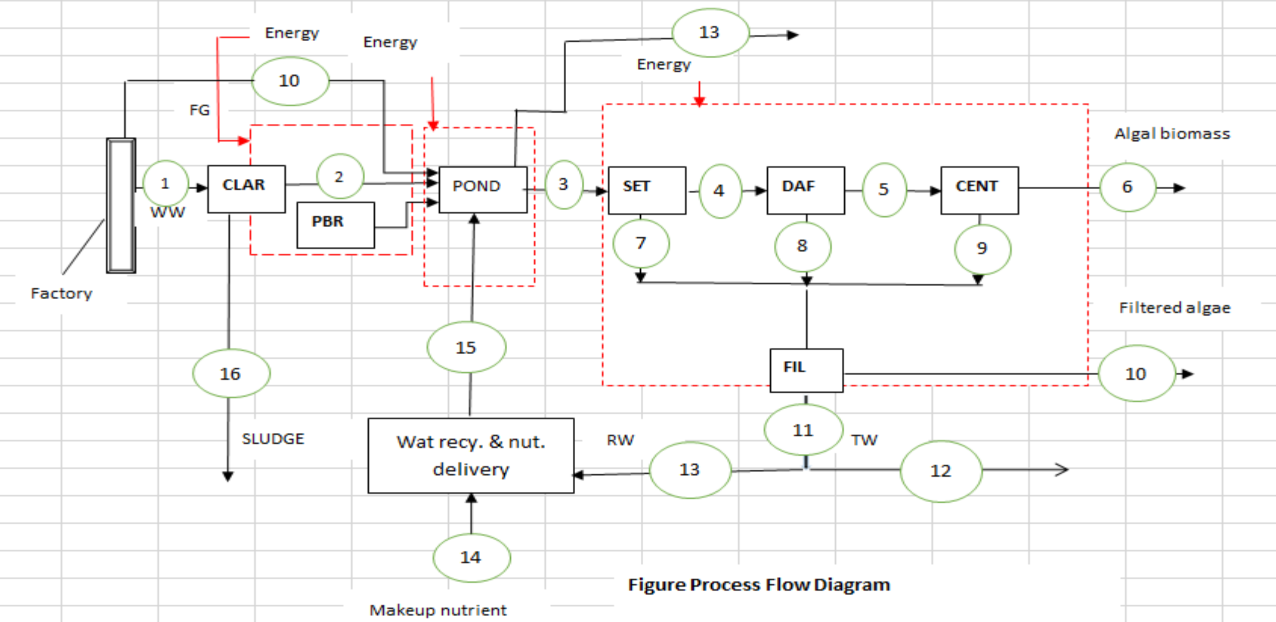

Figure Process Flow Diagram

Stream No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cmponent																
water(km3/yr	268.5	260.5	241	11.5	1.7	0.4	229.6	9.7	1.3	12.0	228.6	228.6	1.7	0	0	8
BOD5 (mgO2/L)	1200	828	207	207	207	207	207	207	207	207	207	207				372
COD(mgO2/L	2200	1540	447	447	447	447	447	447	447	447	447	447				660
TN(t/yr)	15	12	1	_	_	_	_	_	_	_	_	_	_	7.91	7.91	3
TP(t/yr	10	7.4	1.48	_	_	_	_	_	_	_	_	_	_	_	_	2.6
Oil& grea (	60	21	< 21	< 21	< 21	< 21	< 21	< 21	< 21	< 21	< 21	< 21	_	_	_	39
CO2 (t/yr	_	_	_	_	_	_	_	_	_	272.1	40.8	_	_	_	_	_
Agae(t/yr)	_	_	120.5	114.5	103.0	98.1	6	11.5	5.2	22.6	0	0	0.0	_	_	_

Figure C. Material flow in the integrated Process

Appendix D: Material and Energy balance Equations

Sugar cane production in the field:

$$\boldsymbol{C = P_{sc} \times A_{sc}} \tag{D.1}$$

Where C is cane production (ton/year) in Metahara sugar cane factory, P_{sc} is productivity of cane (tons/ha year), and A_{sc} is area for the cane cultivation (ha).

Values for the productivity of cane and the area for the cane cultivation are given in Table 1.

Total bagasse production in the sugar mill:

$$\boldsymbol{m_{tb} = 0.14 m_{cap} \times OD} \tag{D.2}$$

Where m_{tb} is total bagasse production (t/year), m_{cap} is mill capacity (tons of cane per day, TCD)

And OD is operation days of the factory per year.

The mill capacity and the operation days are given in Table 1.

Excess bagasse production in the sugar mill:

$$\boldsymbol{m_{be} = 0.155 m_{tb}} \tag{D.3}$$

Where m_{tb} is total bagasse production (tons/year), and m_{be} is production of excess bagasse

Heat content of bagasse

$$\boldsymbol{Q_b = 7893 m_b} \tag{D.4}$$

Where Q_b is heat content of the bagasse (BTU), and m_b is mass of bagasse (lb)

Heat content of the bagasse is given in Table 1.

Mass production of flue gases at the sugar mill:

$$\boldsymbol{m_{fgb} = 7.14m_{tb}} \quad \textbf{(D.5)}$$

Where m_{fgb}, is the production of flue gas at mill/boilers (tons/year) and m_{tb} is the total bagasse production (tons/year).

Production of CO_2 at the sugar mill/boiler:

$$\boldsymbol{m_{bCO2} = 1.72m_{tb}} \quad \textbf{(D.6)}$$

m_{bCO2} is production of the CO_2 at the boilers and m_{tb} is the total bagasse production (tons/year)

Waste water production at the sugar mill:

$$\boldsymbol{m_{ww} = 0.2m_{cap} \times OD} \quad \textbf{(D.7)}$$

Where m_{ww} is the waste water production at the sugar mill (tons/year), m_{cap} is the mill capacity (tons of cane per day,TCD), and OD is operation days of the factory per year

Molasses production at the ethanol plant:

$$\boldsymbol{m_{m} = 0.032m_{cap} \times OD} \quad \textbf{(D.8)}$$

m_m is the molasses production at the ethanol factory (tons/year**)** m_{cap} is the mill capacity (tons of cane per day,TCD), and OD operation days of the factory per year

Ethanol production at the ethanol plant:

$$\boldsymbol{q_{EtOH} = 0.23m_{m}} \quad \textbf{(D.9)}$$

q_{EtOH} is the ethanol production at ethanol factory (m^3/year), m_m is the molasses production at ethanol factory (tons/year**)**

Production of carbon dioxide from the fermenters of the ethanol plant:

$$\boldsymbol{m_{fCO2} = 0.2m_{m}} \quad \textbf{(D.10)}$$

m_{fCO2} is the production of CO_2 at fermenter (tons/year), and m_m is the molasses production at the ethanol factory (tons/year)

Production of vinasse at ethanol plant:

$$q_{tv} = 2.3m_m \tag{D.11}$$

q_{tv} is total vinasse production (m^3/year), and m_m is the molasses production at the ethanol factory (tons/year)

Microalgae Cultivation in the pond:

$$Comp_{alg} = C_{106}\,H_{181}O_{45}N_{15}P \tag{D.12}$$

Where $Comp_{alg}$ is the composition of algae used in the modelling

Requirements of nutrients by the algae:

$$m_c = m_{calg}/m_{alg} \times Mwt_{CO2}/Mwt_C \tag{D.13}$$

$$m_N = 0.087m_{alg} \tag{D.14}$$

$$m_P = 0.0128m_{alg} \tag{D.15}$$

Where m_c is the mass of carbon required by microalgae, m_{calg} is mass of carbon in algae, m_{alg} is mass of algae is Mwt_{CO2} is of molecular weight of carbon dioxide, Mwt_C is molecular weight of carbon, m_N is mass of nitrogen required by microalgae as per the composition of algae, and m_p is mass of phosphorus required by algae as per the composition of algae.

Appendix E: Material and energy flows in the process

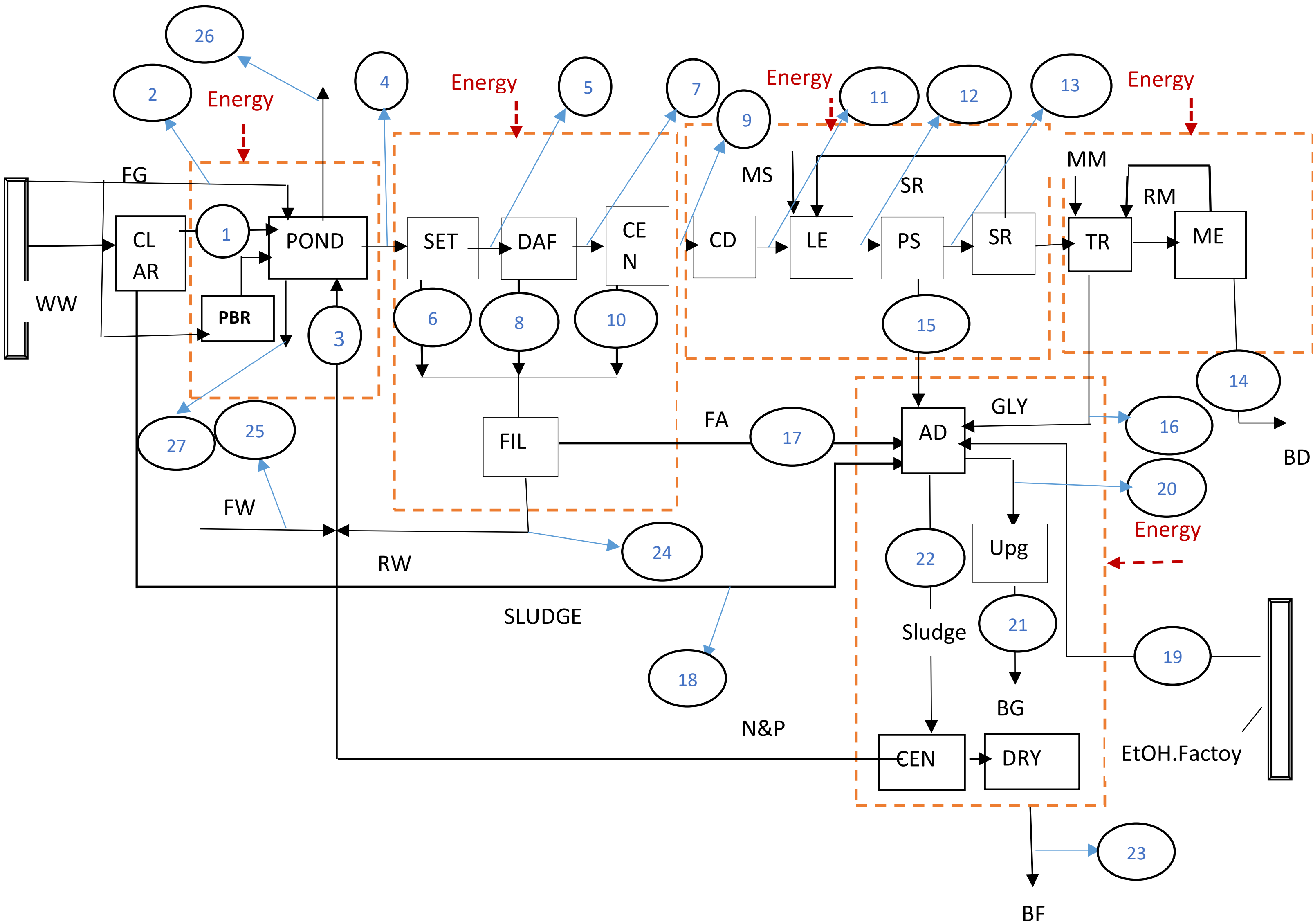

Figure E-1 Process Flow Diagram

Material Flow in the Integrated Process

1. Input parametres

Parameter	Q_{ww}	X_N	X_P	K_N	K_P	K_{CO2}	$Mwt,_{CO2}$	$Mwt,_C$	f_C	f_N	f_P	%H	%O	P_B	R	A_{TOT}
	[m^3/day]	[kg/kg]	[kg/kg]	[none]	[none]	[none]	[kg/kmoles]	[kg/kmoles]	[kg/kg]	[kg/kg]	[kg/kg]	[wt%]	wt%	kg/m^2/day	[kg/kg]	[m^2]
Values	1042	0.012	0.0074	0.95	0.8	0.85	44	12	0.527	0.087	0.0128	7.5	29.83	0.025	0.84	225923
Parameter	A_{SUR}	r_{evp}	r_1	r_2	r_3	ρM	Xc_1	Xc_2	Xc_3	%filt	ρ_{ww}	ρ_{agae}	C_{CD}	C_{EXT}	C_{TR}	%W,LEA
	[m^2]	[none]	[kg/kg]	[kg/kg]	[kg/kg]	[kg/m^3]	[kg/kg]	[kg/kg]	[kg/kg]	wt%	[kg/m^3]	[kg/m^3]	[kg/kg]	[kg/kg]	[kg/kg]	[wt%]
Values	1000	0.00691	0.95	0.9	0.95	792	10	60	250	100	1000	800	0.9	0.8	0.8	90
Parameter	% oil	R_{GB}	ρEtOH	EtOH-loss	Lip-loss	%W	%gly	%Meth	Pur-gly	υ_A	υ_{LEA}	υ_{GLY}	υ_{SLD}	υ_M	r_{COD}	V_{EtOH}
	wt%	[kg/kg]	[kg/m3]	[kg/day]	[kg/day]	[wt%]	[wt%]	[wt%]	[kg/kg]	[kg/kg]	[kg/kg]	[kg/kg]	[kg/kg]	[kg/kg]	kg/kg	[m^3/day]
Values	0.3	0.1	789	0.0052	0.05	0.005	0.0024	0.002	0.85	0.73	0.63	0.85	0.99	0.99	0.65	29.1
Parameter	Y_A	Y_{LEA}	Y_{GLY}	Y_{SLD}	Y_M	Y_{COD}	$r_{N,AD}$	$r_{P,AD}$	M_{SLD}	V_{VIN}	M_{COD}	pur. of BG	$M_{N,VIN}$	$M_{P,VIN}$	P_B	V_M
	[m^3/kg]	[m^3/kg]	[m^3/kg]	[m^3/kg]	[m^3/kg]	[m^3/kg]	[kg/kg]	[kg/kg]	[kg/day]	[m^3/day]	[kg/day]	[kg/kg]	[kg/day]	[kg/day]	kg/m^2/day	[m^3/day]
Values	0.43	0.43	0.43	1.01	0.53	0.344	0.16	0.21	1750	396	16731	0.84	1	0.087	0.025	0.10

2. Material flow in each stream (1-27)

Components / Stream no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nitrogen (kg/day)	13	_	505	491	467	25	420	47	399	21	399	399	40	_
Phosphorus (kg/day)	8	_	71	72	69	4	62	7	59	3	59	59	6	_
CO2 (kg/day)	_	9277	_	_	_	_	_	_	_	_	_	_	_	_
Biomass (kg/day)	_	_	_	5648	5366	282	4829	537	4588	241	241	241	_	_
Water (m3/day)	1042	_	_	11296	530	10766	74	455	13	62	13	13	1	_
Disrupted biomass (kg/day)	_	_	_	_	_	_	_	_	_	_	4129	_	_	_
Undisruptedbiomass (kg/day)	_	_	_	_	_	_	_	_	_	_	459	459	_	_
Extracted oil (kg/day)	_	_	_	_	_	_	_	_	_	_		991	_	_
LEA (kg/day)	_	_	_	_	_	_	_	_	_	_	4129	3138	_	_
Sludge from prim. WWT(m3/day)	_	_	_	_	_	_	_	_	_	_		_	_	_
Filtered algae (kg/day)	_	_	_	_	_	_	_	_	_	_	1	_	_	_
COD (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Biodiesel (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	753
Glycerol (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Crude biogas (km3/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Upgraded biogas (km3/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Biofertilizer (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Components / Stream no.	15	16	17	18	19	20	21	22	23	24	25	26	27
Nitrogen (kg/day)	359	_	0	_	396	_	_	673	168	_	_	_	_
Phosphorus (kg/day)	53	_	0	_	34	_	_	143	71	_	_	_	
CO2 (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	
Biomass (kg/day)	3597	_	1	_	_	_	_	_	_	_	_	_	
Water (kg/day)	11	_	_	27	368	_	_	407	_	11284	4446	1561	3812
Disrupted biomass (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_
Undisruptedbiomass (kg/day)	459	_	_	_	_	_	_	_	_	_	_	_	_
Extracted oil (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_
LEA (kg/day)	3138	_	_	_	_	_	_	_	_	_	_	_	_
Sludge from prim. WWT(m3/day)	_	_		32	_	_	_	_	_	_	_	_	_
Filtered algae (kg/day)	_	_	1	_	_	_	_	_	_	_	_	_	_
COD (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_
Biodiesel (kg/day)	_	_	_	_	_	_	_	_	_	_	_	_	_
Glycerol (kg/day)	_	75		_	_	_	_	_	_	_	_	_	_
Crude biogas (m3/day)	_	_	_	_	_	8081	_	_	_	_	_	_	_
Upgraded biogas (m3/day)	_	_	_	_	_	_	6788	_	_	_	_	_	_
Biofertilizer (kg/day)	_	_	_	_	_	_	_	_	168	_	_	_	_

Determination of mass flow rate of recycle nitrogen by iteration (all units are in kg/day)														
	1	3	4	9	11	11	12	12	15	14	16	17	17	19	22
It.no.	N_{FED}	N_{RECY}	$M_{TOT,algae}$	$M_{After,harv}$	$M_{disrupted}$	$M_{undisrupted}$	M_{oil}	M_{LEA}	$M_{N,RIS}$	M_{BD}	M_{GLY}	M_{FILT}	$M_{N,FILT}$	$M_{N,VIN}$	$M_{N,AD}$
1	12.5	0.0	136.5	110.9	99.8	11.1	24.0	75.9	7.6	18.2	1.8	25.6	2.2	396.0	340.9
2	12.5	255.7	2928.1	2378.4	2140.5	237.8	513.7	1626.8	162.2	390.4	39.0	549.8	47.8	396.0	509.1
3	12.5	381.8	4305.8	3497.4	3147.6	349.7	755.4	2392.2	238.5	574.1	57.4	808.4	70.3	396.0	592.1
4	12.5	444.1	4985.6	4049.6	3644.6	405.0	874.7	2769.9	276.2	664.8	66.5	936.1	81.4	396.0	633.1
5	12.5	474.8	5321.1	4322.1	3889.9	432.2	933.6	2956.3	294.8	709.5	71.0	999.0	86.9	396.0	653.3
6	12.5	490.0	5486.7	4456.6	4010.9	445.7	962.6	3048.3	304.0	731.6	73.2	1030.1	89.6	396.0	663.3
7	12.5	497.4	5568.4	4522.9	4070.6	452.3	977.0	3093.7	308.5	742.5	74.2	1045.5	91.0	396.0	668.2
8	12.5	501.1	5608.7	4555.7	4100.1	455.6	984.0	3116.1	310.7	747.9	74.8	1053.0	91.6	396.0	670.6
9	12.5	503.0	5628.6	4571.9	4114.7	457.2	987.5	3127.1	311.8	750.5	75.1	1056.8	91.9	396.0	671.8
10	12.5	503.9	5638.4	4579.8	4121.8	458.0	989.2	3132.6	312.4	751.8	75.2	1058.6	92.1	396.0	672.4
11	12.5	504.3	5643.3	4583.8	4125.4	458.4	990.1	3135.3	312.6	752.5	75.2	1059.5	92.2	396.0	672.7
12	12.5	504.5	5645.7	4585.7	4127.1	458.6	990.5	3136.6	312.8	752.8	75.3	1060.0	92.2	396.0	672.8
13	12.5	504.6	5646.9	4586.7	4128.0	458.7	990.7	3137.3	312.8	752.9	75.3	1060.2	92.2	396.0	672.9
14	12.5	504.7	5647.4	4587.1	4128.4	458.7	990.8	3137.6	312.9	753.0	75.3	1060.3	92.2	396.0	672.9
15	12.5	504.7	5647.7	4587.4	4128.6	458.7	990.9	3137.8	312.9	753.1	75.3	1060.4	92.3	396.0	673.0
16	12.5	504.7	5647.9	4587.5	4128.7	458.7	990.9	3137.8	312.9	753.1	75.3	1060.4	92.3	396.0	673.0
17	12.5	504.7	5647.9	4587.5	4128.8	458.8	990.9	3137.9	312.9	753.1	75.3	1060.4	92.3	396.0	673.0
18	12.5	504.7	5648.0	4587.6	4128.8	458.8	990.9	3137.9	312.9	753.1	75.3	1060.4	92.3	396.0	673.0
19	12.5	504.7	5648.0	4587.6	4128.8	458.8	990.9	3137.9	312.9	753.1	75.3	1060.4	92.3	396.0	673.0
20	12.5	504.7	5648.0	4587.6	4128.8	458.8	990.9	3137.9	312.9	753.1	75.3	1060.4	92.3	396.0	673.0

Fig. E.2 Determination of mass flow rate of recycle nitrogen

Energy Requirements

1. Cultivation step

1.1. Input parametres

C_{PW}	C_{PP}	C_{PF}	C_{FI}	A_{TOT}	Q_{WW}	Q_{EFF}	$CO_{2,FED}$
(kw/ha)	(kwh/m3)	(kwh/m3)	(kwh/kg-CO2)	(ha)	(m3/day)	(m3/day)	(kg/day)
2	0.024	0.048	0.0222	23	1042	11296	9277

1.2 Energy Requirements

E_{PW}	E_{PP}	E_{PF}	E_{FI}
(Kw)	(kwh/day)	(kwh/day)	(kwh/day)
46	25.008	542.208	205.9494

2.Harvesting step

2.1 Input parametres

C_{DAF}	C_{cent}	C_{filt}	$Q_{W,DAF}$	$Q_{W,cent}$	M_{filt}
(Kwh/m^3)	(Kwh/m^3)	(kwh/kg)	(m^3/day)	(m^3/day)	(kg/hr)
0.1	5	0.01	455	62	1

2.2. Energy requirements

E_{DAF}	E_{cent}	E_{filt}
(kwh/day)	(kwh/day)	(kwh/day)
45.5	310	0.01

3. Extraction step

3.1. Input parametres

C_{hom}	$C_{E,ext}$	$C_{TH,ext}$	M_{Hom}	M_{oil}
(kwh/kg)	(kwh/kg)	(kwh/kg)	(kg/day)	(kg/day)
0.2	0.276	1.3	4129	991

3.2. Energy requirements

E_{Hom}	$E_{E,ext}$	$E_{TH,ext}$
(kwh/day	(kwh/day	(kwh/day)
825.8	273.516	1288.3

4. Tranasesterification step

4.1. Input parametres

$C_{E,tr}$	$C_{TH,tr}$	M_{BD}
(kwh/kg)	(kwh/kg)	(kg/day)
0.00038	0.68	753

4.2. Energy requirements

$E_{E,tr}$	$E_{TH,tr}$
(kwh/day	(kwh/day)
0.28614	512.04

5. Biogas prodcution step

5.1. Input parametres

$C_{E,Bg}$	$C_{TH,BG}$	$C_{E,upg}$	T_S	V_{BG}
(kwh/kg)	(kwh/kg)	(Kwh/m^3)	(kg/day)	(Km^3/day)
0.085	0.22	0.17	37516.2	7520

5.2 Energy requirements

$E_{E,BG}$	$E_{TH,BG}$	$E_{E,UPG}$
(kwh/day	(kwh/day	(kwh/day)
3188.88	8253.56	1278.4

Fig. E.3 energy requirements for the integrated process

Abbreviations in the flow diagram (Figure E-1)

WW- Waste water, **FG**- Flue gas, **CLAR**- Clarification, **PBR**-photo-bioreactor, **Ev. water**-Evaporated water, **SET**-settling, **DAF**-dissolved air floatation, **CEN**-Centrifugation, **CD**- cell disruption, **LE**-lipid extraction, **PS**-phase separation, **SR**-solvent recovery/recycle, **TR**-transesterification, **ME**-methanol evaporation, **RM**-recycle methanol, **MM**- makeup-methanol, **MS**- make up solvent, **FIL**-filtration, **FA**-filtered algae, **FW**-fresh water, **RW**-recycle water, **AD**-anaerobic digester, **GLY**-glycerol, **UPG**-upgrading, **BD**-biodiesel, **BG**-biogas, **N**-nitrogen, **P**-phosphorus, **DRY**- drying

Appendix F: cost estimation data and estimation

Table F. 1. Average Chemical Engineering Plant Cost Index (CEPCI) and Consumer Price Index (CCI)

Year	CECPI	CPI
1996	381.7	156.9
1997	386.5	160.5
1998	389.5	163.0
1999	390.6	166.6
2000	394.1	172.2
2001	394.3	177.1
2002	395.6	179.0
2003	402.0	184.0
2004	444.2	188.9
2005	468.2	195.3
2006	499.6	201.6
2007	525.4	207.342
2008	575.4	215.303
2009	521.9	214.537
2010	550.8	218.056
2011	585.7	224.939
2012	584.6	229.594

2013	567.3	232.957
2014	576.1	236.736
2015	556.8	237.017
2016	541.7	240.007
2017	567.5	245.12

	IRR	Service life		Total annual cost		Revenue1	vol. of biodisel	**MBSP**	**NPV**
	0.1	20		899441		731898	209222	**2.173**	**-0.0001**
Years	Cash flow	IRR	Dep.rate		PV				
0	-2445005	0.1	1		-2445005				
1	287189	0.1	0.9091		261081.2				
2	287189	0.1	0.8264		237346.6				
3	287189	0.1	0.7513		215769.6				
4	287189	0.1	0.6830		196154.2				
5	287189	0.1	0.6209		178322				
6	287189	0.1	0.5645		162110.9				
7	287189	0.1	0.5132		147373.6				
8	287189	0.1	0.4665		133976				
9	287189	0.1	0.4241		121796.3				
10	287189	0.1	0.3855		110723.9				
11	287189	0.1	0.3505		100658.1				
12	287189	0.1	0.3186		91507.38				
13	287189	0.1	0.2897		83188.53				
14	287189	0.1	0.2633		75625.94				
15	287189	0.1	0.2394		68750.85				
16	287189	0.1	0.2176		62500.77				
17	287189	0.1	0.1978		56818.89				
18	287189	0.1	0.1799		51653.53				
19	287189	0.1	0.1635		46957.76				
20	287189	0.1	0.1486		42688.87				
				Sum	-7.4E-05				

Figure F.1. Estimation of the MBSP ($/L) using spread sheet