INTEGRATED BIOETHANOL AND BIOELECTRICITY PRODUCTION FROM GHANAIAN SEAWEED BIOMASS

BY

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi

or any other educational institution, except where due acknowledgment is made in the thesis.

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DEDICATION

This thesis is dedicated to the memory of my uncle, the late Kwabena Okrah for his support, encouragement and inspiration.

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ABSTRACT

This study investigated the potential of integrating bioethanol and bioelectricity production technologies as an efficient means of maximising seaweed biomass utilization. It particularly sought to examine optimal conditions for the production of bioethanol from selected seaweeds, the bioelectricity potential of seaweed residue using microbial fuel cells and the sustainability of the integrated bioethanol and bioelectricity technologies on seaweeds. The motivation for this work is the growing concerns over the dire food securities issues that could occur from the continued use of edible biomass such as maize, cassava and sugarcane in commercial bioethanol production. The study was conducted through the screening of pretreatment methods, optimisation of dilute acid and enzymatic hydrolysis, screening of yeasts strains and

screening of ethanol production pathways with seaweeds as substrates. It also included the evaluation of power generation, internal resistances and substrate consumption from microbial fuel cells fed with seaweed bioethanol residue and; an Energy Return on Investment analysis of various seaweed bioenergy production scenarios. The study established that the optimal ethanol yields for Ghanaian seaweeds, *U. fasciata*, *H. dentata* and *S. vulgare* were 5.06, 2.44 and 3.69% dry matter, respectively. This was obtained via the SHF pathway through enzymatic hydrolysis with an optimal cellulase dosage of 8 filter paper unit/g dry biomass and fermentation with *S. cerevisiae* SI17, C8T17 or PT17 yeast strains. The study also found residues from seaweed bioethanol production to be efficient substrates for use in microbial fuel cells since, it yielded power densities between 0.46 and 0.50 W/m^3 which were comparable to sodium acetate by up to 52.62%. The integrated approach to seaweed biomass utilization was considered successful since waste generation was reduced to as low as 24.43% from a potential 79% from seaweed bioethanol production alone. It further established that the production of both bioethanol and bioelectricity from Ghanaian seaweeds would be sustainable based on the Energy Return on Investment value of 4.2 obtained after a Life Cycle Assessment of the bioenergy processes. The study further established that the production of bioethanol alone from seaweeds would not be sustainable commercially despite a net gain in energy from its Life Cycle Assessment.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
ATP	Adenosine Triphosphate
BM	Ball milling
BP	British Petroleum
CBP	Consolidated bioprocessing
CBU	Cellobiase unit
CE	Coulombic efficiency
CIA	Central Intelligence Agency
CNG	Compressed Natural Gas
COD	Chemical Oxygen Demand
CSIR	Centre for Scientific and Industrial Research
DC	Direct current
DCW	Dry cell weight
DM	Dry matter
E10	10% Ethanol Blended Fuel
E85	85% Ethanol Blended Fuel
ECG	Electricity Company of Ghana
EIS	Electrochemical Impedance Spectroscopy
EROI	Energy Return on Investment
FAO	Food and Agriculture Organization
FPU	Filter Paper unit
HMF	Hydroxymethyl furfural
HPLC	High Performance Liquid Chromatography
HR	High range
HSD	Honest Significant Difference
HTT	Hydrothermal treatment
IEA	International Energy Agency
IHRHW	Integrated-hydroxy-radicals-and-Hot-water-pretreatment
ISO	International organization for Standardization
KCCM	Korean Culture Centre of Microorganisms
KCTC	Korean Collection for Type Cultures
KNU	Kilo Novo Units
KNUST	Kwame Nkrumah University of Science and Technology
KOH	Potassium Hydroxide

LCA	Life Cycle Assessment
LPG	Liquefied Petroleum Gas
MEC	Microbial Electrolysis Cell
MFC	Microbial Fuel Cell
N	Normality
NAD+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide
NCYC	National Collection of Yeast Cultures
OD	Optical density
PAHBAH	4-Hydroxybenzoic acid hydrazide
PAP	Plasma-assisted pretreatment
PTFE	Polytetrafluoroethylene
R_2	Correlation coefficient
SHF	Separate Hydrolysis and Fermentation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous Saccharification Fermentation
STEX	Steam explosion
SUB-A	Pure substrate
SUB-B	Mixed substrate
TRS	Total reducing sugars
UK	United Kingdom
UN	United Nations
USA	United States of America
USD	United States dollar
VS	Volatile solids
WO	Wet oxidation
WRI	Water Research Institute

CHAPTER 1. INTRODUCTION

1.1 Background of the study

Fuels have become an indispensable source of energy needed in operating various combustion engines mostly found in private and commercial vehicles. In industries, it is used in operating various machinery for generating heat, electricity and moving mechanical parts. The commonest forms of fuels used in Ghana for the aforementioned purposes include; gasoline (petrol), diesel, kerosene, liquefied petroleum gas (LPG) and compressed natural gas (CNG). These forms of liquid fuels are all derivatives of crude oil and natural gas commonly referred to as fossil fuels.

The global reserves-to-production ratio for 2015 on crude oil stood at 50.7 (BP, 2016). This ratio refers to the proven oil reserves at the end of 2015 divided by the production for the year indicating the length of time that those reserves would last if the current production rate were to continue. In a nutshell, the global proven oil reserves of 1,697.6 billion barrels would be depleted in 50.7 years at the 2015 crude oil production rate (BP, 2016). These projections are rather worrying since the global oil consumption grew to 1.9 million barrels per day for 2015 or 1.9% which is nearly double the recent historical average growth rate of 1%. This is also significantly larger than the oil consumption for 2014 of 1.1 million barrels per day (BP, 2016).

In 2015, the total petroleum products consumed in Ghana was approximately 65,000 barrels per day which comprised of 33% gasoline and 54% diesel with the rest representing LPG, kerosene and other fuels. Ghana's Jubilee field produced 38.8 million barrels of crude oil for the year 2015 at a daily production rate of 105,935 barrels (Energy Commission, 2016). Ghana's proven crude oil reserves as at January 2016 was an estimated 660 million barrels (CIA, 2016). The country therefore has a reserves-to-production ratio of 17.01 based on the 2015 production data. This indicates that in approximately 17 years Ghana's proven crude oil reserves would be completely depleted if no new reserves are discovered. The need for the development and promotion of alternative sources of fuel is therefore very urgent both on a global and local scale if the current annual global consumption of crude oil continues to grow at a rate of 1.9% or higher.

Fossil fuels currently account for over 80% of the global total primary energy supply (IEA, 2013). Its combustion results in the release of greenhouse gases such as carbon

dioxide, nitrogen oxides, sulphur oxides, carbon monoxide and volatile organic compounds (Van den Hende *et al*., 2012). Carbon dioxide is the greenhouse gas of the most significant concern since it is considered to trap heat in the earth's atmosphere causing the phenomenon referred to as "global warming" (Borines *et al*., 2013). The concentration of carbon dioxide in the atmosphere was estimated as 380ppm in 2010 and was projected to increase to 450 ppm by 2020 if measures are not taken to mitigate its emission (Kraan, 2013).

The growing concerns over greenhouse gas emissions, growing energy demand coupled with fuel insecurity resulting from the rapid depletion of fossil fuel reserves has led to the extensive development of various renewable energy technologies (Adams *et al*., 2009). Since crude oil and natural gas are considered non-renewable sources of fuel, the most sustainable alternatives currently are energy from the sun, wind, water and biomass. These renewable sources of fuel and energy currently provide the best solutions to the rapid depletion of our proven oil reserves. Solar, wind and hydro-energy technologies are used typically in the generation of heat and electricity. Biomass is currently the only form of renewable energy capable of producing commercial liquid fuels (Adams *et al*., 2009). Fuels produced from biomass include bioethanol, biodiesel, biogas, biobutanol and bio-oil. These are collectively referred to as biofuels.

Biofuels are currently being used commercially in fuel blends with gasoline and diesel especially in the US and Brazil (Borines *et al*., 2013). They are used in blends such as the E10 (contains 10% v/v biofuel) and E85 (contains 85% v/v biofuel). The former is available for use in any combustion engine and the latter specifically used in flex-fuel engines (Kim and Dale, 2006). Bioethanol is the most widely used transport biofuel globally with a combined total production of 86 billion litres in the year 2010 (Chakraborty *et al*., 2013).

Brazil, U.S. and Canada are the leading producers of bioethanol (Baeyens *et al*., 2015). The largest major use of bioethanol is as fuel for engines and also as a fuel additive (Baeyens *et al*., 2015). Commercial bioethanol production has been limited to edible feedstock such as sugarcane, corn, sugar beet and rapeseed (Chakraborty *et al*., 2013). In Ghana, commercial bioethanol is produced from cassava which is also cultivated as

a staple food in the country (Ghana Trade Portal, 2016). The continued use of edible feedstock for commercial bioethanol production could result in issues over food security, competition for arable land and fresh water use and the excessive use of pesticides and fertilizers (Kraan, 2013).

Edible biomass such as sugarcane, corn, sugar beet and cassava used in the production of bioethanol are referred to as first generation biomass. The concerns over food security surrounding first generation biomass has led to the consideration of lignocellulosic biomass such as wood chips, agro residue and other non-edible biomass collectively referred to as second generation biomass. These do not compete with edible biomass for use as food. They however require harsher conditions for pretreatment and hydrolysis due to the presence of high concentrations of lignin in their cellular structure (Trivedi *et al*., 2013). They also compete with edible biomass for the use of arable land and fresh water for their cultivation. These concerns have stifled the commercial development of lignocellulosic biomass as a substrate for bioethanol production and has also led to the development of the third generation biomass. Third generation biomass refers to algal biomass which includes macroalgae and microalgae (Khambhaty *et al*., 2012).

Macroalgae commonly referred to as seaweeds, is higher in carbohydrates than microalgae and is considered a good substrate for bioethanol production (Meinita *et al*., 2015). The main benefits derived from the use of seaweeds over terrestrial biomass include: higher biomass production rate per unit area; higher growth rate; no competition with food crops for land use; requires no agricultural input such as fertilizers, pesticides and water; and is also cost-effective in pre-treatment (Jones and Mayfield, 2012).

Bioethanol production from seaweed is known to utilise the carbohydrate fraction which constitutes 30-60% of the entire biomass (Hong *et al*., 2014). Of the carbohydrate fraction, 50-70% is often converted to fermentable reducing sugars which is usable (Kostas *et al*., 2015). Bioethanol has been produced from seaweed species belonging mainly to the genera *Kappaphycus*, *Gelidium*, *Gracilaria*, *Sargassum*, *Ulva* and *Laminaria* (Hargreaves *et al*., 2013; Chirapart *et al*., 2014).

Seaweeds have also been used briefly as substrates in bioelectricity production using microbial fuel cells (MFCs) (Velasquez-Orta *et al*., 2009). MFCs are bioelectrochemical systems used in the production of bioelectricity through the activity

of electrogenic bacteria (Logan *et al*., 2006). This technology if fully developed commercially could support in subsidizing the electrical energy deficits in developing countries such as Ghana. Energy deficits in Ghana's energy sector are caused by the over-reliance on thermal facilities which also depend on depleting fossil fuel reserves (ECG, 2013). Other energy deficits include; the absence of electricity in some rural communities and the current high electricity demand by consumers as against the supply from providers (ECG, 2013).

The production of bioethanol alone as an alternative fuel from seaweeds leaves large amounts of organic rich residues made up of residual carbohydrates, proteins and lipids available as waste. These could serve as adequate substrates for microbial fuel cells in the production of bioelectricity. The development of the bioethanol and bioelectricity technologies directly respond to targets 1 and 2 of the *UN Sustainable Development Goal 7: Affordable and Clean Energy* which states, "By 2030, ensure universal access to affordable, reliable and modern energy services" and "By 2030, increase substantially the share of renewable energy in the global energy mix", respectively (UN, 2015). The technologies would also directly respond to the renewable energy policy goals of Ghana which includes: achieving a 10% contribution of modern renewables (excluding large hydro and wood fuels) in the electricity generation mix by 2020; reducing the demand on wood fuels from 72% to 50% by 2020; and promoting the development and use of biomass technologies such as biofuels and waste-to-energy (Renewable Energy Act, 2011).

This study therefore seeks to integrate two unique biotechnologies in the form of the bioethanol production and microbial fuel cell technologies in a biorefinery approach to co-produce ethanol and electricity, respectively from selected Ghanaian seaweeds while assessing their waste for soil amendment qualities. Emphasis will be on process conditions which will optimally harness the carbohydrates fraction of the biomass for bioethanol while the residual biomass would be used in bioelectricity production.

1.2 Problem statement

Global fossil fuel reserves are depleting at an alarming rate due to the growing consumption of petroleum products. Renewable alternative fuels such as bioethanol from biomass is therefore urgently needed. Developing countries such as Ghana could

face dire food security issues if edible biomass such as maize, cassava and sugarcane continues to be used in the production of bioethanol. Seaweeds (macroalgae) are therefore recommended in this study as suitable substrates for bioethanol production. The production of bioethanol alone from seaweeds leave large amounts of organic rich residues (30-75% of seaweed biomass) made up of residual carbohydrates, proteins and lipids available as waste. This waste if not controlled would decay to release greenhouse gases. A biorefinery approach which involves the application of multiple technologies on the same biomass will therefore have to be employed in the processing of seaweeds.

1.3 Objectives of the study

1.3.1 Main objective

To assess the potential of integrating bioethanol and bioelectricity production technologies as an efficient means of maximising seaweed biomass utilization.

1.3.2 Specific objectives

1. To examine the optimal bioethanol production yield from seaweed biomass through the variation of its processing conditions.
2. To determine the bioelectricity production potential of seaweed residue from bioethanol production.
3. To use life cycle assessment to investigate the sustainability of producing both bioethanol and bioelectricity from seaweeds in an integrated approach.

1.4 Research hypothesis

1. The ethanol yield from Ghanaian seaweeds is influenced by hydrolysis parameters, fermentation pathway and yeast strain selection.
2. Residues obtained from the production of bioethanol from Ghanaian seaweeds are efficient substrates for bioelectricity production in microbial fuel cells.
3. The integrated processing of bioethanol and bioelectricity from seaweeds is a sustainable bioenergy production process.

1.5 Significance of the study

Bioethanol is considered a viable alternative renewable fuel to help reduce the consumption of fossil fuels and also reduce the emission of greenhouse gases. The current use of edible biomass such as sugarcane, maize and cassava in commercial bioethanol production could cause dire food security issues especially in developing countries such as Ghana. This study introduces Ghanaian seaweeds as an efficient alternative biomass which can be processed using the biorefinery approach to maximise its use. Specifically, the study has the following significance:

- This study is expected to establish the prospects of Ghanaian seaweeds as a substrate for bioethanol production and its residue as a substrate for bioelectricity production.
- This study hopes to maximise the efficiency of seaweed biomass use, minimize waste and also improve its economic value through the integrated application of the bioethanol and bioelectricity production technologies.
- This study will also establish the optimal process conditions under which the highest yield of bioethanol from Ghanaian seaweeds can be obtained.
- The data and information obtained would serve as an adequate basis for ethanol producing industries to consider the transition from the use of edible terrestrial biomass to algal biomass as feedstock.
- The study hopes to establish an efficient biorefinery process pathway to which various forms of biomass can be applied for their efficient use and improved economic value.
- The study hopes to determine whether the proposed biorefinery technologies for seaweed processing would be sustainable.

1.6 Limitations of the study

- This study was performed solely on an analytical laboratory scale basis. The direct transfer of process conditions from this scale to the commercial scale may not result in similar yields of the target products.
- All samples and materials used in this study were collected solely in Ghana. Their effect, results and observations were therefore reported relative to conditions in described in this text only. Replication of this study in other climatic regions may therefore not yield the same results.

- This research is limited to the use of a double chamber MFC configuration only. The use of other configurations will not necessarily yield the same results for parameters to be measured and calculated.
- Electrical circuits vary greatly in their components and outputs. The results are therefore reported relative only to the electrical circuit constructed for this research and other similar circuits.

1.7 Organisation of the research

This research involved the collection of performance data on the hydrolysis and fermentation of selected seaweed species under various conditions to obtain the optimal condition for high bioethanol yield. The performance of various hydrolytic catalysts and fermenting organisms used were evaluated during the bioethanol production process. Residues from the bioethanol production processes were recovered, characterised and introduced into microbial fuel cells as substrates for bioelectricity generation. Electrical, electrochemical and biochemical analytical tools were used to evaluate the performance of the residues during the bioelectricity production process. Effluents from the MFCs were examined for components useful in soil amendment. The integrated biorefinery processes were evaluated for their sustainability as against other bioenergy processes. Conclusions and derivations were drawn from data trends along with measured and calculated parameters relevant to the study.

1.8 Conceptual framework of the study

The entire concept of the study is built around the biorefinery approach to biomass conversion. This approach emphasizes the use of multiple technologies in the processing of biomass primarily to reduce waste and add more value to the biomass. In this study, bioethanol and bioelectricity production processes are integrated to obtain ethanol (C_2H_6O) and electric current (e^-) as shown in Figure 1-1.

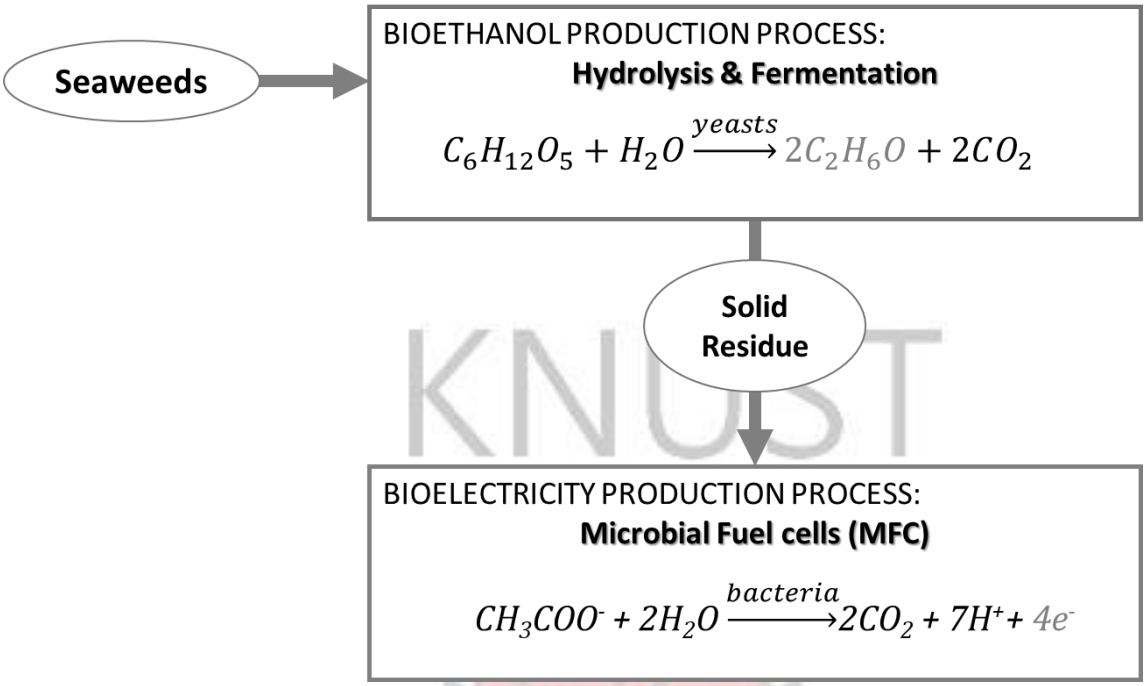

Figure 1-1. Conceptual framework used in seaweed processing in this study

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Renewable energy systems such as wind turbines and photovoltaic systems are known to be most suitable in the production of heat and electricity (Adams *et al*., 2015). Biomass however, is unique in its capacity to produce not only heat and electricity but also transport fuels and other useful platform chemicals (Adams *et al*., 2015). Despite its importance, the use of biomass in energy production faces a lot of challenges especially in the competing use of arable land for food crop production. The prospect of marine biomass as an alternative substrate for bioenergy production is highest in Europe, Africa and the Americas where it is rarely cultivated for food (Adams *et al*., 2015). The succeeding sections seek to outline the current developments made in the use of seaweeds as sole source of substrate in bioethanol and bioelectricity production technologies. Emphasis would be on the current methods used especially in the hydrolysis and fermentation stages of bioethanol production. Some sections would also offer some insight into bioelectricity generation using the microbial fuel cell

technology. The concluding sections would briefly review some integrated biorefinery processes.

2.2 Overview on biofuel production from algae

Biofuels which refers generally to fuels produced from biomass, are considered the most viable and eco-friendly alternatives to fossil fuels currently available. They include; bioethanol, biodiesel, biogas and bio-butanol. Bioethanol and biodiesel are the most widely used commercial transport biofuels globally. Unfortunately, the commercial production of these fuels come from edible biomass such as maize, sugarcane, cassava, soybean, rapeseed and palm oil. Several studies have recommended the use of macroalgae which is high in carbohydrates and microalgae which is high in lipids as substrates in the production of bioethanol and biodiesel, respectively (John *et al*., 2011).

Generally, the processes used in bioethanol production from biomass includes pretreatment, hydrolysis, fermentation and distillation (Borines *et al.*, 2013). The pretreatment process is done through physical, chemical and or biological methods to increase the efficiency of the hydrolysis process especially when the catalyst to be used is an enzyme. Hydrolysis is done to breakdown the complex sugars in the biomass to simple sugars. The fermentation process involves the use of an organism such as fungi or bacteria to convert the simple sugars to ethanol. The ethanol is recovered through a distillation process along with an optional dehydration process to improve the quality of the product.

Biodiesel refers to methyl esters of fatty acids obtained through the transesterification of triglycerides from biomass. It has similar characteristics to conventional petroleum diesel due to similarities in their higher heating value, flash point and kinematic viscosity. The higher heating of biodiesel can be as high as 41 MJ/kg which is comparable to the 43 MJ/kg from petroleum diesel (Demirbas, 2009). The common production process involves the extraction of the oils with a solvent from the biomass followed by the transesterification of the extracted oil using a short chain alcohol such as methanol. This study will focus primarily on the production of bioethanol largely because it can be economically and efficiently produced from seaweeds (Cho *et al*., 2013, Chen *et al*., 2015).

Currently, biofuels have been grouped into three based on the type of biomass used in its production namely the first, second and third generation biofuels. The firstgeneration biofuels are produced primarily from edible feedstock such as maize, soybean, palm oil, sugarcane and cassava (Borines *et al.*, 2013). The secondgeneration biofuels are produced from non-edible biomass such as agro-residues, wood, municipal solid wastes and other lignocellulosic biomass (Kemausuor *et al*., 2014). The third-generation biofuels are produced from marine biomass such as micro and macroalgae (seaweeds) (Daroch *et al*. 2013).

Marine biomass accounts for over 50% of primary biomass produced globally but has been the least harnessed for various applications (Adams *et al*., 2011). It is grouped into two, namely macro and microalgae. Both groups have been used in the production of various biofuels. Microalgae has been explored predominantly as substrate for biooils and biodiesel while macroalgae has been used mainly in bioethanol and biogas production (Adams *et al*., 2011). This study would focus on the prospects of macroalgae commonly known as seaweeds.

2.3 Seaweeds as substrates for bioethanol production

Commercial bioethanol is currently produced predominantly from maize, sugarcane and cassava. This has caused an on-going global debate over the use of these substrates as food as against fuel. For developing countries like Ghana, the continued used of biomass such as maize and cassava, which are staple foods across the country, for fuel production could cause serious food security issues. There is therefore the need for efficient alternative substrates which are not cultivated as food in the sub-region. Seaweeds are being proposed in this study largely because they have higher growth rate and yield per hectare than terrestrial biomass; do not compete with food crops for land use and; do not require agricultural inputs such as fertilizers and fresh water (Cho *et al*., 2013). They are also being considered as a viable substrate for bioethanol production in particular because of their high carbohydrate content, ranging between 25-70% (Cho *et al*., 2013).

Marine macroalgae commonly known as seaweeds are plant-like multicellular organisms that live attached to hard substrata such as rocks in coastal areas (Ab Kadir *et al*., 2014). Their basic structure consists of a thallus, which forms the body of the organism and a holdfast, a structure on its base which allows it to be attached to hard

surfaces such as rocks near the shoreline of coastal areas. Brown seaweeds are the largest in size, growing up to 4m in length for some species. Green and red seaweeds are smaller ranging from a few centimetres in some species to a meter in others (Schultz-Jensen *et al*., 2013).

The seaweed industry was valued at USD 5.5-6 billion in the year 2003 (McHugh, 2003). Its use as food accounted for up to 5 billion of this amount while other uses such as hydrocolloids production was valued at about USD 1 billion. There were also small contributions from its use as fertilizer and animal feed additive. According to the FAO (2013), 8.2 and 15.8 million tons of brown and red seaweed, respectively were produced in the year 2013. This was valued at USD 1.3 billion and 4.1 billion for the brown and red seaweeds, respectively. For the green seaweed 14,739 tons valued at USD 15.7 million was produced globally in the year 2013 (FAO, 2013). The enormous difference in the production values of the brown and red from the green seaweed can be attributed to the valuable hydrocolloids such as alginate, carrageenan and agar found only in the red and brown seaweeds.

The structural differences found between land-based plants and seaweeds gives seaweeds an advantage of a higher yield per hectare. In comparison to land-based plants, seaweeds have an average yield per hectare per year of 730,000 kg while sugarcane, sugar beet, maize and wheat have 68,260; 47,070; 4,815 and 2,800 kg, respectively (Adams *et al*., 2009). The high yield from seaweed in general is attributed to the low energy required in the formation of its supporting tissue during growth. Seaweeds can also absorb nutrients across its entire surface and can be cultivated three dimensionally in water (Adams *et al*., 2009).

2.4 Composition of seaweeds

Seaweeds are composed of carbohydrates, proteins, lipids and minerals which ranges from 30-60%, 10-40%, 0.2-3% and 10-40%, respectively (Lee *et al*., 2014). Besides their unique and varying composition, seaweeds have been grouped into three, based on their pigmentation. They are rhodophyceae (red seaweeds), phaeophyceae (brown seaweeds) and chlorophyceae (green seaweeds) based on their pigments rphycoerythrin, chlorophyll and xanthophyll, respectively (Borines *et al.*, 2011).

Seaweed composition has been found to vary based on several factors such as the season, availability of nutrients, water salinity and availability of sunlight (Dahiya,

2015). The seaweed component of primary importance to bioethanol production is the carbohydrates (polysaccharides), since they currently form the only fraction that can be fermented to ethanol. Generally, seaweeds are composed of large fractions of complex sulphated polysaccharides which are uniquely different in each group serving as their cellular storage and structural support tissue (Chirapart *et al*., 2014). The subsequent sections will seek to highlight the various types of polysaccharides (carbohydrates) in the three groups of seaweeds and their cellular structure.

2.4.1 **Polysaccharides in green seaweeds (chlorophyceae)**

The polysaccharides found in green seaweeds include ulvan, starch and cellulose. These polysaccharides range between 30-60% of the entire biomass. Ulvan found mainly in seaweeds belonging to the genera *Ulva*, is made up of various oligosaccharide units of L-rhamnose-3-sulfate and D-xylose-2-sulfate (Lee *et al*., 2014). Ulvan is a water-soluble hydrocolloid with a myriad of applications in the food, pharmaceutical and chemical industries primarily as a gelling agent (Trivedi *et al*., 2016). It is generally considered the weakest in the family of hydrocolloids found in seaweeds.

Starch and cellulose usually found in terrestrial plant tissue are also found in green seaweeds. They are both made up of monomeric units of glucose but are different in molecular structural configuration. The structural difference is found in the anomeric carbon (C1) configuration which is β- in cellulose and α- in starch (McNamara *et al*., 2015). Cellulose has a very stable crystalline structure made up of a regular linear chain with 1,4-β-glycosidic linkages in parallel linear arrays. Their structural chains have hydrogen and van der Waal bonds which makes them very strong and highly resistant to physical and biological breakdown. In contrast, starch has a loosely bonded helical molecular configuration making it more susceptible to biological and physical degradation (McNamara *et al*., 2015).

2.4.2 **Polysaccharides in red seaweeds (rhodophyceae)**

Red seaweeds are group into two based on the type of phycocolloid found in their structure. They are, carrageenophytes which contain carrageenan and agarophytes which contain agar. Both groups however have the polysaccharide cellulose as a common component. The agar in agarophytes is made up of the sulphated-galactans; D-galactose and 3,6-anhydro-a-lactose. Agar is produced commercially from

seaweeds belonging to the genera *Gelidium* and *Gracilaria.* It is used predominantly as a gelling agent in the food industry (McHugh, 2003). It also has some microbiological and pharmaceutical applications.

Carrageenan found in carrageenophytes consists of linear alternating and repeating sulphated-galactan units of 3-linked β-D-galactopyranose and 5-linked α-Dgalactopyranose (Yun *et al*., 2013). It is extracted in commercial quantities from various species of the genera *Kappaphycus, Chondrus* and *Eucheuma*. Carrageenan is group into three namely *iota, kappa* and *lambda* based on their gelling properties. The *iota* and *kappa* carrageenans form gels with calcium and potassium salts, respectively. The *lambda* carrageenan however forms a viscous solution without gels (McHugh, 2003). Carrageenan is used particularly in the dairy food industry.

2.4.3 **Polysaccharides in brown seaweeds (phaeophyceae)**

Polysaccharides in brown seaweeds are the most diverse among the three groups of seaweeds (Table 2-1). They include laminarin, mannitol, cellulose, alginate and fucoidan. The fractions of these polysaccharides were found to be 0-30% laminarin, 4-25% mannitol and 17-34% alginate in the species *Laminaria hyperborea* (Horn, 2009). Laminarin forms the main storage polysaccharide in most brown seaweeds. It has β-1,3 glucan chains with a high degree of polymerisation especially in the *Laminaria* sp. (Adams *et al*., 2011). Mannitol, a sugar alcohol is formed from the reduction of the monomeric sugar mannose. Its primary function is osmoregulation in the cells in brown seaweeds (Borines *et al.*, 2011).

Laminarin and mannitol content in brown seaweeds varies throughout the year. In a study of the *Laminaria digitata* species, the concentration of the two polysaccharides were reported to be lowest from January to April and August to December at less than 5% of dry biomass. They were however highest in June and July at 32 and 25% dry matter (DM) for mannitol and laminarin, respectively (Adams *et al*., 2011). This rise and fall in concentrations correspond directly to the seasonal changes in the weather. Biomass cells often form cellular storage tissue at a higher rate in the summers, but a decline is often observed in the winters (Adams *et al*., 2011). The hydrolysis of laminarin can be efficiently achieved enzymatically using laminarinase (endo-1,3(4)β-glucanase) and cellulases (endo-1,4(4)-β-glucanase) (Masarin *et al*., 2016). The oxidation to fructose is however required for mannitol by a dehydrogenase for reducing sugar recovery (Borines *et al.*, 2011).

Alginate (or alginic acid) is composed of repeating linear co-polymers of the uronates, β-D-mannuronate and α-L-guluronate which are linked by 1,4-glucosidic bonds in various sequential arrangements (Enquist-Newman *et al*., 2013). They are found in the cell wall and are considered very critical during bioethanol production largely because they could form up to 50% of the entire carbohydrate fraction of the brown seaweed biomass.

Fucoidan, which is often the brown seaweed polysaccharide of lowest concentration, is made up of L-fucose and sulphated ester groups including sulphated fucogalacturonans (Ale and Meyer, 2013). It has been extracted commercially from brown seaweeds from the genera *Undaria*, *Laminaria* and *Sargassum*. There have been several reports of fucoidan possessing some antixodant, anticoagulant, antiviral, therapeutic and other beneficial biological and pharmacological properties (ye Lee *et al*., 2013).

Table 2-1. Composition of sugars in seaweeds

Type of sugar	*Chlorophyceae (Green seaweed)*	*Rhodophyceae (Red seaweed)*	*Phaeophyceae (Brown seaweed)*
Polysaccharide	Mannan	Carrageenan (galactan)	Laminarin
	Ulvan	Agar (agaran and agaropectin)	Mannitol
	Starch	Cellulose (Fibrin)	Alginate
	Cellulose	Lignin	Cellulose
			Fucoidan
Monosaccharide	Glucose	Glucose	Glucose
	Galactose	Galactose	Galactose
	Arabinose	Fucose	Fucose
	Xylose	Xylose	Xylose
	Uronic acid	Rhamnose	Mannose
		Arabinose	Uronic acid

(modified from Chen *et al.,* 2015)

2.5 Cultivation of seaweeds

The choice, availability, and consistent supply of raw materials is critical for the efficient production of biofuels. In the context of bioethanol, the cultivation methods for maize, sugarcane and cassava have been well established and improved for decades. This makes raw material supply a lot easier for bioethanol industries. The selection of seaweeds therefore as an adequate alternative to these crops can only be fully viable if their cultivation methods are also well established. Interestingly, the concept of farming seaweeds has been practiced for decades and is practiced mostly near shore or off shore in various parts of Asia. The concept of off-shore farming or cultivation is not clearly defined since cultivation methods such as tidal flat farms, floating cultivation, ring cultivation and wind-farm systems which are done in the sea are all described as offshore cultivations (Buck and Buchholz, 2004).

The cultivation of seaweeds requires some fundamental conditions. These include: subtidal or sheltered coast; substrata such as the seafloor, ropes or rocks along the beach; photons from the sun for its photosynthetic activity and; nutrients such as nitrogen, carbon and phosphorus. Nutrients such as nitrogen are available in large concentrations in seawater as nitrates and ammonium. Phosphorus is obtained as phosphates which are dissolved into the seawater from the land while carbon is obtained from dissolved CO_2 from the atmosphere. These conditions which can be found naturally along sheltered coasts are good indicators that seaweed can efficiently and sustainably be cultivated with very little effort by way of land use, fresh water use and fertilizer application as required by terrestrial plants.

To successfully cultivate seaweeds, several dynamic environmental factors must be considered. These include temperature, salinity, water quality, nutrient concentrations and the possible presence of toxins (Martins *et al*., 1999). The natural influences of seasonal changes, rainfall patterns and the abundance of sunshine influence the growth pattern of the seaweeds (Lapointe *et al*., 2004). Human activities such as fishing, aquaculture, open defecation and wastewater disposal also play a significant role in the growth of seaweeds (Sanderson *et al*., 2008). These factors largely affect the composition of the seaweeds.

Seaweeds can be obtained through capture in the wild or purposely cultivated. Various cultivation methods have been used in the cultivation of seaweeds since the 1980s. The most common and cost-effective is the attachment of seaweeds or their seedlings to

ropes and nets at the sea shoreline (Peteiro *et al*., 2012). Other controlled methods include growing seedlings indoors or in greenhouse tanks before being transplanted on to ropes in the sea (Peteiro *et al*., 2014). The use of ropes during the cultivation at sea can be done in a horizontal, vertical or concentric arrangement (Buck and Buchholz, 2004; Peteiro *et al*., 2012).

The horizontal rope system was used in the cultivation of the seaweeds, *Undaria pinnatifida* and *Laminaria saccharina*. It yielded 5.9 and 8 kg/m (wet weight) after 5 and 8 months of cultivation, respectively (Peteiro *et al*., 2012). The vertical rope system was also used when growing *Palmaria palmata* and *Saccharina latissima* yielding 1 and 28 kg/m meter (wet weight) of top rope (horizontal) per year, respectively (Sanderson *et al*., 2012). The concentric rope system was used on *L. saccharina* yielding 4 kg/m (wet weight) after a year (Buck and Buchholz, 2004). Harvesting methods for naturally growing seaweeds include; hand-picking, thalli cutting and boat harvesting (Figure 2-1) (Kirkman and Hendrick, 1997).

The largest producers of seaweeds are from Asia where it is cultivated mainly as food. The world production of aquatic plants which includes seaweeds was at 26.9 million tons in 2013, valued at 6.7 million dollars. China is the world largest producer of seaweeds, producing up to 13.5 million tons in 2013. Zanzibar is the largest seaweed producer in Africa, producing up to 110,000 tons in 2013. Other producers include Phillipines, South Korea, Indonesia, Denmark, France and South Africa (FAO, 2013).

Figure 2-1. Seaweed harvesting through hand picking (right) and with a boat (left)

2.6 Seaweeds along the coast of Ghana (species and distribution)

The 540-km coastline of Ghana is very rich with diverse species of seaweeds growing naturally. Currently, seaweed is not cultivated in Ghana. It has also not been commercially valorised in any form. In countries such as the Philippines, Indonesia and Tanzania, seaweed cultivation has had an enormous socio-economic impact on the livelihood of coastal communities (Valderrama, 2012). It has served as an alternative livelihood for some fishermen and reduced overfishing. Ghana is known to have the largest diversity in algae across West Africa with up to 200 species documented (Bolton *et al*., 2003). This indicates that Ghana has a lot of potential for seaweed production which could improve the livelihood of coastal communities. Algal blooms, which refers to the rapid increase in the population of algae in an aquatic system, is prevalent along the west coasts of Ghana and has been described by residents as a menace since it clogs the nets of fishermen and also causes a foul smell when it decays on the shore (Figure 2-2).

Figure 2-2. Algal bloom along the west coast of Ghana

Species from all the three groups of seaweeds have been identified across the coasts of Ghana (Figure 2-3). Green seaweeds species that have been found included; *Entocladia viridis, Boodlea composite, Bryopsis stenoptera, Caulerpa racemose, Caulerpa sertularioides, Caulerpa taxifolia, Chaetomorpha linum, Cladophora conferta, Codium guineense, Ernodesmis verticillate, Rhizoclonium africanum, Ulva fasciata, Ulva flexuosa* and *Ulva rigida* (John *et al*., 2004).

The red seaweed species found include; *Acrochaetium dasyae, Amphiroa rigida, Bornetia secundiflora, Bostrychia radicans, Bostrychia tenella, Callithamnion granulatum, Ceramium cornutum, Champia vieillardii, Chondria capillaris, Dasya*

baillouviana, Dictyurus fenestratus, Gelidiopsis variabilis, Gelidium corneum, Gelidium crinale, Gracilaria armata, Gracilaria cervicornis, Gracilaria rangiferina (also known as *Hydropuntia dentata*), *Hypnea divaricate, Hypnea musciformis, Iridaea elongate, Laurencia flexuosa, Lomentaria patens, Mesophyllum canariense* and *Naccaria wiggii* (John *et al*., 2004).

Species of brown seaweeds found include; *Asteronema breviarticulatus, Colpomenia sinuosa, Dictyota bartayresiana, Dictyota cervicornis, Dictyota ciliolate, Hincksia mitchelliae, Levringia brasiliensis, Lobophora variegate, Padina antillarum, Padina durvillaei, Padina Mexicana, Ralfsia expansa, Rosenvingea intricate, Saccorhiza polyschides, Sargassum filipendula, Sargassum vulgare* and *Sphacelaria brachygonia* (John *et al*., 2004).

The distribution of these seaweeds has been along both the east coast and the west coast of Ghana. A recent survey by the Centre for Scientific and Industrial Research-Water Research Institute found *U. fasciata, H. dentata, H. musciformis, P. durvillaei* to be the most densely distributed along the beaches of towns on the east coast such as Old Ningo, Sakumono, Tema New Town, Prampram, and Ahwiam (Addico, 2015). They also found a similar distribution of these same species but with *S. vulgare* being the most dominant on the west coast in towns including Butre, Mumford, Apam, Komenda, Aboadze, Shama, Takoradi and Lower Dixcove (Addico, 2016). There were small traits of *Ulva lactuca*, *Hypnea cervicornis*, *Padina antillarum*, *Sargassum fluitans* and *Sargassum Natans* also identified in these areas.

Figure 2-3. Some seaweed species found along the coast of Ghana (left to right: G. cervicornis, P. antillarum, U. rigida)

2.7 Seaweed biomass handling and pre-treatment

Handling of seaweed biomass after harvesting is considered very critical for any bioconversion process. Poor handling could lead to the accelerated decay of the seaweed especially during transportation and storage. It could also lead to process

contamination from debris such as sand, aquatic plants and animals which are often harvested unintentionally. The primary means of handling seaweed after harvest are washing, sorting, drying and size reduction. These processes also serve as some form of biomass pretreatment.

Seaweeds are often washed immediately with water after harvesting or just before processing to remove debris, salt crystals, sand and any other impurities (Chirapart *et al*., 2014; Talebnia, 2015). In most studies, it has often been done with distilled water (Kim *et al*., 2015) and tap water (Adams *et al*., 2015). Interestingly, washing has been reported to have a considerable effect on the properties of the seaweed biomass. In a study to determine the effect of washing or otherwise on the brown seaweed, *L. digitata*, it was observed that there was a change in its carbohydrates content (Adams *et al*., 2015). A loss of up to 49.3% DM of laminarin (a polysaccharide) was observed after washing continuously under tap water. This is considerably high, but the combined effect of the presence of debris, salts and sand in the process streams of a commercial bioethanol plant could also have damaging effect on equipment such as pipes, tanks and pumps. The overall effect of not washing on capital intensive equipment certainly makes seaweed washing an important necessity.

Drying of the seaweed is also considered an important handling process since the moisture content of freshly harvested seaweeds is known to be in the range of 85-90% (Chen *et al*., 2015). Drying methods for seaweeds used in previous studies include sun drying (Cho *et al*., 2013), oven drying (Ge *et al*., 2011) and freeze drying (Adams *et al*., 2015). All these individual drying methods have unique effects on the properties of the biomass. A study compared the effect of oven drying, frozen-oven drying and freeze drying on the composition of *L. digitata*. It was reported that the carbohydrate, laminarin which is found in large concentrations in brown seaweeds was 109.4, 108.0 and 147 mg/g dry biomass for oven dried, frozen-oven dried and freeze-dried seaweed, respectively (Adams *et al*., 2015). Freeze drying turned out to be the most efficient retaining the bulk of laminarin in the biomass. The economic benefits of freeze drying over the others would need to be compared to conclusively recommend it over the other drying methods. The drying process is often done by seaweed farmers prior to packaging and transportation so the commercial-scale feasibility of freeze drying method for peasant farmers may have to be evaluated.

The sun drying method even though difficult to control, is quite an economical, convenient and sustainable option for seaweed farmers. A loss in pigmentation or decolourization of the seaweed may occur because of the exposure to sunlight but its overall effect on the carbohydrate content is currently unknown. The use of non-dried seaweeds has not been considered in literature largely because the seaweeds can only be stored and transported economically in the dry form.

Size reduction or grinding of seaweeds is also a critical handling process and an efficient pretreatment method since it increases the surface area of the biomass. Seaweed substrate sizes reported in previous studies for bioethanol production range between 20 and 200 mesh (Ge *et al*., 2011, Cho *et al*., 2013). This enhances the activity of catalysts used in the subsequent hydrolysis and fermentation processes of bioethanol production. Size reduction also minimizes the bulk volume of biomass to facilitate its easier transportation and storage.

2.8 Hydrolysis of seaweeds

The hydrolysis process in bioethanol production is one of the most limiting stages in the entire production process since it is the stage where the sugars to be converted to ethanol is obtained. Hydrolysis simply refers to cleavage or division through the addition of water molecules. In the context of complex sugars (polysaccharides), it involves the use of a water molecule by a catalyst to break the glycosidic linkages within polymeric sugars (di-, tri-, oligo- or polysaccharide) to their monomeric form (monosaccharides or reducing sugars) as seen in Figure 2-4. During the cleavage of sugars, a hydrogen atom (H^+) is gained by one part of the polymeric structure whiles the other gains a hydroxyl group (OH^-) (Figure 2-4). Thus, the separation continues until all polymeric units are reduced to their individual monomeric form.

Hydrolysis of seaweeds for bioethanol production involves the breakdown of polysaccharides (complex sugars) such as cellulose, laminarin, ulvan, alginate, carrageenan, mannitol and agar to simple sugars (monosaccharides) such as glucose, galactose, rhamnose, mannose, fucose, xylose and arabinose for fermentation to ethanol (Lee and Ofori-Boateng, 2013).

Numerous methods have been used in various studies for the hydrolysis of seaweeds for bioethanol production. To date, the effect of each individual method on the various groups of seaweeds is yet to be analysed. The methods that have been used in seaweed

hydrolysis includes dilute acid thermal (Abd-Rahim *et al*., 2014; Yazdani *et al*., 2015; Lee *et al*., 2015), dilute alkaline thermal (Trivedi *et al*., 2013; Van der Wal *et al*., 2013), enzymatic (Kim *et al*., 2013a; Tan and Lee, 2014; Puspawati *et al*., 2015), thermal (Yazdani *et al*., 2015; Gao *et al*., 2015), ball milling (Schultz-Jensen *et al*., 2013), hydrothermal (Schultz-Jensen *et al*., 2013), and ultrasound (Karray *et al*., 2015). These methods can broadly be grouped into physical, physicochemical and enzymatic methods. Several studies have employed a combination of these methods on the same biomass to maximise sugar recovery. The succeeding sections would examine these methods in detail while evaluating their efficiency.

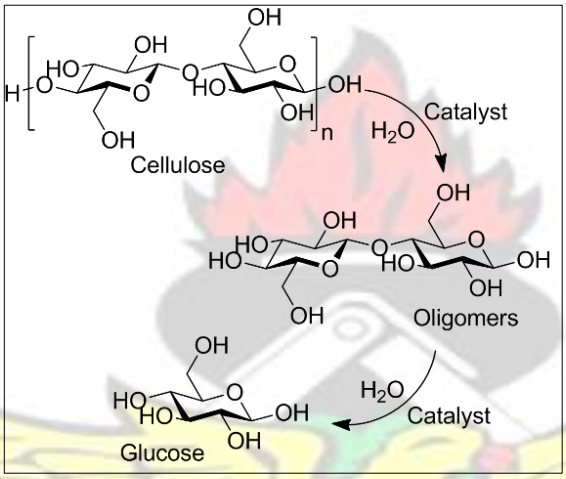

Figure 2-4. Structural breakdown of polysaccharides to monosaccharides during hydrolysis

2.8.1 **Physicochemical hydrolysis of seaweeds**

The most widely used modes of hydrolysis for seaweeds are the physicochemical methods. They are considered efficient, lower in cost and faster in reaction time than other methods (Ho *et al*., 2013). Dilute acid thermal hydrolysis is the commonest physicochemical method currently used in seaweed bioethanol research. Sulphuric and hydrochloric acids are the two most extensively used chemical catalysts in this form of hydrolysis (Kostas *et al*., 2015; Ge *et al*., 2011; Meinita *et al*., 2015). Several studies within which acid catalyst have been used can be found in Table 2-2. The total reducing sugar (TRS) yields in these studies were found to be between 100-400 mg/g DM, a good indicator of the suitability of seaweeds for bioethanol production.

A significant challenge associated with the use of acid catalysts is the formation of the by-products of 5-hydroxymethylfurfural (HMF) and furfural. HMF is formed from the dehydration of hexoses (C6 sugar monomers) such as glucose and galactose while

furfural is formed from the dehydration of pentoses (C5 sugar monomers) such as xylose and arabinose, respectively (Ra and Kim, 2013). HMF dehydrates further to form levulinic acid. HMF and furfural inhibit the activity of fermenting organisms such as yeasts by impeding protein and RNA synthesis (Ra and Kim, 2013).

Activated carbon filters have been used in a few studies for the treatment of hydrolysates to reduce the concentration of these inhibitors prior to the fermentation process (Hargreaves *et al*., 2013; Meinita *et al*., 2013). In one of such studies, the hydrolysate from *Gelidium amansii* was filtered through an activated carbon bed. The HMF concentration in the hydrolysate decreased to 5 g/l from an initial 30 g/l (Hargreaves *et al*., 2013). Another study controlled the effect of HMF by using the yeast strain *Scheffersomyces stipitis* (KCTC 7228) to convert HMF to the furfural alcohol 2,5-bis-hydroxymethylfuran which has no known adverse effect on glucose fermentation to ethanol (Ra and Kim, 2013).

The use of chemical catalysts is considered cost effective largely because there is a well-established market for its production and use. These catalysts are also used in dilute concentrations ranging from 0.06 to 0.9 M (Trivedi *et al*., 2013; Ge *et al*., 2011). The use of acid catalyst may however require a neutralization process using a base such as NaOH or KOH before the fermentation process to obtain a suitable pH required for efficient yeast fermentation (Khambhaty *et al*., 2012).

To improve the recovery of reducing sugars from seaweeds, the dilute acid thermal hydrolysis has been optimized in several studies (Borines *et al*., 2013; Meinita *et al*., 2013). The key variables examined include substrate concentration, reaction time, reaction temperature and catalyst concentration. Several of studies examined these same variables on similar seaweeds but obtained different optimal conditions. Factors that could account for these variations include; the differences in the type, composition and molecular structure of the polysaccharides in the species used. Significant differences have also been found in the concentrations of polysaccharides of the same species harvested at different seasons in the year (Adams *et al*., 2011).

Dilute alkaline hydrolysis, the alternative to acid hydrolysis, requires the use of a base such as NaOH and KOH as catalysts. There are quite a few studies on this type of hydrolysis because relatively higher concentrations of base catalysts are required to achieve efficiencies comparable to acid catalysts (Van der Wal *et al*., 2013; Karray *et al*., 2015; Tan and Lee, 2014). Also, the same base catalysts are used in the extraction

of the hydrocolloids agar and carrageenan found in the seaweeds under similar conditions to that used in hydrolysis (Kumar *et al*., 2013, Masarin *et al*., 2016). This could lead to the formation of a viscous gel which is not favourable for fermentation.

To avoid this a biorefinery approach was used by Tan and Lee (2014) on the red seaweed, *Eucheuma cottonii*. The hydrocolloid, carrageenan was first extracted before its residue was treated with 1% H_2SO_4 and 1% NaOH at 120 ℃ for 30 min before enzymes, cellulase (15 FPU/g dry biomass) and β-glucosidase (52 CBU/g dry biomass) were applied at 50 ℃ for 72 hours. The glucose recovery after enzymatic hydrolysis were 90.5% and 80.2% for acid and base catalysts, respectively (Tan and Lee, 2014).

Unfortunately, dilute alkaline hydrolysis also yields by-products from sugar dehydration. These are of the form 2-hydroxy-3-methyl-2-cyclopenten-1-one, 2,5dimethyl-4-hydroxy-3(2H)-furanone and hydroxyacetone (Shen *et al*., 2015). The effect of these by-products on yeast fermentation are currently unknown. Neutralization with acids is also required for the use of base catalysts before yeast fermentation.

Generally, the economics and efficiency of sugar recovery from dilute acid and alkaline thermal hydrolysis makes them very viable but their sustainable use raises some questions. This is because the catalyst cannot be easily recovered for recycling in the process stream. There are also environmental concerns over the toxicity of acids and the disposal of salts from the distillate bottoms.

Table 2-2. Comparison of various hydrolysis conditions and their sugar yields

Seaweed species	Hydrolysis conditions	TRS yield	Reference
Sargassum sp.	Dilute acid (10% w/v DM, 3.4-4.6% H_2SO_4, 115 °C, 90 min) Enzymatic (50 FPU cellulase/g DM, 250°CBU cellobiase/g DM, 50 °C, 100 rpm, 48 h)	120 mg/g DM	Borines *et al*., 2013

Laminaria japonica	Dilute acid (0.1% H_2SO_4, 121°C, 60 min) Enzymatic (10% w/v DM, 45 FPU cellulase/g DM, 55 CBU cellobiase/g DM, pH 4.8, 50 °C, 150 rpm, 48 h)	277.5 mg glucose/g DM	Ge *et al*., 2011
Kappaphycus alvarezii	Dilute acid (8% w/v DM, 0.2 M H_2SO_4, 110 °C, 90 min) Enzymatic (150 FPU cellulase/g DM, pH 5.5, 50 °C, 150 rpm, 48 h)	34.28 g/l (acid) 49.92 g/l (enzymatic)	Abd-Rahim *et al*., 2014
P. palmata	Dilute acid (10% w/v DM, 0.2 M H_2SO_4, 121 °C, 15 min)	164.3 mg/g DM 70.6 mg galactose/g DM	Mutripah *et al*., 2014
U. fasciata	Dilute acid (5% w/v DM, 1% H_2SO_4, 100 °C, 60 min) Enzymatic (2% cellulase/g DM, 45 °C, 150 rpm, 36 h)	113.68 mg/g DM	Trivedi *et al*., 2013
Gelidium latifolium	Dilute acid (12% w/v DM, 0.2 M H_2SO_4, 130 °C, 15 min)	34.43 g/l galactose 2.4 g/l glucose	Meinita *et al*., 2015
Gracilaria (0.01g cellulase/g DM, pH	Dilute acid (20% w/v DM, 0.1 N H_2SO_4, 121 °C, 60 min) 4.8, 50 °C, 100 rpm, 6 h)	315 mg glucose/g DM 277 mg galactose/g DM	Wu *et al*., sp. 2014 Enzymatic
Gracilaria verrucosa	Enzymatic (10% w/v pulp, 20 FPU cellulase/g DM, 60 U cellobiase/g DM, pH 5, 50 °C, 150 rpm, 36 h)	38.93 g/l	Kumar *et al*., 2014
U. lactuca	Dilute acid (10% w/v DM, 5% H_2SO_4, 121 °C, 15 min)	5.30 g/l glucose	Kostas *et al*., 2015
Gelidiella acerrosa	Dilute acid (10% w/v DM, 0.2 M H_2SO_4, 121 °C, 15 min)	72.5 mg/g DM 33.3 mg galactose/g DM	Mutripah *et al*., 2014

2.8.2 **Enzymatic hydrolysis of seaweeds**

The use of enzymes in converting polysaccharides to simple sugars is regarded as one of the most efficient hydrolysis methods. This is because it has a high conversion yield, non-toxic activity and produces no inhibitors (Tan and Lee, 2014). The most common enzymes used in the saccharification of seaweeds are cellulases (Borines *et al*., 2013; Ge *et al*., 2011; Wu *et al*., 2014). These cellulases are often secreted by several organisms for their natural metabolic activity. Fungi, known to secrete cellulases, include species from the genera, *Schizophillum, Penicillium, Phanerochaete, Fusarium* and *Trichoderma.* Bacteria, also capable of secreting cellulases, include those from the genera, *Clostridium, Cellulomonas, Thermonospora, Ruminococcus, Erwinia, Acetovibrio* and *Streptomyces* (Gupta and Verma, 2015).

Cellulases have been grouped into three based on their exclusive action on biomass during hydrolysis. These are endoglucanases, exoglucanases and β-glucosidases (Gupta and Verma, 2015). The action of endoglucanases (also known as endo 1,4-Dglucanhydrolase) occurs randomly at amorphous sites in the cellulose chain releasing oligosaccharides of varied length through the insertion of water molecules in the 1,4β bond. Their incision of cellulose fibres leaves reducing and non-reducing ends which are also susceptible to further breakdown (Quiroz-Castañeda and Folch-Mallol, 2013). Exoglucanases act on these reducing and non-reducing ends of the cellulose fibres releasing cellobiose units from the free chain ends. This forms 40 to 70% of the action of cellulases on biomass. In the aqueous phase, β-glucosidases breakdown the units of cellobiose and cellodextrins to release glucose (Ndimba *et al*., 2013).

There are also enzymes which are considered hemi-cellulolytic in their action on biomass. These are complex mixtures of individual enzymes including endo-1,4-β-Dxylanases, exo-1,4-β-D xylocuronidases, α-L-arabinofuranosidases, endo-1,4-β-D mannanases, β-mannosidases, acetyl xylan esterases, α-glucoronidases and αgalactosidases (Bhatia *et al*., 2012). These are very favourable for seaweeds due to the diversity of individual polysaccharides other than cellulose found in seaweeds which includes; laminarin, mannitol, alginate, agar, carrageenan and ulvan (Chen *et al*.,

2015). Seaweed polysaccharides are known to contain α-(1,3), α-(1,3)-(1,4), and α(1,3)-(1.2) glycosidic linkages. Amylases have also proved efficient in the saccharification of seaweeds. Waste product from salted *U. pinnatifida* was hydrolysed

with 1.4 KNU/ml of termamyl 120L, an endoamylase with broad pH tolerance. A reducing sugar yield of 32 g/100g total sugars was obtained (Ra *et al*., 2014).

Several commercial brands currently on the market have been used in various studies on seaweed biomass. These include Celluclast 1.5L, Novozyme 188, Cellic Ctec I and II. These enzyme brands have been used either alone or in a mixture with others to improve the recovery of reducing sugars (Hong *et al*., 2014; Wu *et al*., 2014). Kumar *et al*. (2013) used a mixture of cellulase from *Trichoderma reesei* (ATCC 26921) and β-glucosidase from *Aspergillus niger* (Novozyme 188) to hydrolyse pulp from *G. verrucosa* (residue after agar extraction). A reducing sugar yield of 0.87 g/g cellulose was obtained after the enzymes were applied at 50 ℃ for 42 hours while shaking at 150 rpm (Kumar *et al*., 2013).

Most studies involving the use of enzymatic hydrolysis on seaweeds have been combined with physicochemical hydrolysis methods (Gao *et al*., 2015; Wu *et al*., 2014). These physicochemical biomass pretreatments support enzymatic hydrolysis by increasing the biomass reaction surface area for more efficient action by enzymes to improve the reducing sugar yield (Ge *et al*., 2011). Borines *et al.* (2013), achieved this by first treating *Sargassum* sp. with 4% H_2SO_4 at 115 °C for 90 min after which 50 FPU/g of cellulase enzyme was added and incubated at 50 ℃ at 100 rpm for 48 hours. A reducing sugar concentration of 17.65 g/l was obtained.

Kim *et al*. (2011a) studied the recovery of reducing sugars from the species, *U. lactuca, G. amansii, L. japonica,* and *Sargassum fulvellum.* Using dilute HCl (0.2 M) and H_2SO_4 (0.1 M) at 121 ℃ for 15 min as pretreatment processes, 9.8 and 11.6% DM, respectively of reducing sugars was recovered from *G. amansii*. It was further hydrolysed with a mixture of the commercial enzymes, Celluclast 1.5L (endoglucanase) and Viscozyme L to obtain a reducing sugar yield of 56.6% DM (Kim *et al*., 2011a).

In another study also using the seaweed *G. amansii* as substrate, dilute H_2SO_4 (94 mM) was used as chemical catalyst for the pretreatment of the seaweed at 121 ℃ for 60 min. This was followed by the application of a mixture of the enzymes Celluclast 1.5L (0.168 U/ml seaweed slurry) and Viscozyme L (0.024 U/ml seaweed slurry) at 50 ℃ for 24 hours at pH 5.5 and a shaking speed of 150 rpm. A reducing sugar concentration of 43.5 g/l from a total carbohydrate concentration of 75.8 g/l was obtained at a

conversion efficiency of 57.4% (Ra *et al*., 2013). The commercial enzyme, Celluclast 1.5L was again applied to *K. alvarezii* in another study after it had been pretreated optimally with dilute H_2SO_4 at 110 °C for 90 min. An initial reducing sugar concentration of 34.28 g/l was obtained after the dilute acid pretreatment. The reducing sugar concentration increased to 49.92 g/l after the enzyme was applied at 50 °C for 48 hours at a shaking speed of 150 rpm and a pH of 5.5 (Abd-Rahim *et al*., 2014).

These studies emphasize the substantial variation in reducing sugars when acid and enzymatic hydrolysis methods are used concurrently. The various hydrolysis methods are therefore not only competing but also complimentary in their action on biomass. Even though the combined effect of acid and enzymatic hydrolysis is significant, there are recurring questions about the sustainable use of both technologies mainly because of the costs involved in their combined application. The drawbacks from the use of enzymes is the need for support from pretreatment processes, its high cost, difficulty in its recovery from process streams and hydrolysis times of 48 to 72 hours (Talebnia, 2015; Gupta and Verma 2015).

2.8.3 **Other methods of hydrolysis**

Numerous alternative methods of pre-treatment and hydrolysis to dilute acid thermal and enzymatic have been applied to seaweeds to recover reducing sugars. These are used as substitutes to alleviate any concerns associated with the use of the conventional acid and enzymatic hydrolysis or in combination with them to maximise the yield of reducing sugars. Examples of these alternative treatments include; autoclave treatment, hot water wash, gamma irradiation, wet oxidation, and ultrasonication.

Autoclave treatment was used on the *G. amansii* species in a study by Kim *et al*., 2015. This was done by heating the substrate in an autoclave at 121 °C for 80 min. This was followed by enzymatic hydrolysis with cellulase (8.0 mg/g biomass) and β-glucosidase (4.0 mg/g biomass) at 37 °C for 24 hours with varying substrate loads. The reducing sugar yields for the autoclave treated and untreated seaweeds were however comparable at 0.58 and 0.53 kg/kg dry biomass, respectively. A significant difference was however observed in the glucose fraction of the reducing sugars recovered. This was 0.45 and 0.22 kg/kg dry biomass for the autoclave treated and the untreated seaweed, respectively. This suggests that the autoclave treatment has a strong bias for

the hydrolysis of glucans other than the galactans also found in the red seaweed, *G. amansii* (Kim *et al*., 2015).

An assessment of the effect of gamma irradiation on the hydrolysis of the brown seaweed, *Undaria sp*. was done in a study by Yoon *et al*., (2012). Gamma Irradiation was used previously in the depolymerisation of sugarcane bagasse, chaff, sawdust and wheat straw (Yoon *et al*., 2012). In these cellulosic biomass, radiation-induced reactions are initiated through the rapid localilzation of the energy absorbed within the molecules producing highly active radicals that cause secondary degradation through reactions such as chain scission (Khan *et al*., 2006). Hydrated samples of *Undaria sp*. were irradiated at 22 ℃ at dosage levels between 10 and 500 kGy (kiloGray) at a rate of 10 kGy/hr. The reducing sugar concentration increased from 0.017 g/l at a 0 kGy to 0.048 g/l at a dose level of 500 kGy. The irradiated samples were treated further in 1% H_2SO_4 at 121 ℃ for 3 hours. The reducing sugar concentration for samples irradiated at 500 kGy increased to 0.235 g/l (Yoon *et al*., 2012). This implies that indeed gamma irradiation combined with acid hydrolysis those have a significant effect on sugar recovery.

Another unique hydrolysis method is the application of the Integrated-hydroxylradicals-and-Hot-water-pretreatment (IHRHW) in combination with enzymes on the brown seaweed, *Macrocystis pyrifera* (Gao *et al*., 2015). The hydroxy radicals were obtained using the Fenton or Haber-Weiss reaction in vivo. These free radicals are known to degrade the hydrogen bonds within the cellular structure of biomass by targeting carbohydrates and lignin (Wang *et al*., 2003). The entire treatment was done in a two-step process. The seaweed biomass was first pre-treated with hot water at 100 ℃ which was followed by the Fenton reaction then to enzymatic hydrolysis. The recovery of glucose from the biomass was at 24.1 and 58.6 g/g DM for the untreated and IHRHW treated seaweed, respectively (Gao *et al*., 2015). This indicates that the sugar recovery efficiency was maximised through the combination of treatments.

Schultz-Jensen *et al*. (2013), subjected *C. linum* to hydrothermal pretreatment (HTT), wet oxidation (WO), steam explosion (STEX), plasma-assisted pretreatment (PAP) and ball milling (BM) in a single study to determine their effect on glucan yields. These treatments are usually applied to terrestrial biomass. The study further examined their overall effect on ethanol yield through the SSF process. The overall glucan yields

recorded were 74, 64, 50, 38 and 36 g/100g DM for WO (at 200 °C, 12 bar for 10 min), HTT (at 200 °C, 15.5 bar for 10 min), STEX (at 210 °C for 5min), PAP (at room temperature for 60 min) and BM (for 18 hours) respectively (Schultz-Jensen *et al*., 2013). These results clearly recommend WO, STEX and HTT as the effective options for the pretreatment process but considerations over the use of energy and materials would need to be assessed further. Issues of sustainability would also have to be considered through the analysis of the Energy Return on Investment (EROI) for each process.

The effect of acid catalysis, thermoalkaline, ultrasonication and enzymatic hydrolysis on the species *U. rigida* was examined by Karray *et al*. (2015). The concentration of reducing sugars obtained were 3.62, 2.88, 2.53 and 7.3 g/l for acid catalysis, thermoalkaline, ultrasonication and enzymatic hydrolysis, respectively (Karray *et al*., 2015). This clearly suggests that ultrasound treatment cannot be considered efficient in the release of sugars as against the conventional acid and enzymatic hydrolysis. Ultrasound can be used to disrupt cells to release cell contents at a shorter time.

To minimize the issues of toxic catalysts, long reaction times and high catalysts costs associated with the use of physicochemical and enzymatic hydrolysis, hydrothermal conditions using subcritical water was used in a study on the brown seaweed, *Saccharina japonica* (Meillisa *et al*., 2015). This method employs the use of subcritical water which refers to moisture under high pressure with temperatures between its boiling point (100 °C) and critical point (374 °C) to modify the physical features of the solvent through changes in its viscosity, surface tension and dielectric constant (Meillisa *et al*., 2015). These solvent properties support the breakdown of the biomass. At a temperature of 180 °C and at a pressure of 13 bar a glucose concentration of 0.43 g/l was obtained in the study. This sugar recovery shows that the hydrothermal condition can only be used to compliment the conventional methods of hydrolysis.

2.9 Biomass fermentation pathways

The fermentation stage is a major limiting process in bioethanol production since it is during this process that the reducing sugars released after hydrolysis are converted to ethanol by fermenting organisms. The efficiency of this process is often decided by two critical factors. These factors which are of major concern to ethanol producers are the selection of the fermenting organism(s) and its ideal operating conditions. The

finding and selection of fermenting organisms capable of converting both hexose and pentose forms of reducing sugars is also currently a major challenge in the industry (Talebnia *et al*., 2010).

The yeast species, *Saccharomyces cerevisiae* is the most extensively used and studied organism for sugar fermentation to ethanol. Various strains of this fungi with different features have been developed and used (Ota *et al*., 2013). *S. cerevisiae* readily ferments hexose sugars such as glucose, mannose and fructose using the Embden-MeyerhofParnas pathway and ferments the hexose, galactose using the Leloir pathway (Van Maris *et al*., 2006). However, most strains of *S. cerevisiae* do not readily ferment pentoses such as xylose, rhamnose and arabinose even though they are abundant in most biomass used in ethanol production. Seaweeds in particular are known to contain various fractions of hexoses, pentoses and some uronic acids which needs to be fermented all together to improve their viability as substrates in ethanol production.

2.9.1 **Hexose fermentation by *S. cerevisiae***

Glucose is the most abundant hexose sugar in seaweeds and can be assimilated by the yeast strain *S. cerevisiae*. *S. cerevisiae* contains within its cell structure a complex system of 32 hexose transporters with different substrate specificities and affinities (Van Maris *et al*., 2006). These transporters are all capable of transferring glucose using facilitated diffusion across the plasma membrane based on the concentration gradient. The dissimilation of glucose after absorption into the membrane is via the Embden-Meyerhof-Parnas glycolytic pathway (Ji *et al*., 2016).

In this pathway (Figure 2-5), glucose is oxidised to two pyruvate units which results in the formation of two ATP (Adenosine Triphosphate) units per unit glucose. Energy is given since this reaction is exothermic. This energy is used to bind inorganic phosphates to ADP (Adenosine Diphosphate) and to convert NAD+ (Nicotinamide Adenine Dinucleotide) to NADH. The NADH is formed by the enzyme, glyceraldehyde-3-phosphate dehydrogenase. The two pyruvates are further oxidised to two acetaldehyde units giving off two CO_2 units as by-products (Ji *et al*., 2016). The two acetaldehydes are then converted to two ethanol units using H+ ions from NADH which is reduced to NAD+. Glucose is often not the only hexose sugar found in hydrolysates. *S. cerevisiae* is however capable of efficiently fermenting mannose and fructose via a similar pathway. This can only be achieved if a functional transporter

specific to those sugars is present in the plasma membrane. Enzymes are also required to link the metabolism of the hexose to the main glycolytic pathway while maintaining a closed redox balance.

Although majority of wild strains of *S. cerevisiae* cannot assimilate xylose and other pentoses, some yeast strains have been identified with the capacity to do this. The yeasts strains, *Pachysolen tannophilus*, *Brettanomyces naardenensis*, *Candida shehatae*, *Candida tropicalis*, *Candida tenuis*, *Pichia segobiensis* and *Pichia stipitis* were first found to have the capacity to ferment xylose in the 1980s. These xylosefermenting yeast rely on two oxidoreductases, xylose reductase and xylitol dehydrogenase in the xylose metabolism pathway.

The successful identification of these xylose fermenting yeasts indicate that more and more culture collections can be screened to build stronger culture libraries for yeast fermentation. Screening of cultures along with some metagenomic (genetic material recovery) studies can be used to identify enzyme secretions from the activity of the yeast strains which give optimal features required for effective ethanol production. The metabolic engineering of the more common *S. cerevisiae* with heterologous enzymes and transporters from pentose fermenting species can also be considered.

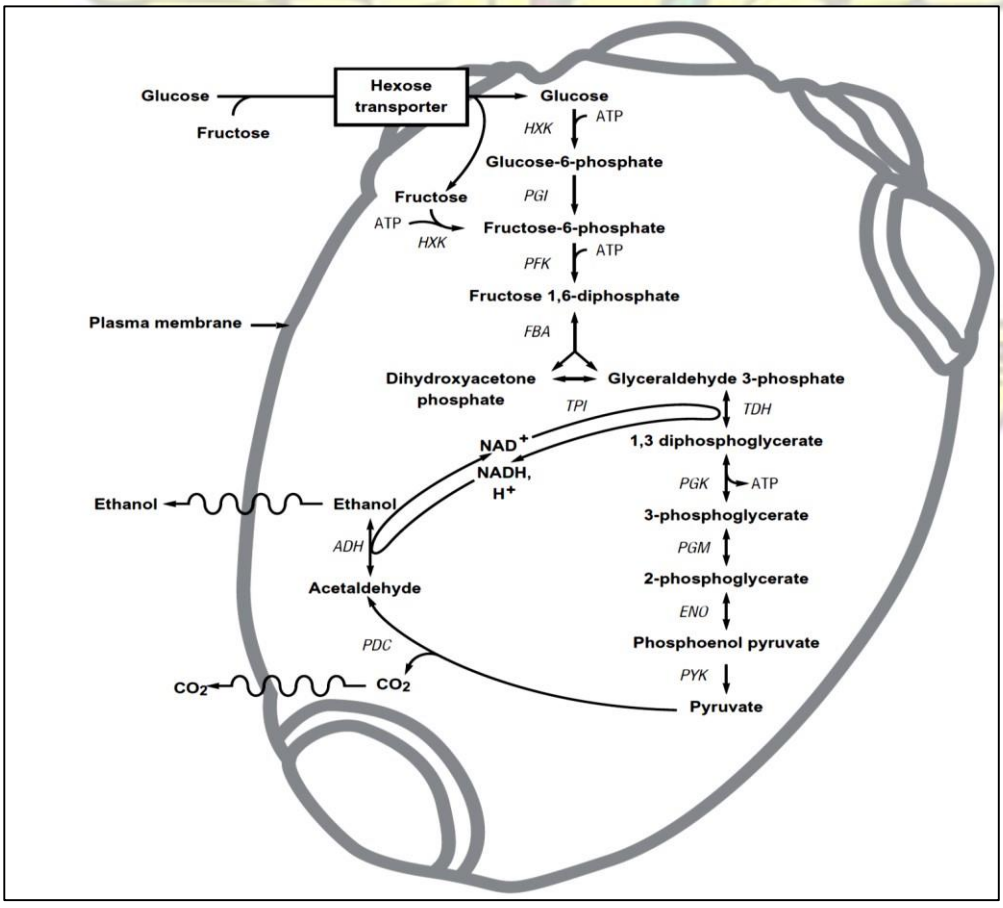

Figure 2-5. Embden-Meyerhof-Parnas glycolytic pathway for sugar utilization (Ji et al., 2016)

2.9.2 **Organisms used in seaweed fermentation**

Various species of bacteria and fungi have been used in various studies for the fermentation of reducing sugars to ethanol. Organisms reported to have been used in fermenting sugars from seaweeds include various strains of *S. cerevisiae* (SchultzJensen *et al*., 2013; Yazdani *et al*., 2015; Tan and Lee, 2014), *Pichia angophorae* (Cho *et al*., 2013; Kim *et al*., 2013b), *P. stipitis* (Schultz-Jensen *et al*., 2013; Kim *et al*., 2014) and *Escherichia coli* (Ota *et al*., 2013). The fungi, *S. cerevisiae* is the most commonly used fermenting organisms in bioethanol production since most strains are low in cost and readily available. Various strains of *S. cerevisiae* have been synthesized but the commonest strains are the baker's and brewer's yeast which can be purchased on the open market.

S. cerevisiae is often used by commercial bioethanol production industries with sugarcane, maize and cassava as raw material because these raw materials are high in hexoses (C6 monosaccharides) such as glucose, galactose, fructose and mannose. The commercialization of cellulosic and algal biomass as substrates for bioethanol production may have to resort to strains with the combined capacity to hydrolyse both hexose sugars and pentose sugars (C5 monosaccharides) such as xylose and arabinose. A fermenting organism capable of converting both hexoses and pentoses to ethanol is the yeast, *P. stipitis* (Takagi *et al*., 2015). This yeast strain was used by Takagi *et al*., (2015) on the seaweeds, *Ulva sp., Gracilaria sp.* and *Costaria. costata* to obtain ethanol yields of 0.08, 0.07 and 0.05 g/g reducing sugar.

P. angophorae (also known as *Ambrosiozyma angophorae*) is another unique yeast strain found to be capable of fermenting mannitol (a sugar alcohol found in brown seaweeds) (Adams *et al*., 2011). It also releases the enzyme laminarinase which catalyzes the hydrolysis of laminarin in brown seaweeds. This strain was used in a study to ferment reducing sugars from the brown seaweed, *S. latissima* producing an ethanol yield of 8.86 µl/ml seaweed slurry (Adams *et al*., 2011).

In another study, three different fermenting organisms were compared, *Saccharomyces paradoxus, P. angophorae* and *E. coli* (Ota *et al*., 2013). These organisms were fed with mannitol and glucose as sole substrates. Ethanol yield concentrations of 10.2, 11.5 and 11.0 g/l were obtained with mannitol as substrate while 8.9, 9.3 and 9.2 g/l was

obtained with glucose as substrate for *S. paradoxus*, *P. angophorae* and *E. coli*, respectively (Ota *et al*., 2013). Indeed, all three organisms were efficient in their recovery of ethanol from the selected substrates which indicates that they would be useful organisms for fermenting brown seaweeds.

The selection of yeast strains is often done with a target substrate in mind but some studies have rather acclimated common yeast strains to various types of sugars to improve their selectivity and absorption of those sugars. This tool is known to improve the activity of the acclimated organism in the conversion of hydrolysates which are most often heterogenous. This was done in a study with the yeast strains, *S. cerevisiae* (KCCM 1129) and *P. angophorae* (KCTC 17574) in a two-stage fermentation cycle. *P. angophorae* was acclimated during culturing in 50 g/l mannitol solution for 24 hours before inoculation for fermentation (Ra and Kim, 2013). The acclimated yeast gave an ethanol recovery of 0.30 g/g mannitol as compared to 0.13 g/g mannitol from nonacclimated yeast (Ra and Kim, 2013). This study clearly highlights significant benefits in the acclimation of fermenting organisms to specific target substrates. This approach can be considered for pentose sugars such as xylose and rhamnose which are considered challenging to ferment.

2.10 Bioethanol production process pathways

The hydrolysis and fermentation stages together form the major limiting processes in bioethanol production. Their individual and combined efficiencies therefore determine the final ethanol yield. Two major bioethanol production pathways can be defined based on how the hydrolysis and fermentation processes occur. They may either occur sequentially which is referred to as *Separate Hydrolysis and Fermentation* (SHF) or concurrently which is referred to as *Simultaneous Saccharification and Fermentation* (SSF).

2.10.1 Separate Hydrolysis and Fermentation (SHF)

The SHF pathway is the most commonly used primarily because of the flexibility it offers in the selection of the hydrolysis methods to be used. It is also favourable since the optimum operating conditions for the selected hydrolysis catalyst and fermenting organism can be used. The SHF involves, the hydrolysis of the seaweed biomass and the recovery of the reducing sugars typically through filtration or centrifugation (separation of the liquid hydrolysate containing the reducing sugars from the solid

residue). This is followed by the inoculation of the fermenting organism(s) into the hydrolysate and the adjustment of the operating conditions for the fermentation process. The hydrolysis and fermentation processes may however take place in the same reactor.

The SHF pathway was used in the conversion of the red seaweed *G. tenuitipitata* (Chirapart *et al*., 2014). The seaweed was first hydrolysed with an acid before fermentation with *S. cerevisiae* to obtain an ethanol yield of 0.042 g/g reducing sugars (Chirapart *et al*., 2014). This same approach was used in the conversion of the brown seaweed, *U. pinnatifida* using the yeast *P. angophorae* as the fermenting organism to obtain an ethanol yield of 0.33 g/g reducing sugars (Cho *et al*., 2013). The SHF was also used in a biorefinery approach with agar extraction residue from *G. verrucosa* as substrate. An ethanol yield of 0.43 g/g reducing sugars was obtained with *S. cerevisiae* as fermenting organism (Kumar *et al*., 2013).

2.10.2 **Simultaneous Saccharification and Fermentation (SSF)**

In the SSF approach to bioethanol production, the enzymes and fermenting organisms for saccharification (hydrolysis) and fermentation, respectively are added to the fermentation broth in the same reactor at the same time. The typical seaweed fermentation broth for SSF consists of the biomass, enzymes such as cellulases, dry yeast, yeast extract, peptone and citrate buffer (Kim *et al*., 2015). The biggest advantage the SSF process has over SHF is the reduction in cost from the use of a single reactor for both saccharification and fermentation. The overall ethanol yield is however limited since the optimal yield for both processes is not used due to their wide variation (Sarkar *et al*., 2012). The typical temperatures for saccharification and fermentation is 50 ℃ and 30 ℃, respectively however the SSF is typically done at 37 ℃ which is suitable for both enzymatic and yeast activities (Ge *et al*., 2011; Kim *et al*., 2015). The two processes are therefore always operated with a common operating condition which is efficient for both.

The SSF process was used in the conversion of the brown seaweed, *S. japonica* (ye Lee *et al*., 2013). The seaweed was first treated with 0.06% sulfuric acid at 170 ℃ for 15 min prior to the SSF. In the SSF, the enzymes (cellulase and β-glucosidase) and the yeast *S. cerevisiae* were added to the hydrolysate to form the fermentation broth before a stepwise incubation at temperatures from 37 to 46 °C for 48 h while shaking at 130 rpm. The ethanol concentration obtained was 6.65 g/l (ye Lee *et al*., 2013).

The SSF is considered the highest yielding ethanol production pathway in comparison with SHF. This assertion was examined in a study with the red seaweed, *G. amansii*. An ethanol concentration of 3.78 mg/ml and 3.33 mg/ml was obtained for the SSF and SHF, respectively (Kim *et al*., 2015). The SSF process was done at 37 ℃ for 24 h while the SHF process was done at 37 ℃ for 24 h during hydrolysis and; 30 ℃ for 12 h during fermentation. The results from this study indicates that the SSF yield was only marginally higher than that in the SHF. The advantages of SSF over SHF therefore requires further investigation since the efficiencies of both processes are quite comparable.

2.10.3 **Other bioethanol production pathways**

Apart from the SSF and SHF processing pathways, other bioethanol production pathways that have been used include; *Simultaneous Saccharification and Cofermentation* (SSCF), and *Consolidated Bioprocessing* (CBP). In the SSCF pathway, the liquid hydrolysate and the solid residue are combined into a single stream after hydrolysis to which the fermenting organism is added. This was used in a study on the red seaweed, *K. alvarezii*, which had a galactose rich liquid fraction and a cellulose rich solid fraction, to obtain an ethanol concentration of 64.3 g/l. The conventional SSF process was applied to the same liquid and solid fractions of *K. alvarezii* separately in the same study to obtain an ethanol concentration of 38 and 53 g/l, respectively (Hargreaves *et al*., 2013). Co-fermentation in the SSCF can also be achieved with multiple fermenting organisms in the same medium targeting different reducing sugars. Some organisms capable of co-fermenting different substrates via the SSCF process include *S. cerevisiae*, *E. coli*, *Z. mobilis*, *P. tannophilus*, *C. shehatae* and *P. stipitis* (Sarkar *et al*., 2012).

The CBP process occurs simultaneously in a single reactor with the combination of polysaccharides recovery, monosaccharides recovery and the fermentation of hydrolysates to ethanol. The CBP process requires the use of a single or combined consortium of microbes to achieve all these processes simultaneously. Microbes capable of performing CBP include; *C. thermocellum*, *Neurospora crassa*, *Fusarium oxysporum* and *Paecilomyces sp*. (Gupta and Verma, 2015).

2.11 Bioethanol recovery processes

Ethanol produced during fermentation are found in a ternary mixture with other components such as the solid residue from the biomass, residual reducing sugars, residual complex sugars, enzymes (if applied) and the fermenting organisms. The ethanol is separated from this mixture primarily through distillation and dehydration processes. The distillation process is considered by most ethanol production industries as the most energy intensive process in bioethanol production due to the high amount of heat energy required for vaporizing the ethanol from the fermentation broth (Alvarado-Morales *et al*., 2013).

Commercial ethanol production plants rely on distillation columns in combination with dewatering systems such as molecular sieves to obtain ethanol concentrations higher than the ethanol-water azeotrope (Fasahati and Liu, 2014). Commercial distillation units are capable of distilling broths to the ethanol-water azeotropic point of 95.6% after which separation via distillation is no more feasible. Dehydration techniques such as vacuum distillation, pressure swing, membranes and molecular sieves are required to improve the concentration of the distillate to greater than 99% which forms fuel grade ethanol (Baeyens *et al*., 2015). Molecular sieves can be considered as the most economical dewatering process since it exploits the difference in molecule size between water and ethanol to selectively adsorb water using adsorbents such as zeolite (Baeyens *et al*., 2015). These adsorbents can be regenerated repeatedly for reuse through drying.

2.12 Bioelectricity production using Microbial Fuel Cells

The concept of bioelectricity generation via electron transfer using microorganisms was first discovered by Potter in 1911 using *E. coli* while Cohen in 1931 built the first actual microbial fuel cell (Ieropoulos *et al*., 2005). The extensive research on MFCs however, has only been done in the last two decades due to the growing demand for sustainable renewable energy technologies. These systems have distinct advantages and prospects over current bioenergy generation systems such as anaerobic digestion, pyrolysis and gasification. Their biggest advantage over other bioenergy generation systems is their direct and simultaneous generation of electricity and treatment of wastewater with no intermediate conversion stages (Pham *et al*., 2006).

Microbial fuel cells are bio-electrochemical systems which converts the chemical energy in largely organic compounds to electrical energy catalysed by microorganisms under anaerobic conditions (Pham *et al*., 2006). Microbes referred to as electrogenic bacteria oxidise organic compounds at the anode of MFCs to release electrons, protons and carbon dioxide (Equation 1). The electrons are collected at the anode and transferred through an external circuit to a load then to the cathode where it is reduced in the presence of protons and oxygen to form water (Equation 2) (Kim *et al*., 2008; Franks and Nevin, 2010).

$$Anode\ reaction: C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (1)$$

$$Cathode\ reaction: 24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O \quad (2)$$

The entire working principle of MFCs is built on the oxidative action of bacteria on organic substrates forming a negative terminal and the reductive action of electrons and protons by a catholyte forming a positive terminal. This creates a potential difference (voltage) which drives the resulting electric current through the circuit for the generation of power. The current generated in MFCs is a direct current (DC) which is unidirectional between the positive and negative terminals.

MFCs form part of a larger group of biological systems known as bio-electrochemical systems. These are systems within which electric current is released through the activity of microorganisms. They include microbial fuel cells (Figure 2-6) and microbial electrolysis cells (MECs). In MECs, the current produced at the anode is used to drive other reactions at the cathode to generate other useful products such as hydrogen and hydrogen peroxide (Logan *et al*., 2006).

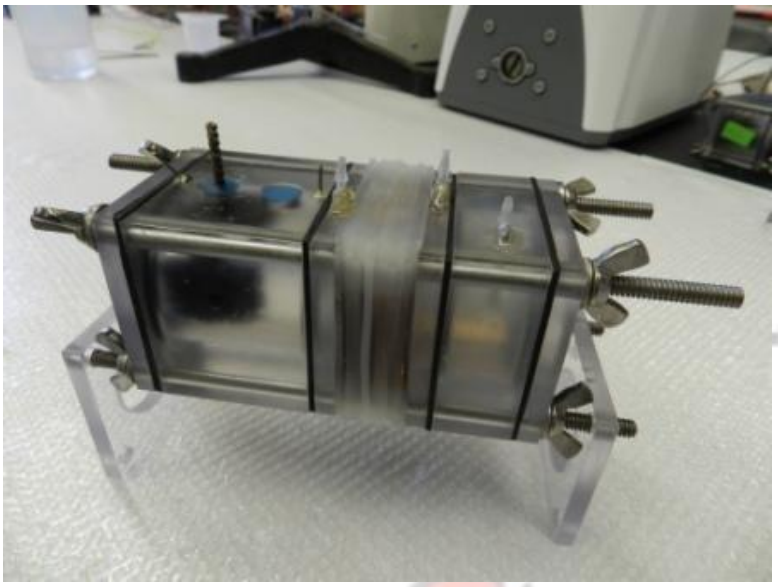

Figure 2-6. A double chamber microbial fuel cell

2.12.1 **Components and Operation of MFCs**

MFCs have been designed and configured in a myriad of ways by researchers to maximise current generation, reduce cost and minimize electric charge losses. The basic components in all configurations are an anode in an anaerobic compartment, an aerobic cathode, electrolytes (for both anode and cathode) and a separator for charge transfer between electrolytes (Figure 2-7) (Freguia *et al*., 2009). Configurations available include; single chamber, double chamber and tubular MFCs which can be operated in a fed-batch mode or continuous mode (Figure 2-7).

The anode compartment in all configurations contains an electrode and an electrolyte (anolyte). The electrode in the anode compartment serves as the site for biofilm formation, substrate oxidation, and electron transfer (Logan *et al*., 2006). The anolyte is the medium which contains the substrate and the inocula. In the anolyte, a biofilm is formed during inoculation from pure or mixed microbial cultures. Mixed cultures are often preferred due to their ability to handle a broad range of substrates and their high resistance to process disturbances (Rabaey *et al*., 2005). The biofilm is made up of extracellular polymeric tissue with surface adhering microbial culture. It contains various species of electrogenic bacteria which initiates the oxidation of the substrate to release electrons, protons and carbon dioxide (Toutain *et al*., 2004).

Bacteria found in biofilms of MFCs include; gram negative pure cultures such as *Geobacter sulfurreducens*, *Shewanella oneidensis*, *Pseudomonas aeruginosa* along with gram positive cultures of *Clostridium acetobutylicum* and *Enterococcus faecium*

(Read *et al*., 2010). The electrons released at the anode by these microbes in the biofilm are deposited on to the solid electrode surface and conducted to the external circuit load. Electron transfer occurs directly by the bacteria using its conductive cellular appendages such as pilli and cytochrome-c. It can also occur indirectly through exogenous or endogenous soluble molecules called redox electron shuttles (mediators) such as methylene blue and humic substances (Reguera *et al*., 2005; Rabaey *et al*., 2005). These are secreted by bacteria or added by the MFC user.

Cathodes in MFCs primarily function as the electron acceptor. They consist of an electrode and an electrolyte (catholyte). The selection of a sustainable electrolyte with favourable reduction kinetics is a major challenge in the commercial development of MFC cathodes. The most common electrolytes are atmospheric oxygen and potassium ferricyanide (Liu *et al*., 2012; Wei *et al*., 2012). Other alternative electrolytes include potassium permanganate and potassium dichromate.

Atmospheric oxygen is considered the cheapest catholyte for MFCs but its poor reduction kinetics means a solid catalyst is required on the electrode surface. Platinized graphite is an example of such a cathode which uses atmospheric oxygen as catholyte in an MFC with platinum coating as its catalyst (Gil *et al*., 2003; Jang *et al*., 2004). Potassium ferricyanide is considered as one the most efficient electron acceptors in MFCs especially for double chambered MFCs, recording power densities up to eight times higher than oxygen-based cathodes (Wei *et al*., 2012). They are often preferred because they do not require any catalyst on the electrode surface. Bio-cathodes which uses microbes and some plant species in the cathode chamber to accept electron charges have also been reported (Clauwert *et al*., 2007). These offer advantages in lower cathode operation cost and possible de-nitrification properties (He and Angenent, 2006).

The anode and cathode of MFCs are physically divided by a separator or membrane. This prevents the mixing of electrolytes since the microbes in the anode operates anaerobically while the aerobic cathode may contain a catholyte which is toxic to bacteria. The separator also regulates the movement of ions between electrolytes. The ideal separator or membrane should be able to impede oxygen and electron transfer while permitting efficient proton transfer. Materials used as separators in MFCs include cation exchange membrane, anion exchange membrane, bipolar membranes, microfiltration membranes, ultrafiltration membranes, salt bridge, glass fibres and

porous fabrics. Cation (proton) exchange membranes such as the Nafion 117 (Dupont Co., USA) is the most commonly used in MFCs due to its high selectivity for various cations (Mauritz and Moore, 2004). The cost of membranes remains a major hurdle for the development of scale-up MFC units for commercial power generation.

The performance of MFCs is often measured by their power density, current density, coulombic efficiency, substrate removal efficiency and internal resistances. These parameters evaluate not only power generated but also the efficiency of the substrate conversion process. Power and current densities refer to the power and current per unit anode electrode surface area (W/m^2 and A/ m^2) or per unit anodic volume (W/m^3 and A/m^3) of the MFC, respectively (Logan, 2007). Coulombic efficiency refers to the amount of substrate recovered as electric current while substrate removal efficiency refers to the amount of substrate oxidised relative to the initial substrate introduced into the anode. Resistances in MFCs generally impede the flow of current causing a reduction in the overall current and power densities. These internal resistances are often measured via electrochemical impedance spectroscopy (EIS) which measures the dynamic system response without interrupting the regular operation of the MFC (Logan, 2007).

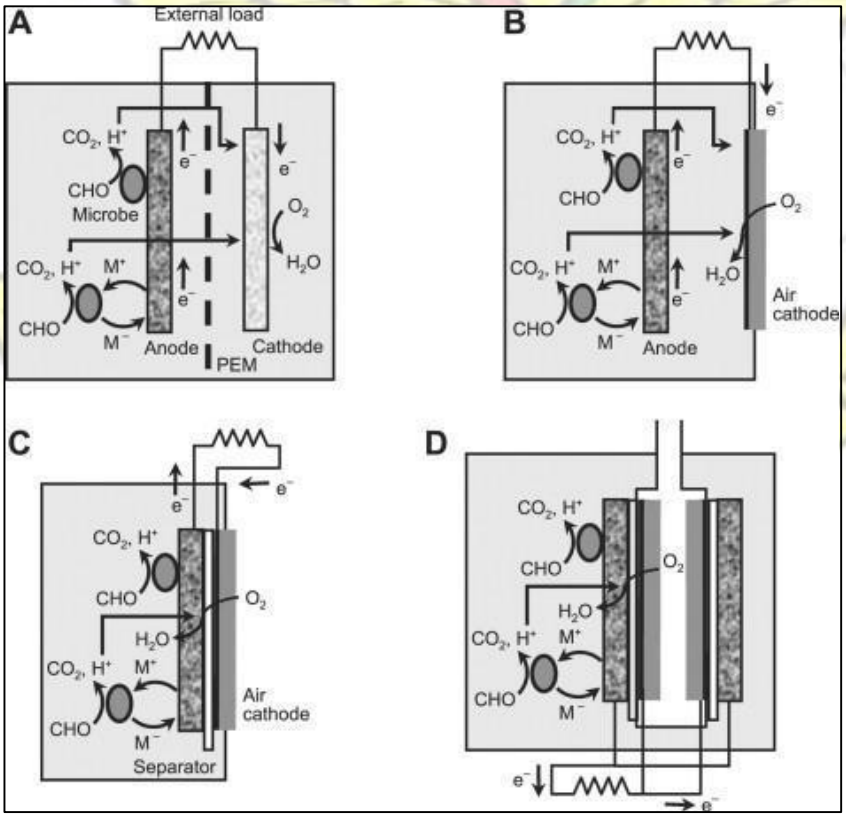

Figure 2-7. Four different configurations of microbial fuel cells (A: double chamber, B: single chamber, C: single chamber with separator, D: tubular)

2.12.2 **Substrates used in MFCs**

An important feature of MFCs can be found in their ability to degrade an enormous variety of substrates. Substrate selection is very vital since it is the principal component which is degraded to obtain the electric current. Substrates that have been used in MFCs range from pure compounds to various mixtures such as those from domestic and industrial wastewater. They include acetate, glucose, starch, cellulose, pyridine, phenols, proteins and lipids (Ren *et al*., 2008; Zhang *et al*., 2009; Freguia *et al*., 2009). The enormous diversity in substrate utilization by microbes in MFCs makes it a potentially important tool for application in various biorefineries.

Acetate has been the most preferred substrate in MFCs particularly during inoculation to induce the growth of electrogenic bacteria since it is inert to fermentation and methanogenesis at ambient conditions (Aelterman, 2009). According to Sun *et al*. (2015), MFCs initiated during inoculation with acetate generates higher coulombic efficiencies and power densities due to a high relative abundance of up to 13.9% for electrogenic bacteria such as *G. sulfurreducens* and *Desulfuromonas acetexigen* in its biofilm (Sun *et al*., 2015). Acetate also forms the end-product of various microbial metabolic pathways for complex carbon-based organic compounds. A power density of 1.74 W/m^3 was reported for an H-shaped double chamber MFC operating with acetate as primary substrate and faecal sludge as secondary substrate (Offei *et al*., 2016).

In a comparison between glucose (a pure compound substrate) and anaerobic sludge (a mixed compound substrate) as sole substrate for a membrane-less MFC, power densities of 0.161 W/m^2 and 0.0003 W/m^2 were obtained for the glucose and anaerobic sludge, respectively (Hu, 2008). This wide disparity was attributed to the presence of competing biological processes such as fungal fermentation and methanogenesis in the anaerobic sludge as well as the complexity of the degradation pathway. This wide difference indicates a possible challenge for scale-up MFCs which cannot rely on pure substrates such as glucose and acetate. Glucose certainly represents the ideal situation while anaerobic sludge represents the real situation as to substrates that are readily available in large quantities for use by MFCs. Nonetheless, successful power recoveries for heterogeneous substrates such as corn stover waste, brewery wastewater

and starch processing wastewater have been demonstrated. Power densities of 0.371 W/m^2 (Zuo *et al*., 2006), 0.528 W/m^2 (Feng *et al*., 2008), 0.239 W/m^2 (Lu *et al*., 2009), 12.8 W/m^3 (Zhang *et al*., 2008) have been reported for air-cathode MFCs operating with corn stover, brewery wastewater, starch processing wastewater and landfill leachate, respectively. Apart from these listed, more substrates have been explored and are still being explored in MFC research. This study will explore the prospects of seaweed residue after bioethanol production as sole source of substrate for MFCs.

2.12.3 **Seaweeds as substrates in MFCs**

The use of seaweeds as substrates in MFCs for power generation is quite new with limited research activity on it to date. However, various pure organic compounds found in seaweeds have been specifically used as substrates in MFCs. These include; glucose, cellulose, galactose, fucose, galacturonic acid, glucuronic acid, mannitol, starch and xylose (Pant *et al*., 2010; Sun *et al*., 2015). The sugar alcohol, mannitol is the only pure substrate studied which is uniquely found in brown seaweeds in large concentrations. The study by Catal *et al.* (2008) using mannitol as sole substrate in an air-cathode (single chamber) MFC with non-wet proofed carbon cloths as electrodes yielded a current density of 0.58 mA/cm^2.

From available literature, three studies have uniquely studied the use of seaweeds as substrates in MFCs. Velasquez-Orta *et al.* (2009), studied the use of the green seaweed *U. lactuca* as substrate for a single chamber air-cathode MFC (25 ml) with graphite fibre brush anodes and platinized cathode. The maximum power and current densities obtained were 0.76 W/m^2 (215 W/m^3) and 0.20 mA/cm^2, respectively. Wang *et al*. (2012) studied the green seaweed, *Enteromorpha prolifera* as substrate for a single chamber cylindrical air-cathode MFC (27 ml) with acid-treated carbon cloth as anode and Vulcan-PTFE-treated carbon cloth as cathode. This species is considered a major problem in some parts of China due its frequent blooms which creates a foul smell along the beaches. The maximum power density, coulombic efficiency and substrate removal efficiency were 1.03 W/m^2, 69.1% and 76.1%, respectively. Gadhamshetty *et al*. (2013) were the first to report the use a brown seaweed as a substrate in MFCs. They used the species *L. saccharina* in a double chamber MFC (200ml) with graphite felt as electrodes in both the anode and cathode. The maximum power and current densities obtained were 0.25 W/m^2 and 0.9 A/m^2, respectively (Gadhamshetty *et al*., 2013).

Even though reports on the use of seaweeds (macroalgae) are very limited, reports on the use of microalgae however is quite numerous. Microalgal species that have been used in MFCs include *Scenedesmus* sp. (Cui *et al*., 2014) and *Chlorella vulgaris* (Velasquez-Orta *et al*., 2009). The compositional differences in micro and macroalgae (up to 50% protein in *C. vulgaris* and 60% carbohydrates in *U. lactuca*) was reflected marginally in their power densities of 277 W/m^3 (*C. vulgaris*) and 215 W/m^3 (*U. lactuca*) when compared in a study (Velasquez-Orta *et al*., 2009). Generally, the performance of seaweeds as substrates in MFCs is considerably high and can be compared to conventional substrates such as acetate. Its use in this study in the biorefinery approach therefore has substantial merit.

2.13 Review of integrated biorefinery processes

The integrated production of biofuels is a direct application of the biorefinery concept. This concept relies on the use of multiple technologies to produce high value products from a single biomass. It is considered an efficient, economical and sustainable approach to biomass conversion (Talebnia, 2015). Through the biorefinery concept most components of a single biomass can be converted to useful products.

Biorefineries integrate various biomass conversion technologies to produce fuels, power, heat and other value-added products from biomass. These refineries have evolved over the last two decades in several phases. Phase I biorefineries convert a single raw material to a single product. Phase II converts a single raw material using multiple processing tools to obtain a broad range of products. Phase III biorefineries, commonly referred to as integrated biorefineries use a wide range of raw materials and technologies simultaneously or sequentially to produce a wide range of valuable products (Pande and Bhaskarwar, 2012). Some integrated biorefineries use various feedstocks and technologies to produce biofuels as main products along with coproducts such as platform chemicals, heat and power (Pande and Bhaskarwar, 2012).

Several studies have used the integrated biorefinery approach to maximise the use of the biomass and improve both economic and process sustainability. This approach was used in the processing of the green seaweed, *C. linum* to co-produce bioethanol and biomethane in a single study (Yahmed *et al*., 2016). A bioethanol yield of 0.41 g/g reducing sugar (0.093 g/g pretreated seaweed) was obtained after the pretreatment, enzymatic hydrolysis and fermentation of the seaweed biomass. The enzymatic hydrolysis was done with a crude enzyme from *Aspergillus awamori* at 45 °C for 30

hours while the fermentation was done with *S. cerevisiae* at 28 ℃ for 48 hours while shaking at 150 rpm. The fermentation broth was then distilled to recover the ethanol while the residue, referred to as vinasse, was used as the feed for anaerobic digestion. The anaerobic digestion of the vinasse which was done at 38 ℃ in a 0.5 l digester for 30 days yielded 0.26 l/gVS of biomethane (Yahmed *et al*., 2016). The final waste generated was 0.3 g/g of the biomass which represents a substrate utilization of up to 70%. This approach did indeed enhance the use of the substrate.

Ashokkumar *et al*. (2017) also made a similar attempt with the biorefinery approach. They considered the integrated conversion of the brown seaweed *Padina tetrastromatica* to both biodiesel and bioethanol. The crude lipids content was first extracted from the biomass using various solvents to obtain a yield of 8.15% w/w biomass. This was processed further through transesterification (the process of exchanging the organic group R' of an ester with the organic group R' of an alcohol) to obtain a final biodiesel yield of 78 mg/g biomass. The residual biomass after lipids extraction was hydrolysed and fermented (using baker's yeast) to obtain a bioethanol yield of 161 mg/g residual biomass (Ashokkumar *et al*., 2017). This study demonstrated that the integration of biodiesel and bioethanol production processes on a single seaweed biomass can efficiently harness both the lipid and carbohydrate fraction which could form up to 70% of the entire biomass.

These studies highlighted, and several others demonstrate some considerable benefits from the use of integrated processing technologies. The most obvious feature is the increased use of the substrate and the minimization of waste generated. The possible integration of bioelectricity generation and bioethanol production is quite a promising novel biorefinery approach which would be examined in this study on seaweeds.

CHAPTER 3. METHODOLOGY

3.1 Seaweeds selection and pre-processing

The selection of the seaweed species in this study was very critical since these species could be recommended for both commercial scale capture and cultivation by potential Ghanaian farmers. The seaweed species selected for use as substrates in bioethanol and bioelectricity production were based on the following criteria (Danish National Environmental Research Institute, 2011):

1. The species were representative of the three groups of macroalgae to examine the variation of the target product yields between species from the different groups. This is because the components and cellular structure between seaweed groups have been found to differ enormously (McHugh, 2003).
2. The species must have a fast growth rate per unit hectare to obtain high biomass production. This would enhance its viability as an alternative to terrestrial plants.
3. There must be experienced, well-established and economical cultivation methods. This is because the transfer of cultivation technologies to local farmers along Ghanaian coasts is critical in ensuring regular substrate availability for commercial-scale biorefineries.
4. The species must be efficiently harvestable after cultivation to facilitate regular supply.
5. The species must be domestic to Ghanaian coasts since the introduction of a new and possibly invasive species could adversely affect the surrounding ecosystem.

Based on these criteria, the species selected for this study were: *Ulva fasciata* (a green seaweed), *Hydropuntia dentata* (a brown seaweed and an agarophyte) and *Sargassum vulgare* (a brown seaweed) (Figure 3-1). The three seaweeds were harvested by hand picking from both the east and west coast of Ghana in February 2016. *H. dentata* was sampled from Prampram (5.5717° N, 0.1332° W) in the Greater Accra region (east coast of Ghana) at a tide time of 11:14am and tide height of 0.17 m. *U. fasciata* and *S. vulgare* were sampled from Mumford (5.2660° N, -0.7542° W) in the Central region (west coast of Ghana) at a tide time of 9.42am and tide height of 0.13 m. The seaweeds were bagged in polyethylene bags and transported to the laboratory.

The seaweeds were pre-processed using a modified form of the method described in Cho *et al.* (2013). They were washed with sea water and sorted to remove sand, debris and any unwanted material. They were then bagged again in polyethylene bags and stored overnight in a freezer at -15 °C. The seaweeds were sun dried for 3-4 days from an initial moisture content of 80-90% to a final moisture content of 10-13%. The seaweeds were then milled with a pulveriser (Fritsch-Germany) to a particle size of <1 mm. They were subsequently bagged in zip lock bags and stored in a dry cabinet before use.

Figure 3-1. Seaweeds selected for this study (From top to bottom: U. fasciata, S. vulgare, H. dentata)

3.2 Seaweed composition analysis

The seaweeds were characterised for their total carbohydrates, total proteins, lipid content, moisture content, total solids, volatile solids and ash content. The total solids and moisture content were determined as described by Sluiter *et al.* (2008) (adapted from ASTM E1756-01). It involved drying the sample in a convection oven at 105 ℃ continuously until a constant weight was obtained. The loss in moisture is quantified as the moisture content whiles the remaining solid material is quantified as the total solids.

The lipids content was obtained through Soxhlet extraction as described in Borines *et al.* (2013). It involved the heating of the sample in a solvent (petroleum ether) using a Soxhlet extraction apparatus for 16 h. The solvent was recovered through evaporation while the flask containing the lipids extract was cooled and weighed. Total proteins were determined as described in Hames *et al.* (2008). It involved the determination of the nitrogen content using a Hanna Total Nitrogen test kit (Hanna Instruments Inc., USA). The total nitrogen content obtained was then multiplied by a nitrogen-to-protein conversion factor of 6.25 to obtain an estimated protein content for the biomass.

The volatile solids and ash content was determined as described in Sluiter *et al.* (2004) (adapted from ASTM E1755-01). It involved the dry oxidation of the biomass at 575 ℃ until a constant residue weight was obtained. The residue of the biomass after the dry oxidation was quantified as the ash content. The difference between the total solids of the biomass and its ash content was quantified as the volatile solids of the material.

The total carbohydrate was obtained using a modified form of the method described in Van Wychen and Laurens (2015) (adapted from ASTM E1758-01). It involved the sequential hydrolysis of the biomass with 72% sulphuric acid at 30 ℃ for 1 hour and 4% sulphuric acid at 121 ℃ also for 1 hour. The liquid fraction of the hydrolysate obtained was analysed for total sugar using the 4-hydroxybenzoic acid hydrazide (PAHBAH) assay along with a standard glucose curve.

3.3 Monomeric sugars analysis of selected seaweeds

The polysaccharide fraction forms the most important component in any biomass needed for bioethanol production. However only a fraction of the monomeric sugars in the seaweed polysaccharide is fermentable to ethanol by common yeast strains. It

was therefore important to quantify the individual monomeric sugars in the polysaccharides in the selected seaweeds to further determine its strength as a bioethanol substrate. The monomeric sugars in the selected seaweeds were obtained using a modified form of the method described in Van Wychen and Laurens (2015) (adapted from ASTM E1758-01). It involved the sequential hydrolysis of the biomass with 72% sulphuric acid at 30 ℃ for 1 hour and 4% sulphuric acid at 121 ℃ for 1 hour. The liquid fraction of the hydrolysate obtained was analysed for monomeric sugars via High Performance Liquid Chromatography (HPLC).

3.4 Screening of various pretreatments for seaweed hydrolysis

In this study, various pretreatments were examined to assess their relevance in algal ethanol production, their effect on sugar recovery and the best condition that supports enzymatic hydrolysis. Six unique pretreatment conditions were considered and each condition was followed by enzymatic hydrolysis with a commercial cellulase enzyme, Cellic CTec II (Novozymes, Denmark) at 5 FPU/g DM for 72 hours at 50 ℃ with 5% w/v substrate concentration while shaking at 150 rpm in an incubator shaker (Lab Companion SIF5000, Jeio Tech-Korea). The pretreatments used were dilute acid, dilute alkaline, hot buffer, extremely low acid, dry heat and hot water wash with buffer-less and buffered enzymatic hydrolysis as controls. All three pre-processed seaweeds were screened with the pretreatments conditions summarized in Table 3-1.

3.4.1 Dilute acid pretreatment

In this pretreatment, seaweed was added to 10 ml of 0.2 M H_2SO_4 in 100ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line, Germany) at 130 ℃ for 15 min (adapted from Meinita *et al.,* 2015). The mixture was cooled to room temperature after heating. A 100 µl aliquot of the liquid fraction was analysed for total reducing sugars (TRS) using the PAHBAH assay. The pH of the mixture was adjusted to a range of 5-6 with 4.5 M NaOH before enzyme application. The substrate concentration was also adjusted to 5% w/v dry basis with distilled water before enzyme application for hydrolysis.

3.4.2 Dilute alkaline pretreatment

Seaweed was added to 10 ml 0.2 M NaOH in 100ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven

(VWR Dry-Line, Germany) at 130 ℃ for 15 min (adapted from Meinita *et al.*, 2015). The mixture was cooled to room temperature. A 100 µl aliquot of the liquid fraction was analysed for total reducing sugars using the PAHBAH assay. The pH of the mixture was adjusted to a range of 5-6 with 0.2 M H_2SO_4 before enzyme application. The substrate concentration was also adjusted to 5% w/v dry basis with distilled water before enzyme application for hydrolysis.

3.4.3 **Extremely Low acid pretreatment**

Seaweed was added to 10 ml 0.006 M H_2SO_4 in 100ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line, Germany) at 130 ℃ for 15 min (adapted from ye Lee *et al.*, 2013). The mixture was cooled to room temperature. A 100 µl aliquot of the liquid fraction was taken and analysed for total reducing sugars using the PAHBAH assay. The pH of the mixture was not adjusted since it was already in the range of 5-6 before enzyme application. The substrate concentration was adjusted to 5% w/v dry basis with distilled water before enzyme application for hydrolysis.

3.4.4 **Hot Buffer pretreatment**

Seaweed was added to 10 ml 0.05 M Citrate Buffer in 100ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line, Germany) at 120 ℃ for 60 min (adapted from Trivedi *et al.*, 2013). The mixture was cooled to room temperature. A 100 µl aliquot of the liquid fraction was taken and analysed for total reducing sugars using the PAHBAH assay. The pH of the mixture was not adjusted since it was already in the range of 5-6 before enzyme application. The substrate concentration was adjusted to 5% w/v dry basis with distilled water before enzyme application for hydrolysis.

3.4.5 **Dry heat pretreatment**

Seaweed was added to 10ml distilled water in 100 ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line, Germany) at 130 ℃ for 60 min (adapted from Trivedi *et al.*, 2013). The mixture was cooled to room temperature. A 100 µl aliquot of the liquid fraction was taken and analysed for total reducing sugars using the PAHBAH assay. The pH of the mixture was not adjusted since it was already in the range of 5-6 before enzyme

application. The substrate concentration was adjusted to 5% w/v dry basis with distilled water before enzyme application for hydrolysis.

3.4.6 **Hot water wash pretreatment**

Seaweed was added to 10ml distilled water in 100 ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line, Germany) at 50 °C for 90 min (Gao *et al.*, 2015). The mixture was cooled to room temperature. A 100 µl aliquot of the liquid fraction was taken and analysed for total reducing sugars using the PAHBAH assay. The pH of the mixture was not adjusted since it was already in the range of 5-6 before enzyme application. The substrate concentration was adjusted to 5% w/v dry basis before enzyme application. The mixture was further hydrolysed with the commercial cellulase.

3.4.7 **Buffer-less Untreated biomass**

Pre-processed seaweed samples untreated was added to 20 ml distilled water in 100 ml Duran bottles to form a substrate concentration of 5% w/v dry basis. The pH of the mixture was not adjusted since it was in the range of 5-6 before enzyme application. The mixture was hydrolysed with the commercial cellulase.

3.4.8 **Buffered Untreated biomass**

Pre-processed seaweed samples untreated was added to 20 ml 0.05 M Citrate Buffer in 100 ml Duran bottles to form a substrate concentration of 5% w/v dry basis. The pH of the mixture was not adjusted since it was in the range of 5-6 before enzyme application. The mixture was hydrolysed with the commercial cellulase.

Table 3-1. Summary of pretreatment conditions applied to seaweeds

Type of Pretreatment	Conditions				Reference
	Catalyst	Temperature (°C)	Time (min)	Substrate concentration (% w/v)	
Dilute acid	0.2 M H_2SO_4	130	60	10	Meinita *et al.*, 2015
Dilute alkaline	0.2 M NaOH	130	60	10	Meinita *et al.*, 2015

Extremely low acid	0.006 M H_2SO_4	130	60	10	ye Lee *et al.*, 2013
Hot buffer	0.05 M Citrate buffer	130	60	10	Trivedi *et al.*, 2013
Dry heat	Distilled water	130	60	10	Trivedi *et al.*, 2013
Hot water wash	Distilled water	50	90	10	Gao *et al.*, 2015
Buffer-less (control 1)	Distilled water	-	-	-	-
Buffered (control 2)	0.05 M Citrate buffer	-	-	-	-

3.5 Optimization of the dilute acid hydrolysis of the seaweeds

Dilute acid hydrolysis (or pretreatment) is considered the most economical and time saving form of hydrolysis currently available for algal biomass (Mutripah *et al.*, 2014). Acid concentrations as low as 0.006 M and reaction times as low as 15 minutes have been reported with appreciable reducing sugar yields (ye Lee *et al.*, 2013; Meinita *et al.*, 2013). In this study, the interactions between acid concentration, reaction time and reaction temperature in dilute acid hydrolysis were examined to optimize the reducing sugar yields from the three selected seaweeds.

The boundary conditions were kept narrow due to the extensive reports on the optimal conditions for dilute acid hydrolysis of seaweeds. The general full factorial experimental design was used with 3 factors with 3 levels per factor. The factors examined were acid concentration (0.1, 0.2, 0.3 M), reaction time (15, 30, 60 min), reaction temperature (100, 120, 130 min). The experimental design matrix was generated with Minitab 17 statistical software (Appendix A1.1). A total of 27 experimental runs were performed on each seaweed species in triplicates. The interactions between the factors (hydrolysis conditions) and the response variable (TRS) were modelled with the aid of multiple regression analysis using Minitab 17.

For each unit, pre-processed seaweed was added to 10 ml of sulphuric acid solution (of known concentration) in 100ml Duran bottles to form a substrate concentration of 10% w/v dry basis. The mixture was heated in a convection oven (VWR Dry-Line,

Germany) at each specified temperature and time from the experimental design matrix. The mixture was cooled to room temperature and centrifuged at 6000 rpm for 5 min. A 100 µl aliquot of the liquid fraction was taken and analysed for TRS using the PAHBAH assay.

3.6 Optimization of the dilute enzymatic hydrolysis of the seaweeds

Enzymatic hydrolysis is considered the most efficient form of hydrolysis for algal biomass currently available. However various studies have raised concerns over the high cost of enzymes and the long reaction times they require. This study therefore sought to optimize the enzymatic hydrolysis process with emphasis on minimal enzyme application to obtain significantly high sugar yields.

To achieve this the interactions between enzyme dosage, reaction times and substrate concentration were examined to optimize the total reducing sugar yield. The Central Composite Rotational factorial experimental design was used with 3 factors and 3 levels per factor since it is less material and time intensive due to the fewer runs in its design. The factors examined were enzyme (2, 5, 8 FPU/g DM), reaction time (24, 48, 72 h) and substrate concentration (5, 10, 15% w/v dry basis). The experimental design matrix was generated with Minitab 17 (Appendix A1.2). A total of 20 experimental runs were performed on each seaweed species in triplicates. The interactions between the factors (hydrolysis conditions) and the response variable (TRS) were modelled with the aid of multiple regression analysis using Minitab 17.

Pre-processed seaweed was first pretreated with 0.2 M H_2SO_4 at 130 °C in a convection oven for 15 min with a substrate concentration of 20% w/v dry basis (adapted from Meinita *et al.,* 2015). The mixture was cooled to room temperature after heating. The pH was adjusted to the range of 5-6 with 4.5 M NaOH. The amount of enzyme as specified in the experimental design matrix was added to the mixture. The enzyme applied in this study was the Cellic CTec II (Novozyme, Denmark) enzyme, a commercial cellulase whose cellulase activity was determined using a modified form of the method described by Adney and Baker (1996). The final substrate concentration of the mixture was also adjusted to 5% w/v dry basis with distilled water. The mixture was incubated in an incubator shaker (Lab Companion SIF5000, Jeio Tech-Korea) at

50 ℃ while shaking at 150 rpm for the length of time specified in the experimental design matrix.

After the specified time, the mixture was cooled to room temperature and centrifuged at 6000 rpm for 5 min. A 100 µl aliquot of the liquid fraction (supernatant) was analysed for total reducing sugars using the PAHBAH assay. The experimental data obtained was analysed using Multiple regression analysis in Minitab 17 statistical software to obtain the optimal process condition. The optimal condition obtained was validated in separate test runs.

The hydrolysate from the validation run was also cooled to room temperature and centrifuged at 6000 rpm for 5 min. The supernatant (liquid hydrolysate) was decanted and stored for fermentation while the residue was also stored in a -15 ℃ freezer. A 100 µl aliquot of the liquid fraction (supernatant) was analysed for total reducing sugars using the PAHBAH assay.

3.7 Fermentation conditions for seaweed conversion

The fermentation procedures used were adapted from methods described in Chirapart *et al.* (2014) and Cho *et al.* (2013). Since the fermentation of hydrolysates also forms a critical and limiting stage in bioethanol production, the fermentation process in this study emphasized on the selection of high ethanol yielding yeast strains. The fermentation studies included: yeast growth analysis; yeast strain sugar selectivity analysis; SHF of seaweeds and SSF of seaweeds.

3.7.1 Yeast strains used in the study

Four commercial yeast strains and one yeast isolate were purchased for the study (Table 3-2). The commercial strains were *S. cerevisiae* SI17 (Baker's yeast), *S. cerevisiae* C8T17 (Brewer's yeast), *S. cerevisiae* FT17 (Brewer's yeast) and *S. cerevisiae* C8T17 (Brewer's yeast); and the isolate was *A. angophorae* NCYC 2802. The commercial strains were purchased from the open market while the yeast isolate was purchased from the National Collection of Yeast Cultures, United Kingdom. The strain codes attached to the commercial strains were assigned in this study based on their name and year of purchase.

Table 3-2. List of yeast strains used in this study

Yeast strain	Commercial name	Common name	Yeast Identification in this study	Source
S. cerevisiae SI17	Saf-Instant	Baker's yeast	Y1	Saf-Instant, France
S. cerevisiae C8T17	Classic 8 Turbo	Brewer's yeast	Y2	Still Spirits, UK
S. cerevisiae FT17	Fast Turbo	Brewer's yeast	Y3	Still Spirits, UK
S. cerevisiae PT17	Pure Turbo	Brewer's yeast	Y4	Still Spirits, UK
A. angophorae NCYC 2802	NCYC 2802	-	Y5	NCYC, UK

3.7.2 **Yeast culturing and growth analysis**

0.5 g of each of the yeast strains (excluding Y5) was cultured in 200 ml of culture medium made up of 10 g/l yeast extract, 6.4 g/l ammonium sulphate and 20 g/l glucose (adapted from Meinita *et al.*, 2015; Ge *et al.*, 2011). The culture medium was autoclaved at 121 ℃ and cooled to room temperature in ice before the addition of the dry yeast. The inoculated medium was incubated in an incubator shaker at 30 ℃ for 20 h while shaking at 120 rpm (Chirapart *et al.*, 2014).

Due to the small mass and high cost of Y5, < 1mg of it was first sub-cultured in 50 ml of the same cultured medium and under the similar conditions as used in Y1-Y4 but for 44 h. The entire cell colony obtained was then transferred into new 200 ml culture medium and incubated under the same conditions as Y1. Since Y5's starting cell mass was much lower than that in Y1 to Y4, its cell growth yield was expected to be lower.

The cultured yeast cells were harvested after the incubation period by centrifuging the culture medium at 6000 rpm for 10 min. The supernatant was decanted and discarded leaving the yeast cells. The cells were washed 3 times with sterile water. The yeasts cells were suspended in 20 ml sterile water after washing. The yeast cell growth was

obtained by measuring the optical density (OD) of the yeast suspensions of the cultured yeast strains at a wavelength of 600 nm using a spectrophotometer (Genesys 10S VIS, Thermoscientific-USA). The dry cell weight (DCW) was calculated by multiplying the OD by a conversion factor of 0.4 g/l/OD (Lee *et al.*, 2015). The yeast cell growth rate was calculated as:

$$Yeast\ cell\ growth\ rate = \frac{\Delta\ Dry\ cell\ weight}{Culturing\ time} \times 1000 \qquad (3)$$

3.7.3 Sugar selectivity analysis of the selected yeast strains

The selectivity of the 5 yeast strains to various types of monomeric sugars were examined in this study. Two substrate solutions, SUB-A and SUB-B representing a pure and mixed substrate, respectively were prepared. SUB-A was composed of 15 g/l glucose. SUB-B was composed of 5 g/l glucose, 2 g/l galactose, 2 g/l rhamnose, 2 g/l xylose, 2 g/l arabinose, 2 g/l mannitol. The selected monomeric sugars were chosen because of their high concentrations in seaweeds. Fucose and mannose were excluded due to similarities in their yeast fermentation glycolytic pathway with glucose. Glucose therefore adequately represents both sugars.

The substrate solutions were autoclaved at 121 °C for 15 min to prevent microbial contamination. Fermentation was carried out in 100 ml Duran bottles which were sterilized by autoclaving at 121 °C for 15 min. 0.3 g DCW/l of the 5 yeast strains were inoculated separately into 10ml of both substrate solutions with triplicate units for each substrate. The inoculated substrate solutions were incubated at 30 °C for 48 hours while shaking at 120 rpm (Trivedi *et al.*, 2013). At the end of the incubation period, the fermentation broths were centrifuged at 6000 rpm for 5 min to separate the yeast cells and residual solids from the liquid fraction of the broth. The supernatant was analysed via HPLC for residual monomeric sugars.

3.7.4 SHF conditions for seaweed conversion

Seaweed hydrolysates obtained from the hydrolysis optimisation done in section 3.5 and 3.6 were used as the starter medium in the SHF study. All 5 yeast strains selected were considered as inoculum for fermentation. 1 g DCW/l of each cultured yeast strain was inoculated into seaweed hydrolysates to form 9 ml fermentation broths in 100ml Duran bottles. The fermentation broths were incubated at 30 °C in an incubator shaker for 48 h while shaking at 120 rpm (Trivedi *et al.*, 2013). At the end of the incubation

period, the fermentation broths were centrifuged at 6000 rpm for 5 min. Aliquots of the supernatant were analysed via HPLC for fermentation products.

3.7.5 SSF conditions for seaweed conversion

20% w/v dry mass of *U. fasciata*, *H. dentata* and *S. vulgare* seaweeds were first pretreated with 0.2 M H_2SO_4 at 130 °C in a convection oven for 15 min (Meinita *et al.*, 2013). The pre-treated samples were cooled to room temperature and their pH adjusted to the range 5-6 with 4.5 M NaOH. The optimal enzyme dosage (with Cellic Ctec II enzyme) obtained in section 3.6 was applied to the seaweeds. 1 g DCW/l of the 5 cultured yeast strains was also added to the seaweeds. Distilled water was added to the mixture of pre-treated samples, enzyme and yeast to form a final fermentation broth with 5% dry substrate concentration.

The fermentation broths were incubated at 37 °C in an incubator shaker for 48 h while shaking at 120 rpm (Kim *et al.*, 2015). At the end of the incubation period, the fermentation broths were centrifuged at 6000 rpm for 5 min. Aliquots of the supernatants were analysed via HPLC for residual monomeric sugars and fermentation products.

3.8 Analytical methods for sugars and fermentation products

The succeeding sub-sections describes the various materials and methods used in the analysis of monomeric sugars, total reducing sugars and fermentation products. It also includes various formulas used in the computation of yields for monomeric sugars, total reducing sugars and fermentation products as well as their conversion efficiencies.

3.8.1 Analysis of sugars and fermentation products by HPLC

The identification and quantification of monomeric sugars and fermentation products from the seaweeds studied were done using a Shimadzu LC10/20 HPLC equipped with a refractive index detector. Monomeric sugars were analysed in the HPLC on a Rezex RPM column (Phenomenex, USA) operating at a column temperature of 80 °C and a detector temperature of 40 °C with ultrapure water as mobile phase at a flow rate of 0.6 ml/min. The fermentation products were analysed in the HPLC on an Aminex 87H organic column (Biorad Laboratories, USA) operating at a column temperature of 60 °C and a detector temperature of 55 °C with 0.005 M sulphuric acid as mobile phase

at a flow rate of 0.6 ml/min. The HPLC was calibrated with high purity standards of glucose, xylose, mannose, cellobiose, rhamnose, arabinose, fucose, galactose and mannitol for reducing sugars and; ethanol, xylitol, acetic acid, levulinic acid, furfural, lactic acid, glycerol, hydroxymethyl furfural and succinic acid for fermentation products. The sample injection volume for all the analytes were 10 µl. All analytes were filtered with 0.2 µm syringe filters into 1.5 ml crimp vials (with crimp caps) before injection in the HPLC.

3.8.2 **Analysis of total reducing sugars by PAHBAH assay**

The concentration of total reducing sugars in the various hydrolysates obtained were measured using the PAHBAH assay (Du *et al.*, 2010). This assay exploits the reduction effect of the aldehyde group in the structure of reducing sugars on chromogenic agents. In this study, 0.5% w/v of the chromogenic reagent, 4-hydroxybenzoic acid hydrazide in 0.5 M NaOH was reacted with aliquots of the hydrolysates obtained to form a bright yellow colour when heated in a test tube at 100 ℃. The absorbance of the colour formed was measured at a wavelength of 410 nm with a spectrophotometer (Genesys 10S VIS, Thermoscientific-USA). The absorbance obtained was measured against a standard glucose calibration curve to obtain the concentration of total reducing sugars in the hydrolysate.

3.8.3 **Analysis of yields for sugars and fermentation products**

The yields of monomeric sugars, total reducing sugars and fermentation products were calculated relative to the dry mass of the initial biomass used in each experiment. These were calculated using the following equations:

For monomeric sugars,

$$Yield_{monomeric\ sugar} = \frac{[Monomeric\ sugar]}{[Dry\ biomass]} \times 100 \qquad (4)$$

For total reducing sugars,

$$Yield_{TRS} = \frac{[Total\ reducing\ sugars]}{[Dry\ biomass]} \times 100 \qquad (5)$$

For fermentation products (relative to biomass),

$$Yield_{fermentation\ product} = \frac{[Fermentation\ product]}{[Dry\ biomass]} \times 100 \qquad (6)$$

For fermentation products (relative to the TRS),

$$Yield_{fermentation\ product} = \frac{[Fermentation\ product]}{[TRS\ of\ hydrolysate]} \times 100 \qquad (7)$$

For fermentation conversion efficiency (based on the stoichiometric glucose to ethanol conversion factor of 0.512),

$$Conversion\ efficiency = \frac{Yield_{Fermentation\ product}}{0.512} \qquad (8)$$

3.9 Oxidation of residue from seaweed bioethanol production in MFCs

As part of the biorefinery approach to bioethanol production from seaweeds, its residue was used in the production of bioelectricity using microbial fuel cells (MFCs). Residue from bioethanol production can be obtained from two main sources. The first is the solid residue obtained when the liquid hydrolysate is separated immediately after hydrolysis in an SHF pathway. The second can be obtained as bottoms from the distillation of the fermentation broth in an SHF or SSF pathway. In this study, the latter was used due to its popularity in commercial scale plants. The solid residue was therefore obtained after the enzymatic hydrolysis of the three-seaweed species for use as substrates in the generation of electricity in MFCs.

3.9.1 Seaweed residue compositional analysis

The seaweed bioethanol residue was analysed for total solids, moisture content, volatile solids and ash content. The total solids and moisture content were done as described as described by Sluiter *et al.* (2008) while the volatile solids and ash content were determined as described in Sluiter *et al.* (2004).

3.9.2 Inoculum sampling and characterisation

Cow dung from the KNUST Animal Science Department was sampled for use as inocula for the MFCs. The cow dung was sampled solely for purposes of inoculation and not as an alternative source of substrate. It was therefore diluted with tap water and filtered through a cheese cloth to minimize the total solids load.

3.9.3 MFC Configuration

An H-shaped double chamber MFC obtained from the Department of Biotechnology and Biomedicine (Technical University of Denmark; Lyngby, Denmark) was used for the study (Figure 3-2). It was composed of two cylindrical acrylic cylinders with a

volume of 300 ml each. The two chambers were connected with two acrylic tubes of inner diameters of 30 mm each. A cation exchange membrane Nafion™ N117 (Fuel Cell Earth LLC, USA) with an area of 9.62 cm^2 was placed between the two smaller acrylic tubes. The two chambers were sealed with a flat circular 40-mm diameter acrylic plate tightened with rubber rings (as gasket) and screw bolts. The anode chamber was kept completely sealed when the MFC was in operation since the substrate oxidation process occurs under anaerobic conditions. The cathode chamber however had a 2-mm diameter opening in its seal for air flow.

Activated carbon (from Palm kernel shells) beds were used as electrode material for both the anode and cathode with a stainless-steel rod in the centre of each bed as an electric current collector to the external load. An electric circuit was formed by linking a 1000 Ω resistor as the load between the anode and cathode with two copper wires.

Figure 3-2. H-shaped Double-chamber MFC used in bioelectricity generation

3.9.4 **Inoculation of MFCs**

The anode chamber of each MFC used was inoculated with 220 ml of cow dung solution. 3 g/l of sodium acetate was included to serve as the primary source of substrate for the mixed microbial culture in the solution. The cathode chamber was filled with 220 ml of 0.1 M potassium ferricyanide solution (as electron acceptor). The inoculation was done at ambient temperature conditions (27-32 °C) for 10 days. During

inoculation, voltage-time data was collected with a PicoLog ADC-20 data logger (Pico Technology Cambridgeshire-UK) connected between the MFCs and a computer. The voltage-time data was used to assess the strength of the biofilm formed on the electrode surface during the inoculation process. Both anolyte and catholyte were decanted from the MFCs at the end of the inoculation period.

3.9.5 **Operation of MFCs with seaweed residue**

A 220-ml substrate solution made up of distilled water and 4.5 g/l (dry weight) seaweed residue was introduced into the anode chamber immediately after the inocula was decanted from the anode to form the anolyte. A fresh batch of catholyte (220 ml of 0.1M potassium ferricyanide solution) was also used. The pH, temperature, total dissolved solids, solution conductivity and salinity for both electrolytes were measured before their use. The pH and temperature were measured using a pH meter (VWR PH110, VWR-Leicestershire, England) while the total dissolved solids, solution conductivity and salinity were measured using a conductivity meter (VWR CO 310, VWR-Leicestershire, England). The initial and final CODs of each anolyte were measured using a COD kit (Hanna COD HR, Hanna Instruments Inc., USA)

Four identical MFCs were used in the study. Residues from each of the three-seaweed species were introduced into separate MFCs and the fourth MFC was filled with 4.5 g/l sodium acetate solution as a control unit. All the MFCs were operated in 3 fedbatch cycles to ensure the processes were reproducible. The cycle time for each batch was 5 days. After each cycle, the previous substrate solution was decanted before fresh substrate solution of the same concentration was added. During each cycle voltagetime data was monitored with a PicoLog ADC-20 data logger to monitor the performance of the units. The internal resistance of the MFCs were measured when steady voltage was attained using a Potentiostat.

3.10 Analytical methods for MFC performance

The overall performance of the MFCs was examined by monitoring power generation, substrate consumption and internal resistances for the cells. Power generation was monitored by measuring cell voltage with time, and by calculating power and current densities based on the maximum cell voltage obtained under the load. Internal resistances which causes losses in power generated was also monitored by measuring

the overall ohmic resistance using Electrochemical Impedance Spectroscopy (EIS). Substrate consumption was monitored by measuring the substrate removal and coulombic efficiencies at the end of each fed-batch cycle.

3.10.1 **Analysis of power generation**

In this study, power generation was monitored by measuring cell voltages across the load with time. The maximum cell voltage (V) measured, along with the external resistance (R) and anolyte volume were used to calculate the current and power densities (based on Ohm's law) as:

$$Current\ density\ \left(\frac{A}{m^3}\right) = \frac{\left(\frac{V}{R}\right)}{Anolyte\ volume} \qquad (9)$$

$$Power\ density\ \left(\frac{W}{m^3}\right) = \frac{\left(\frac{V^2}{R}\right)}{Anolyte\ volume} \qquad (10)$$

3.10.2 **Analysis of internal resistances by EIS**

The power generated by the MFCs is known to be limited by internal resistances within the system. The internal resistances monitored in this study was the overall ohmic resistance. This was measured using EIS (Offei *et al.,* 2016, Sun *et al.,* 2016). This test was conducted when the MFCs reached steady state operation (stable peak voltage) with a potentiostat (Gamry G750, Gamry Instruments Inc., USA) and an EIS software on a computer. The potentiostat was connected in a two-electrode mode to determine the overall impedance.

The EIS involves superimposing a sinusoidal signal with a small amplitude over a wide range of frequencies to the applied potential of a working electrode of an MFC. A Nyquist plot is generated in the Potentiostat software interface as the output of the EIS. The plot is composed of the real impedance on the x-axis and the imaginary impedance on the y-axis. Each point on the plot corresponds to the measured impedance at each frequency examined. The EIS was conducted in this study in a galvanostatic mode at an AC amplitude of 0.3 mA; frequency range of 20 kHz to 0.1 Hz and; 6 points per decade. The MFCs were analysed for internal resistance under open circuit conditions.

3.10.3 **Analysis of substrate consumption**

The substrate consumption was monitored by calculating the substrate removal coulombic efficiencies. Substrate removal efficiency measures the percentage of the substrate that was used up in the entire process while coulombic efficiency measures the percentage of the substrate that was consumed as electric current. In the study, the initial and final CODs were measured using a COD kit (Hanna COD HR, HannaUSA). This was used to calculate the substrate removal efficiency and coulombic efficiency of the MFCs. The substrate removal and coulombic efficiencies were calculated as:

$$Substrate\ removal\ efficiency = \frac{\Delta\ COD}{Initial\ COD} \times 100 \quad (11)$$

$$Coulombic\ efficiency = \frac{M_s \int_0^{t_b} I\ dt}{F b_{es} v_{An} \Delta COD} \quad (12)$$

M_s is the molecular weight of the substrate, F is Faraday's constant, ΔCOD is the change in substrate concentration over the batch cycle (COD is used as a measure of substrate concentration), t_b is the cycle time, b_{es} is the moles of electrons produced per mole of substrate consumed, v_{An} is the volume of anolyte and I is the average current produced within the operating time (Logan, 2008).

3.10.4 **Analysis of MFC effluent**

The MFC effluent was obtained as the content of the anode decanted at the end of each operating fed-batch cycle. This was characterized for total nitrogen (Hanna Total Nitrogen HR Test vials, Hanna Instruments Inc., USA), total phosphorus (Hanna Checker Phosphorus HR kit, Hanna Instruments Inc., USA) and ammonia content (Hanna Checker Ammonia HR kit, Hanna Instruments Inc., USA).

3.11 Statistical analysis

All experiments in this study were conducted in triplicates. All results are presented as mean and standard deviation computed using Microsoft Excel®. The statistical significance of variation between means of results obtained were evaluated by Oneway ANOVA, Two-way ANOVA and Tukey's multiple comparison test (a Post-Hoc test) using GraphPad Prism 6 statistical software. The experimental designs in this study were generated using Minitab 17 statistical software. The results for dilute acid and enzymatic hydrolysis optimisation (section 3.5 and 3.6) were analysed further using multiple regression analysis in Minitab 17 to obtain regression model equations along

with contour plots which describes the interactions between the variables studied. The optimal conditions for both dilute acid and enzymatic was also obtained using stepwise regression analysis.

3.12 Substrate utilization assessment

A material balance analysis was used to examine the overall usage of the threeseaweeds for both bioethanol and bioelectricity production based on results obtained in the study. The material usage for bioethanol production was defined as the mass of total reducing sugars released from the biomass after hydrolysis for conversion to ethanol relative to the initial biomass i.e.

$$Substrate\ usage_{bioethanol} = \frac{Mass\ of\ seaweed\ TRS}{Initial\ dry\ mass\ of\ seaweed} \times 100 \qquad (13)$$

The material use for bioelectricity production via MFCs was defined as the mass of unused substrate (recovered as MFC effluent) relative to the initial dry mass of seaweed bioethanol production residue used i.e.

$$Substrate\ usage_{bioelectricity} = \frac{\Delta\ seaweed\ residue\ dry\ mass\ per\ batch}{Initial\ seaweed\ residue\ dry\ mass\ per\ batch} \times 100 \qquad (14)$$

3.13 Life cycle assessment of bioenergy production from seaweeds

Life cycle assessment (LCA) is a tool used to systematically analyse the environmental interactions of a product or service over its entire life cycle. Material and energy balances are used in LCAs to create a detailed inventory of all resource usage, energy consumption and material emissions from all processes (Alvarado-Morales *et al.,* 2013). The life cycle ranges from the conversion of the raw material to the desired product; to the final disposal or degradation of the products and any by-products. In this study, the LCA principles are followed sequentially covering all four phases to assess the sustainability of bioenergy production from seaweeds. The assessment phases include: defining the goal and scope, inventory analysis, impact assessment and interpretation (ISO 14040).

3.13.1 **Goal and scope of the LCA**

The goal of the LCA in this study was to determine the most sustainable pathway to process seaweeds in Ghana using the biorefinery approach. The results from this study can potentially support in decision making related to biofuel and bioenergy policies particularly in Ghana and other tropical regions. The LCA parameter (metric) used in this study as a measure of sustainability was the Energy Return on Investment (EROI). The EROI is generally defined as the ratio of the output energy of a system to its input energy (Murphy and Hall, 2010). This was calculated as the ratio of the lower heating value of energy carriers to the total cumulative non-renewable fossil energy demand (Aitken *et al.*, 2014).

The scope of the LCA for bioenergy from seaweeds was defined by three different bioenergy pathways, *Scenario 1*: Bioethanol production only; *Scenario 2*: Bioethanol and Bioelectricity production (based on MFC technology) and; *Scenario 3*: Bioethanol and Biogas production. The functional unit for comparison was defined as 1 MJ of energy produced by the lower heating value of the energy carriers. The inventory inputs were defined relative to 1 ha of seaweed cultivated. For each input a base case scenario was used.

3.13.2 **Bioenergy process system model description**

The bioenergy process system begins in all scenarios with the cultivation of seaweeds at near shore of the open sea using the bottom planting approach. During bottom planting, the thalli (the stalk of seaweeds) is prepared and planted by a diver 1 km from a landing point. The biomass is harvested using a fishing vessel when thalli is mature. Harvesting was assumed to be twice in year. Harvesting is followed by pre-processing (drying and milling), conversion to biofuels and bioenergy (fermentation/electrogenic substrate oxidation/anaerobic digestion) and its associated by-products. The effluent from the final bioconversion process is dried for use as a fertilizer. Figure 3-3 shows a summary of the various bioenergy process systems considered in this study.

3.13.2.1 Scenario 1: Bioethanol Production

The seaweeds harvested were first dried in the sun for 3-4 days and milled to make them easier to transport to the plant gates for processing. Cellulase enzymes (8 FPU/g dry biomass) and citrate buffer were added to the dried and milled seaweed to form a

slurry for hydrolysis. The slurry formed was hydrolysed at 50 °C for 24 h while agitating at 150 rpm. The temperature of the hydrolysate was cooled to 30 °C. Dry yeast was added, and the slurry allowed to ferment at 30 °C for 72 h while agitating at 150 rpm. The fermentation broth obtained was distilled at 78 °C in a vapour compression steam stripping unit and a vapour compression distillation unit (Alvarado-Morales *et al.,* 2013). The ethanol obtained after distillation was upgraded further to a 99.7% using molecular sieves. The stillage obtained is dried in drying beds and recovered as an organic fertiliser.

3.13.2.2 Scenario 2: Bioethanol and Bioelectricity Production

The same bioethanol production process as described in *Scenario 1* (section 3.13.2.1) was first used to convert the seaweeds in this scenario. However, the stillage obtained after distillation was pumped to microbial fuel cells for direct conversion to electricity. The assumed scale-up model of the MFC was made up 50 cells connected in parallel to maximise the current output based outputs from section 3.9. The cells were connected to a circuit booster unit which delivers an output of 220-230 V. The slurry was loaded into the anode with a substrate concentration of 4.5 kg/m^3 anode volume. Power was assumed to be generated from the substrate over a period of 60 days per ha seaweed harvested based on outputs from section 3.10. The effluent from the MFCs were pumped into drying beds and recovered as organic fertilizer.

3.13.2.3 Scenario 3: Bioethanol and Biogas Production

The same bioethanol production process as described in *Scenario 1* (section 3.13.2.1) was first used to convert the seaweeds. The stillage obtained after distillation was pumped to an anaerobic digester for biogas production. The retention time assumed was 38 days per batch. The volatile solids of the seaweed stillage were assumed to be 83% and the average methane concentration was estimated at 63%. The effluent from the anaerobic digester were pumped into drying beds and recovered as organic fertilizer. In all scenarios energy credits were not assigned to the fertilizer largely due to its primary purpose of soil amendment.

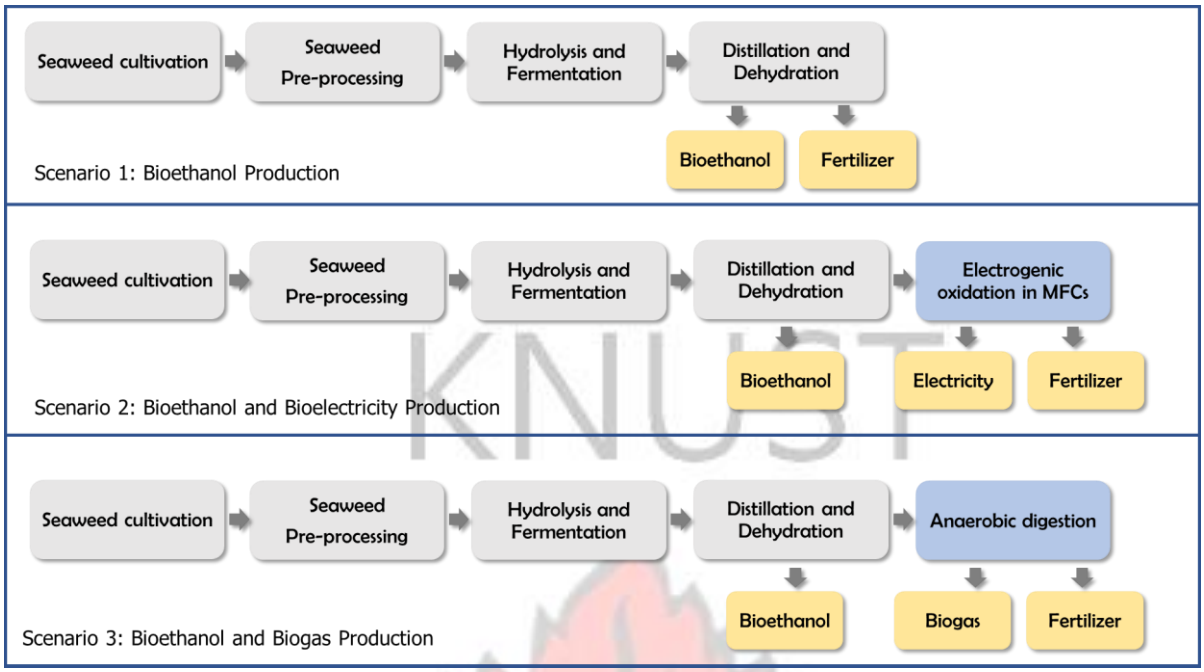

Figure 3-3. Bioenergy process system pathways used in the LCA

3.13.3 **Data acquisition and modelling**

Data was collected from various sources including published literature, experimental analysis and electronic databases (Ecoinvent 2.0). For cases where process data was unavailable, assumptions were made and or process engineering calculations performed to obtain the data. The energy inputs and outputs were calculated and compiled using Microsoft Excel 2016.

3.13.4 **Life cycle inventory**

Biomass properties, bioenergy product yields and their production conditions for the compilation of the life cycle inventory was primarily sourced from experimental work described in chapter 3 of this study. Data for the inventory was also collected from, Tchobanoglous *et al.* (2003), Sinnot and Towler (2009), Alvarado-Morales *et al.* (2013) and Aitken *et al.* (2014). The life cycle inventory has been summarized in Table 3-3 and 3-4 (full details on the LCA inventory can be found in Appendix A13).

Table 3-3. Summary of LCA inventory for inputs to Scenario 1

Scenario	Energy input from each process	Energy values kWh	GJ
Scenario 1 - Bioethanol only	*Seaweed cultivation of 18000 kg on 1 ha*		
	Energy from lighting thallus preparation shed	5.600	0.020
	Energy from diesel for thallus planting	0.322	0.001

	Energy from diesel for harvesting seaweeds	965	0.003
	Pre-processing of 729900 kg of wet seaweed		
	Energy from diesel for transporting seaweeds to plant gate	48.839	0.176
	Energy consumption of the seaweed grinder	244.744	0.881
	Energy consumption of the conveyor (Grinder to Hydrolyser)	0.893	0.003
	Processing of 85870 kg of pre-processed seaweed		
	Energy consumption of Hydrolyser agitator	3413.061	12.287
	Energy consumption by pump (Hydrolyser to Fermenter)	32.436	0.117
	Energy consumption by Fermenter agitator	3413.061	12.287
	Energy consumption by Pump (Fermenter to Distillation unit)	173.923	0.626
	Energy consumption by Compressor	2359.767	8.495
	Total heat energy required for Distillation and Dehydration	8718.250	31.386
	Energy consumption by Pump (Condenser to Storage tank)	0.031	0.0001
	Energy consumption by Pump (Distillation Bottoms to End use)	5.661	0.0204
	Total energy consumed Scenario 1	19381.590	66.303

Table 3-4. Summary of LCA inventory for inputs to Scenario 2 and 3

Scenario	Energy input from each process	Energy values kWh	GJ
Scenario 2 Bioethanol & Bioelectricity	- *Bioethanol production from 85870 kg of dry seaweed*		66.303
	Total energy consumed from Bioethanol Production	19381.590	
	Substrate oxidation of 77283.529 kg of seaweed residue		
	Energy consumed by the Microbial Fuel Cells	0	0

	Effluent treatment (1419.294 m^3 of MFC effluent)		
	Energy consumption by Pump (To Drying beds)	9.667	0.035
	Total energy consumed Scenario 2	18427.221	66.338
Scenario 3 - Bioethanol & Biogas	*Bioethanol production from 85870 kg of dry seaweed*		
	Total energy consumed from Bioethanol Production	19381.590	66.303
	Anaerobic digestion of 77283.529 kg of seaweed residue		
	Energy for mixing the sludge in the Digester	10913.390	39.288
	Energy required to heat the sludge	15439.320	55.582
	Effluent treatment (1423.159 m3 of digestate)		
	Energy consumption by Pump (To Drying beds)	9.695	0.035
	Total energy consumed Scenario 3	44779.967	161.208

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Seaweed composition

In this study, seaweed components that are essential to the bioethanol production process were analysed to examine the suitability of the selected seaweeds as ethanol substrates. Proximate and monomeric sugar analysis were used to examine the seaweeds. Components of major interest were the total solids, moisture content, total carbohydrates and the types of monomeric sugars that form the carbohydrate fraction of the biomass. The succeeding sections will discuss the components found and their relevance to the entire study.

4.1.1 Proximate analysis of selected seaweeds

The composition of the pre-processed seaweeds sampled from the Ghanaian coasts were examined for various constituents. The total solids content ranged between from

82 to 90% after pre-processing through sun drying and grinding (Table 4-1). The sun drying method which is regarded as one of the oldest and simplest methods of drying and biomass preservation was effective in reducing moisture in the seaweeds. A low moisture content is known to prolong the shelf-life of biomass (Dagne Tarle *et al.,* 2015). The high efficiency of the sun drying process could also form an important cost reduction factor for potential seaweed farming in Ghana.

The total carbohydrate fraction of the seaweeds harvested were between 31.2 and 32.6% DM (Table 4-1). There was no significant difference between the carbohydrates content in the three-seaweed species selected (p-value <0.05). This component is of prime importance to bioethanol production since it is the component that is converted to ethanol. The carbohydrate content of *U. fasciata* (31.3% DM), was lower than the 45% and 43% DM reported in Marquez *et al.* (2014) and Trivedi *et al.* (2013), respectively for the same species (Table 4-1). Also, the total carbohydrates of *S. vulgare* (32.6% DM) was much higher than the 19.43% DM reported in MarinhoSoriano *et al.* (2006). The carbohydrate content of *H. dentata* (31.2) compares favourably with the 39% DM reported in Rhein-Knudsen *et al.* (2017). Despite the variation from that reported in other studies, the carbohydrate content in the selected seaweed species can be considered high enough for substantial ethanol recovery of up to 15% DM of the biomass assuming the stoichiometric maximum ethanol recovery of 51.2% of the reducing sugars can be achieved. The selected species were therefore considered as adequate potential substrates for bioethanol production.

The ash content of the seaweeds which generally refers to the inorganic fraction of the biomass was between 27 and 38% DM (Table 4-1). This indicates some potential application of the substrate in soil amendment can be considered through further examination of the ash for nitrogen, phosphorus and some vital trace elements favourable for plant growth. The ash content values for all three seaweeds were within the 2 - 40% DM range for seaweeds reported by Polat and Ozogul (2013).

The protein content was within the range of 10 to 14% DM while lipid content was in the range 1 to 3.2% DM (Table 4-1). The proteins and lipids form part of the volatile solids content of the three seaweeds which were between 51 to 60% DM. This indicates that the seaweeds selected were high in their overall organic fractions and would be well suited as substrates for various biocatalytic conversion processes such as fermentation.

Table 4-1. Composition of the selected seaweeds

Component (% DM)	Seaweed species		
	U. fasciata	*S. vulgare*	*H. dentata*
Total solids (% biomass)[1]	82.420 ±0.003[a]	85.593 ±0.003[b]	90.513 ±0.006[c]
Moisture content (% biomass)[1]	18.467 ±0.001[a]	15.687±0.001[b]	9.960 ±0.005[c]
Volatile solids	55.510 ±0.560	60.240 ±0.610	51.560 ±0.520
Ash content	27.990 ±0.540	27.180 ±0.550	38.720 ±0.720
Total Lipids	1.520 ±0.030	1.020 ±0.020	3.180 ±0.010
Total Proteins	14.380 ±0.000	11.190 ±0.000	10.310 ±0.000
Total Carbohydrates[1]	31.289 ±1.807[a]	32.573 ±0.787[a]	31.159 ±2.093[a]

[1] *Means in the same row with different letters are significantly different (Tukey's HSD, $p<0.05$)*

4.1.2 **Monomeric sugars in the selected seaweeds**

Seaweeds are known to have a very diverse composition of monomeric sugars also referred to as reducing sugars, simple sugars or monosaccharides (Chen *et al.*, 2015). The monomeric sugars cumulatively form the total carbohydrates fraction in seaweeds. It is well reported that most common fermenting organisms for ethanol production prefer hexose monomeric sugars (C-6 sugars) over pentose monomeric sugars (C-5) (Van Maris *et al.*, 2006). In this study the presence of the hexose sugars; rhamnose, fucose, mannose, glucose and galactose were examined in the three seaweeds. The pentose sugars; xylose and arabinose as well as the sugar acid, galacturonic acids were also examined.

The major monomeric sugars found in *U. fasciata* were rhamnose, glucose and xylose (Table 4-2). *S. vulgare* had glucose and fucose as its major monomeric sugars while *H. dentata* had glucose and galactose (Table 4-2). There was no significant difference between glucose content in *U. fasciata* (15.1% DM) and *S. vulgare* (15.3% DM), which formed their largest fraction of monomeric sugar. The glucose fraction for *H.*

dentata was however significantly different at 12% DM. In *H. dentata*, galactose formed its largest monomeric sugar fraction at 13.1% DM.

These results indicate that the hexose fraction of the monomeric sugars was higher than the pentose fraction in all the three seaweeds studied. This was cumulatively between 16 and 27% DM. This is particularly preferred since the hexose fraction of carbohydrates is much easier to ferment via the glycolytic pathway (Van Maris *et al.*, 2006). The difference in hexose and pentose fraction would however impose an added challenge through a possible reduction in ethanol yield unless the fermenting organism chosen demonstrates some significant pentose to ethanol conversion capability. The selection of the fermenting organism would therefore have a critical impact on ethanol yield.

Table 4-2. Monomeric sugar composition of the selected seaweeds

Component (% DM)	Seaweed species[1]		
	U. fasciata	*S. vulgare*	*H. dentata*
Rhamnose	5.379 ±0.154^{a}	-	1.543 ±3.086^{b}
Xylose	7.596 ±1.894^{a}	1.887 ±0.637^{a}	0.952 ±0.221^{a}
Arabinose	-	0.707 ±0.351^{a}	0.634 ±0.035^{a}
Fucose	0.206 ±0.010^{a}	4.019 ±0.322^{b}	-
Mannose	1.238 ±0.264^{a}	1.632 ±0.945^{a}	3.449 ±0.744^{b}
Glucose	15.125 ±0.168^{a}	15.297 ±1.119^{a}	11.978 ±0.302^{b}
Galactose	0.905 ±0.024^{a}	2.901 ±0.216^{b}	13.113 ±2.442^{c}
Galacturonic acid	0.844 ±0.104^{a}	0.603 ±0.105^{a}	0.631 ±0.006^{a}

[1] *Means in the same row with different letters are significantly different (Tukey's HSD, $p<0.05$)*

4.2 Screening of various pretreatments for seaweed hydrolysis

Pretreatment of biomass before enzymatic hydrolysis is often done to disrupt their cellular structure to improve the hydrolytic action of the selected enzyme (Daroch *et al.,* 2013). In this study, six pretreatments were screened along with two controls (water-based and buffer-based) to examine their potential effect on subsequent enzymatic hydrolysis. The pretreatments were applied to all three seaweeds before enzymatic hydrolysis with a commercial cellulase.

4.2.1 Effect of pretreatments on *U. fasciata*

U. fasciata responded best to dilute acid treatment, hot buffer treatment and interestingly, the buffered control (with no treatment) with TRS yields of 21.8, 21.0 and 17.2% DM, respectively with no significant difference between them (Figure 41). The dilute acid and hot buffer, which were done at 130 ℃ for 60 min with 0.2M H_2SO_4 and 0.05 M Citrate buffer, respectively as catalysts, were both considered quite extreme due to the high heat energy required and the toxicity of the catalysts. The buffered control which was not subjected to any pretreatment but was hydrolysed directly in a citrate buffer medium was comparatively milder and less energy intensive.

The results for *U. fasciata* first implies that high temperatures favour structural breakdown, however, in the case of dilute acid hydrolysis higher concentrations of catalysts is required since both the extremely low acid and dry heat treatments were all done at the same temperature. High catalyst loads have been reported to increase the number of active catalytic sites for the substitution reaction by acids (Gensch *et al.,* 2018). The second implication is that buffers used in the hot buffer and buffered medium pretreatment play a significant role especially for the enzymatic hydrolysis that follows pretreatment. Enzymes are known to be very pH sensitive therefore the stable pH provided by the buffers during hydrolysis was expected to improve yield.

The comparatively high TRS yield from the buffered medium which was not pretreated is particularly interesting since it clearly implies that heating may not even be required when using the *U. fasciata* seaweed species. The presence of the polymers; ulvan and starch which have more loosely bonded configuration could account for the ease in hydrolysis of *U. fasciata* (McNamara *et al.,* 2015). The combined cost of pretreatment and hydrolysis from the use of both heat energy and a catalyst would therefore be greatly reduced for *U. fasciata* in bioethanol production.

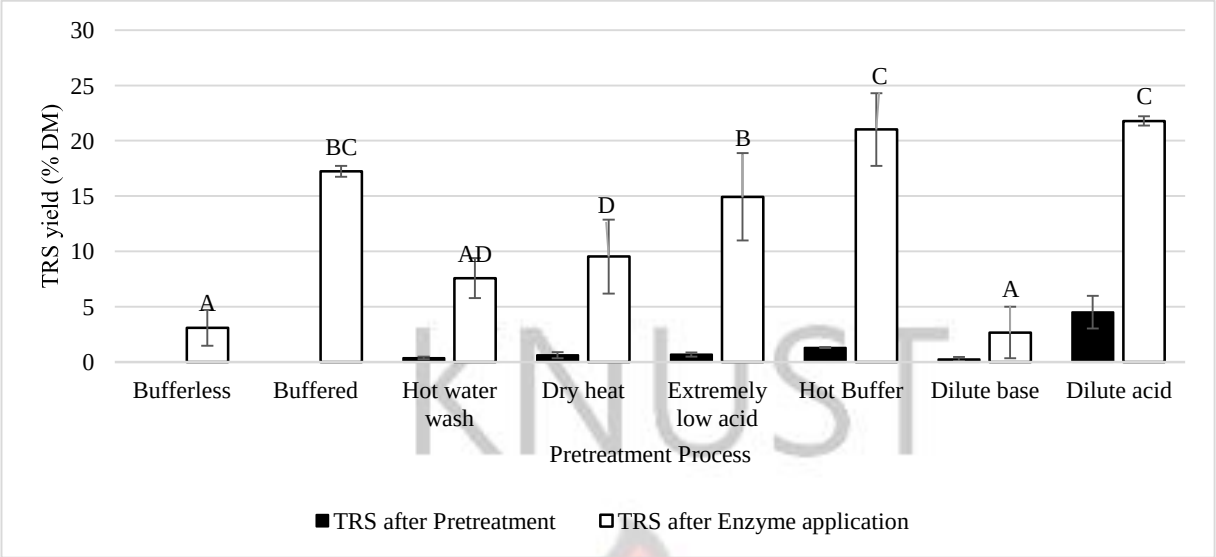

Figure 4-1. Screening of pretreatments on U. fasciata

(Means of the same bar colour with different letters are significantly different; Tukey's HSD, $p<0.05$)

4.2.2 **Effect of pretreatments on *S. vulgare***

S. vulgare responded best to dilute acid and hot buffer treatments with TRS yields of 18.2 and 12.8% DM, respectively with no significant difference between them (Figure 4-2). The change in TRS yield from pretreatment to enzymatic application was also very high from 1.7 to 12.8% DM in the case of hot buffer and from 4.4 to 18.2% DM for the dilute acid. All the other treatments recorded similar TRS yields ranging from 0.7 to 2% DM (Figure 4-2). The change in TRS yields before and after enzymatic hydrolysis for hot water wash, dry heat, extremely low acid and dilute base pretreatments were low, ranging from 0 to 1.3% DM.

The response of *S. vulgare* to the pretreatments is quite similar to the case of *U. fasciata* particularly their preference for heat application. The use of an acid based catalyst in the presence of heat i.e. sulphuric acid as used in the dilute acid pretreatment and citric acid as used in the citrate buffer medium, were both favourable for recovering reducing sugars from *S. vulgare*. The high concentrations of the acids also impact greatly on the cellular breakdown as seen in the change in TRS yields after the enzymes were applied (Figure 4-2).

The structure of *S. vulgare* is considered more stable than *U. fasciata* due to the presence of the hydrocolloid alginate. The weak hydrocolloid ulvan is found in *U. fasciata*. This difference in structural strength could account for the effective hydrolysis of *U. fasciata* after the use of a buffered medium without heat but the same treatment was comparatively ineffective in *S. vulgare*. Heat energy was therefore required in the separation of the 1,4-glycosidic bonds found in *S. vulgare*.

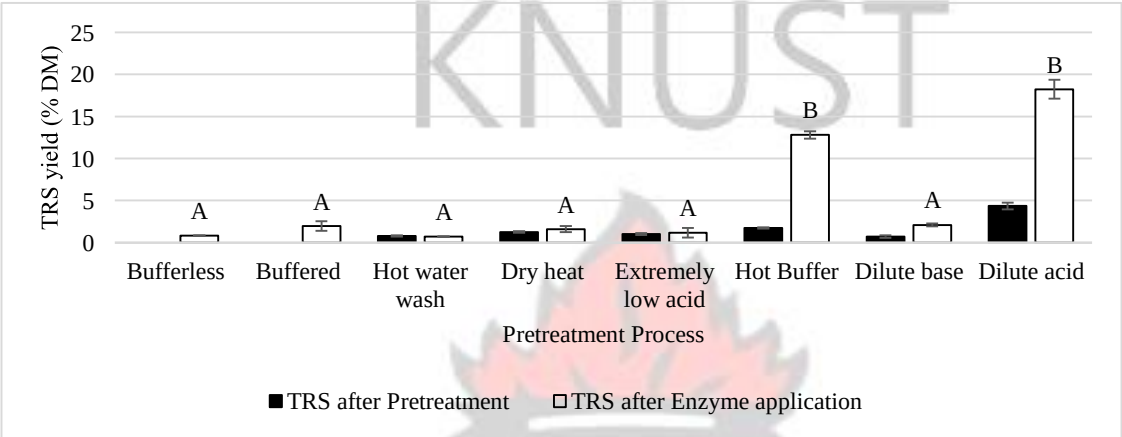

Figure 4-2. Screening of pretreatments on S. vulgare

(Means of the same bar colour with different letters are significantly different; Tukey's HSD, $p<0.05$)

4.2.3 Effect of pretreatments on *H. dentata*

H. dentata responded best to only the dilute acid treatment with a TRS yield of 21.2% DM. This was significantly different from all other treatments as seen in Figure 4-3. *H. dentata* formed a gel after dry heat, extremely low acid, hot buffer and dilute base pretreatments were applied. The gel formation could be attributed to the interactions between the hydrocolloid, agar found in the *H. dentata* species and the OH^- ions in water and bases under high temperatures. This phenomenon of gel formation in water is what gives hydrocolloids their gelling properties for use as thickeners in the food and pharmaceutical industries (Rhein-Knudsen *et al.*, 2017). The gel formation was also reported by Kim *et al.* (2011a) using 0.05-0.2 N $Ca(OH)_2$. These pretreatments could therefore be considered unsuitable for red seaweeds like *H. dentata.*

From the six pretreatments applied, only dilute acid and hot water wash were favourable for use on *H. dentata*. Dilute acid gave a significantly higher yield of 21.2 % DM as against 1.63% DM from the hot water wash (Figure 4-3). Hot water wash was also significantly lower than the buffered medium control which recorded 4.4% DM TRS yield. The TRS recovery before the enzyme was applied in the case of dilute acid pretreatment was also high at 12.58% DM. This implies that the acid catalyst

interacts favourably with the sulphated-galactans predominant in the *H. dentata* which is an agarophyte. Overall, the selection of pretreatment for use on red seaweeds such as *H. dentata* would be extremely limited. This would in turn impact pretreatment and hydrolysis costs for commercial scale bioethanol production.

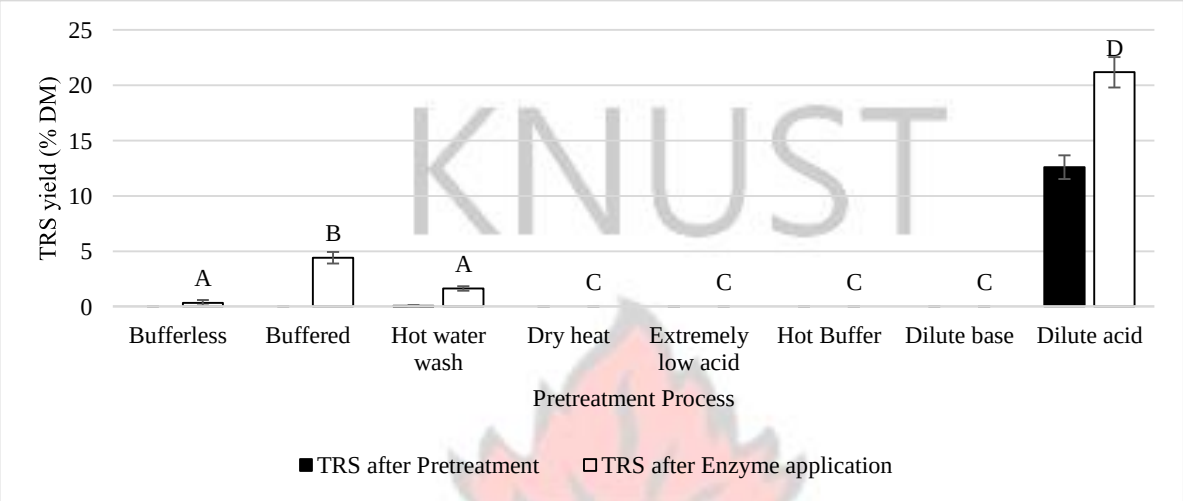

Figure 4-3. Screening of pretreatments on H. dentata

(Means of the same bar colour with different letters are significantly different; Tukey's HSD, p<0.05)

4.2.4 Comparison of pretreatment effects between seaweeds

All three seaweeds responded well to the dilute acid pretreatment with TRS yields between 18 and 22% DM (Figure 4-1, 4-2 and 4-3). This emphasizes its extensive use by numerous seaweed bioethanol production researchers (Meinita *et al.*, 2015). Trivedi *et al.* (2013) adopted a similar approach in screening several pretreatments which included dilute acid, hot buffer, dry and liquid ammonia pretreatments on the *U. fasciata* seaweed. Their study also recorded high TRS yields with a maximum of 20.7% DM from hot buffer pretreatment (Sodium acetate buffer). This compares favourably with the TRS yield from *U. fasciata* (21% DM) obtained in this study.

U. fasciata maintains an important hydrolytic advantage over the other two seaweeds used in the study since it can be efficiently hydrolysed without any pretreatment. Dilute acid pretreatment was selected as the most efficient pretreatment method from the six pretreatments studied. This was applied to all three seaweeds before enzymatic hydrolysis in subsequent parts of this study where enzymes are applied. Even though *U. fasciata* did not necessarily require pretreatment, dilute acid treatment was applied to it for the purposes of uniformity within the study.

4.3 Optimization of the dilute acid hydrolysis of the seaweeds

Dilute acid hydrolysis is considered as one of the most cost-effective and time-saving processes for hydrolysing biomass for sugar recovery (Mutripah *et al.,* 2014). In this study, it was optimised to examine if it could be solely used to efficiently recover the sugars in the seaweed biomass. For the optimisation, the independent variables examined were reaction time, reaction temperature and acid catalyst concentration with TRS as the response variable. The optimisation data was analysed using regression analysis to obtain a set of regression equations. The boundary conditions for the optimisation were based on previous studies. The effects of the independent variables were also examined further using contour plots.

4.3.1 Modelling of TRS recovery from dilute acid hydrolysis

The modelling of the dilute acid hydrolysis process was done to obtain equations that can describe and predict the possible yields from each species within the boundary conditions of the model. All three model equations shown in Table 4-3 were made up of the total reducing sugar yields expressed as a function of the independent variables studied. The model equation obtained for *U. fasciata* was expressed as a second-degree polynomial equation whiles the models for *S. vulgare* and *H. dentata* were first degree polynomials (Table 4-3). The model equation for *H. dentata* also excludes the X_2 term which refers to reaction temperature (Table 4-3). This is an indicator that the reaction temperature has the least effect on the TRS yield for *H. dentata*.

The correlation coefficient (R^2) value which represents the percentage variation that can be explained by the model was highest for *U. fasciata* at 79.9%. *S. vulgare* and *H. dentata* were however lower at 68.8 and 41.7%, respectively. This implies that the models can indeed predict the interactions between the variables under study for each species but with some considerable limitation. The model for *U. fasciata* has the highest adequacy between species in predicting the TRS yield. The relationship between the Y (TRS yield) and X values in all three models were statistically significant at $p \leq 0.005$. The use of fitted models in this study for the dilute acid hydrolysis optimisation of all three types of seaweeds is not only informative, but also novel in comparison with other studies from available literature on bioethanol production from seaweeds.

The optimal conditions for acid hydrolysis using all three-selected species were similar as seen in Table 4-4. However, the reaction temperature of *U. fasciata* must be reduced to 120 °C from 130 °C to obtain its optimal yield. This temperature reduction for only the green seaweed can be attributed to the weaker α-1,4-glycosidic linkages found in the starch fraction of *U. fasciata*. These are easier to breakdown than the β-1,4glycosidic linkages found in alginate fraction of the *S. vulgare* and the β-1,3-glycosidic linkages in agar found in *H. dentata* which are structurally more stable.

Meinita *et al.* (2012) noted a reaction time, temperature and catalyst concentration of 15 min, 130 °C and 0.2 M, respectively as the optimal dilute acid hydrolysis condition from their work on the red seaweed *K. alvarezii*. They obtained a TRS yield of 38.5% DM. The variation between the optimal conditions in this study and theirs can be attributed primarily to the use of the carrageenophyte (a group of red seaweeds), *K. alvarezii*. This is structurally different from the agarophyte, *H. dentata* used in this study due to the presence of the hydrocolloid, carrageenan in carrageenophytes and agar in agarophytes.

Even though considered cost effective, dilute acid hydrolysis optimisation released a maximum TRS yield of 16.3% DM from *H. dentata* from a possible 31% DM. This represents a sugar recovery efficiency of 52.6%. This indicates that a more efficient hydrolysis method may be needed to complement or replace the dilute acid hydrolysis process to maximise sugar recovery. This study therefore examined enzymatic hydrolysis as an alternative.

Table 4-3. Summary of regression models for dilute acid hydrolysis of seaweeds

Seaweed species	Regression model equations[1]	R^2 value (%)	p-value
U. fasciata	$RS = -200.8 - 0.0072X_1^2 + 3.21X_2^2 + 113X_3 - .01316X_2 - 263X_3 + 0.770X_1X_3$	79.92	<0.001
S. vulgare	$TRS = 1.70 + 0.0794X_1 - 0.0361X_2 - 61.4X_3 + 0.709X_2X_3$	68.76	<0.001
H. dentata	$TRS = 14.70 - 0.0970X_1 - 34.6X_3 + 0.950X_1X_3$	41.71	0.005

[1] *X_1 is reaction time (min), X_2 is reaction temperature (°C), X_3 is acid concentration (M)*

Table 4-4. Optimal dilute acid hydrolysis conditions obtained for seaweeds

Seaweed species	Optimal conditi			Model Predicted TRS yield (% DM)	Experimental TRS yield (% DM)
	Time, (min)	Temperature (°C)	Acid concentration (M)		
U. fasciata	60	120	0.3	18.451	16.062 ±1.225
S. vulgare	60	130	0.3	11.012	12.071 ±1.909
H. dentata	60	100 - 130	0.3	15.598	16.319 ±1.051

4.3.2 **Effects of various acid hydrolysis conditions on TRS yield**

The effects of the various independent variables used in the dilute acid hydrolysis process were examined to determine which variable has the most influence on TRS yields as shown in Figure 4-4, 4-5 and 4-6. The results are represented using contour plots which are similar to topographical maps. These plots allow the examination of the relationship between three variables in a two-dimensional view. Two independent variables are represented on the x and y axis of the plot while the response variable (TRS) is represented by the contours (red gradient: low yields, green gradient: high yields).

From the contour plots for *U. fasciata* (Figure 4-4), acid concentration and time had the biggest combined effect on TRS yield. Longer reaction times and higher acid concentrations favoured higher TRS yields of 15% or higher (Figure 4-4). Temperatures below 105 °C were less favourable with yields of less than 9%. This implies that a longer reaction time, higher catalyst loading, and higher temperatures are required to maximise TRS yields from *U. fasciata*.

For *S. vulgare*, acid concentration had the highest influence on TRS yield even though marginal (Figure 4-5). The upper boundary conditions set for the *S. vulgare* optimization may not be favourable since TRS yields greater than 8% were reported after reaction temperatures of 125 °C and catalyst concentrations of greater than 0.26 M (Figure 4-5). This implies that stronger conditions may be required to breakdown the cellular structure of *S. vulgare* to release the reducing sugars.

For *H. dentata*, reaction time was the most influential factor with temperature having no visible effect on TRS yield (Figure 4-6). TRS yields were greater than 12% for reaction times higher than 40 min. This was however limited to acid concentrations greater than 0.15 M. Trends from acid concentration and temperature were generally less predictable for these species.

In all the three seaweeds studied, high temperatures, high catalysts loading and longer retention times favoured TRS yields but with the risk of inhibitor formation. Meinita *et al.* (2015) noted even though these conditions favour high TRS yields, thresholds exist which must not be exceeded. In their study, high temperatures and high acid catalyst loads which exceeded 120 °C and 0.2 M, respectively were noted to cause the formation of high concentrations of sugar degradation products or inhibitors in the form of HMF, furfural and levulinic acid. These inhibitors were however not found in this study even though those thresholds were exceeded. The thresholds of acid hydrolysis must therefore be identified uniquely in each study for each species when dilute acid hydrolysis is used. The TRS yields from this study clearly indicate that the boundary conditions for dilute acid hydrolysis especially for *S. vulgare* must be redefined to possibly maximise yield but this must be done with the risk of inhibitor formation in mind.

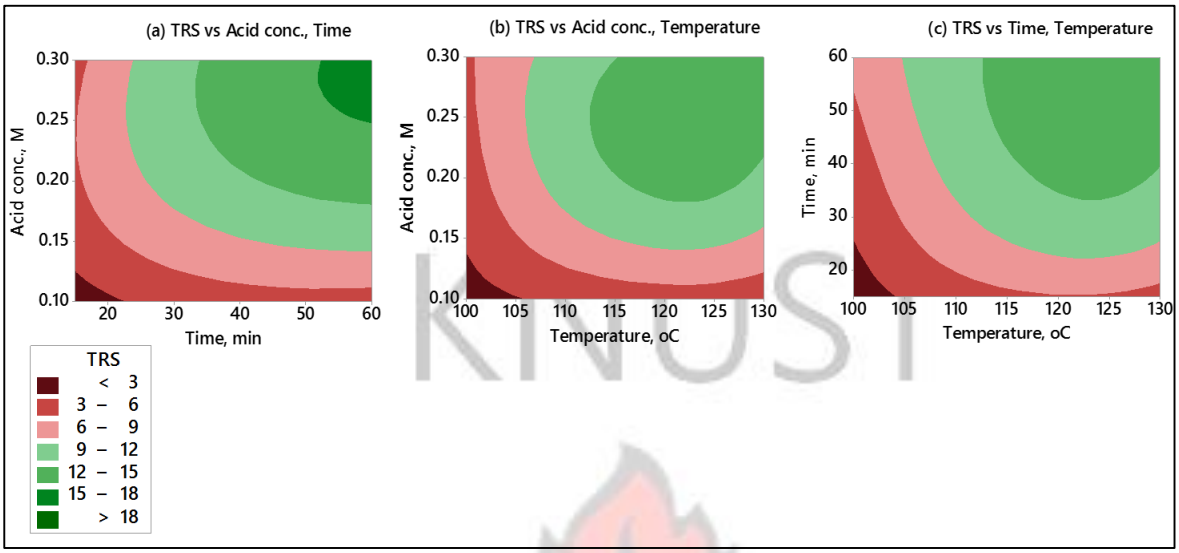

Figure 4-4. Contour plots of the effects of acid hydrolysis various parameters on TRS yield for U. fasciata

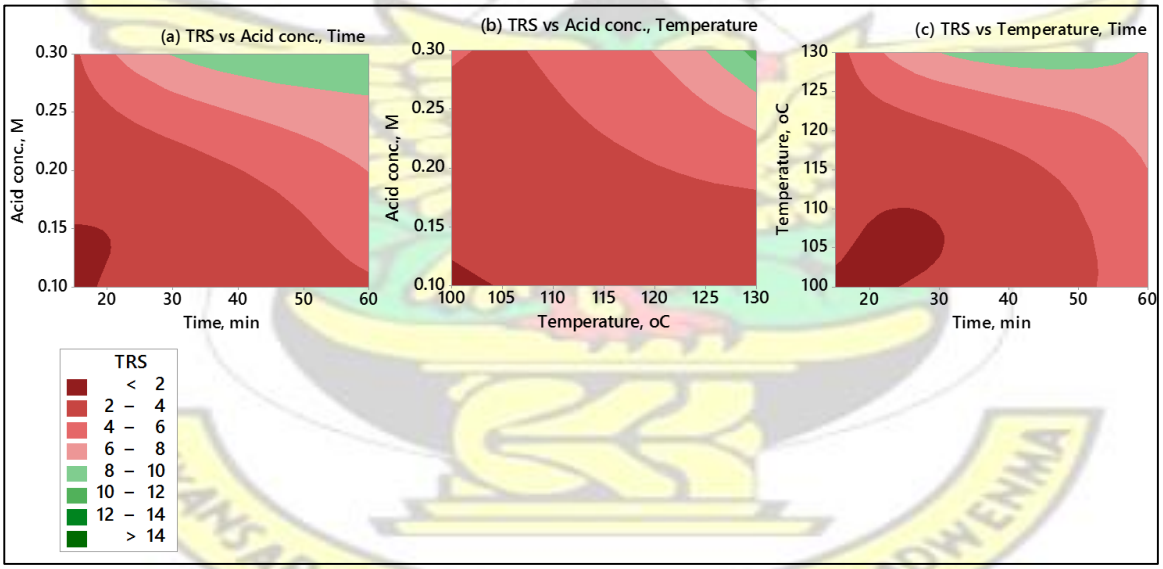

Figure 4-5. Contour plots of effects of various acid hydrolysis parameters on TRS yield for S. vulgare

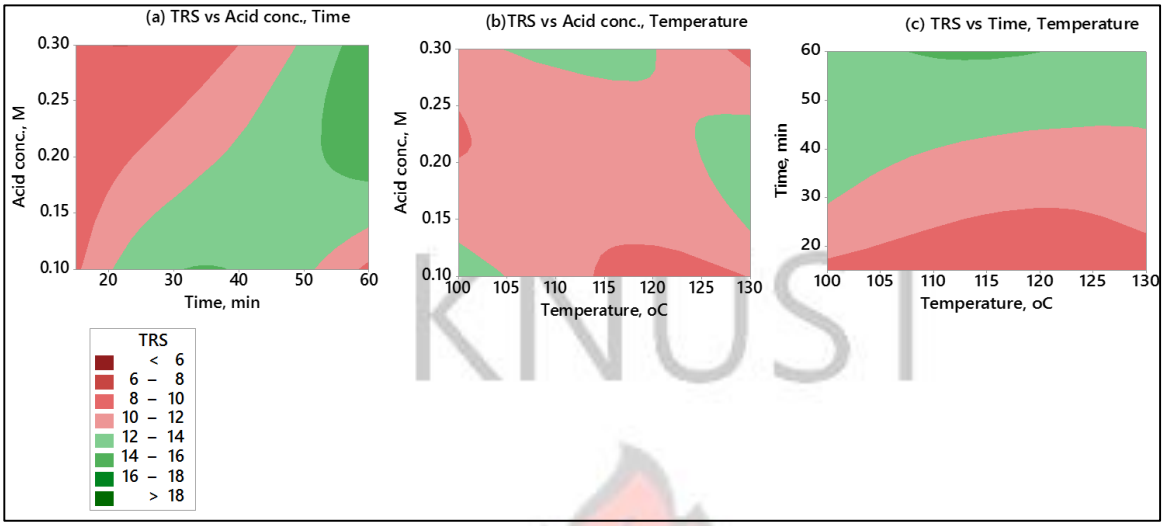

Figure 4-6. Contour plots of effects of various acid hydrolysis parameters on TRS yield for H. dentata

4.4 Optimization of the Enzymatic hydrolysis of the seaweeds

Response surface methodology was used to optimize the enzymatic hydrolysis of pretreated seaweeds. The independent variables considered included substrate concentration (5-15% w/v), enzyme concentration (2-8 FPU/g DM) and hydrolysis time (24-72 h). The optimisation data was further analysed using multiple regression analysis to obtain a set of regression equations that can describe and possibly predict the total reducing sugars for each seaweed species.

The boundary conditions for the optimisation were based on previous studies. The upper limit (8 FPU/g dry biomass) of the enzyme concentration used in this study is one of the lowest enzyme dosage applied in any study from seaweed literature. This limit was selected since one of the biggest challenges in commercial bioethanol production is the cost of enzymes. This study therefore sought to optimize enzymatic hydrolysis with minimal enzyme use. The effects of the independent variables were also examined further using contour plots.

4.4.1 Modelling of TRS recovery from Enzymatic hydrolysis

All three model equations obtained (Table 4-5) were made up of the total reducing sugar yields expressed as a function of the independent variables studied. The models

were also expressed as second-degree polynomial equations for all three seaweeds (Table 4-5). The relationship between the *Y* (TRS yield) and the *X* values in all three models were statistically significant at $p < 0.001$. This is a good initial indicator that the three models will be quite efficient in predicting the reducing sugar yields.

The correlation coefficient (R^2) for *U. fasciata* was the highest between species at 99.36%, a strong indicator of its precision in predicting the TRS yields from the species within the boundary conditions defined. This also indicates that the model can explain the bulk of the variation between variables and that the experimental values obtained are almost fitted perfectly by the model. The models from *S. vulgare* and *H. dentata* were also acceptable with 61.29 and 75.19% as R^2 values even though significantly lower than *U. fasciata* (Table 4-5). This indicates that the models obtained from the optimisation of the enzymatic hydrolysis for all three seaweeds were adequate but that *U. fasciata* is the strongest in describing mathematically the relationship between the variables influencing its enzymatic hydrolysis.

Pilavtepe *et al.* (2013) also used response surface methodology along with regression modelling to described mathematically the relationship between enzyme concentration, substrate loading and reaction time on TRS yields for the green seaweed, *Posidonia oceanica*. Their correlation coefficient of 93.18% was lower than that obtain for the green seaweed, *U. fasciata* in this study. This was however expected due primarily to the variations in the species and their geographical location of harvest.

Interestingly, the same optimal condition of 8 FPU/g DM enzyme dosage, 5% w/v substrate concentration and 24 h hydrolysis time were obtained for all three seaweeds studied (Table 4-6). This implies that the hydrolysis of the seaweeds was favoured by a high enzyme concentration for a shorter time with a lower substrate concentration. Generally, a high enzyme load is reported to result in a high TRS yield due to an increase in the ratio of substrate to enzyme (Pilavtepe *et al.*, 2013). Since a cellulase was used in this study, cellulase loading for similar studies would have to be well defined to efficiently apply the optimal conditions determined in this study especially for a scale-up scenario.

Table 4-5. Summary of regression models for enzymatic hydrolysis of seaweeds

Seaweed species	Regression model equations[1]	R^2 value (%)	pvalue
U. fasciata	$TRS = 25.61 + 1.124X_1 - 1.067X_2 - 0.0432X_3 + 0.02805X_2^2 + 0.001526X_3^2 - 0.02794X_1X_2 - 0.00927X_1X_3 - 0.00580X_2X_3$	99.36	<0.001
S. vulgare	$TRS = 12.55 + 1.019X_1 + 0.922X_2 - 0.1183X_3 - 0.0646X_2^2 + 0.002247X_3^2 - 0.01520X_1X_3$	61.29	<0.001
H. dentata	$TRS = 31.53 + 0.822X_1 - 1.765X_2 - 0.0876X_3 + 0.0530X_2^2 - 0.0585X_1^2 + 0.00549X_2X_3$	75.19	<0.001

[1]*X_1 is Enzyme concentration (FPU/g DM), X_2 is substrate concentration (% w/v), X_3 is time (hours)*

Table 4-6. Optimal enzymatic hydrolysis conditions obtained for seaweeds

Seaweed species	ıal conditions: Enzyme concentration (FPU/g DM)	Substrate concentration (% w/v)	Time (hour)	Model Predicted TRS yield (% DM)	Experimental TRS yield (% DM)
U. fasciata	8	5	24	26.233	26.515 ±1.685
S. vulgare	8	5	24	19.522	20.035 ±0.137
H. dentata	8	5	24	26.822	28.331 ±1.658

4.4.2 **Effects of various enzymatic hydrolysis conditions on TRS yield**

The *U. fasciata* seaweeds recorded TRS yields of < 20% DM at substrate concentrations higher than 7.5% w/v irrespective of the enzyme concentration as shown in Figure 4-7. Similarly, TRS yields of < 20% DM were obtained at substrate concentrations higher than 10% w/v irrespective of the reaction time. This implies that both time and enzyme concentration had the least influence on TRS yields from *U.*

fasciata. This variation could be attributed to an efficient mobility and cleavage of the enzyme to the substrate units to cause their break down due to an enhanced enzyme to substrate ratio. A system with a high solids loading may therefore require more time for efficient hydrolytic activity by the enzyme. It can be inferred from the TRS yields obtained for *U. fasciata* that efficient enzymatic hydrolysis can be achieved over a short period of time with a minimal enzyme dosage.

The same phenomenon was obtained for *H. dentata* and *S. vulgare* where the reaction time and enzyme dosage were the least influential on TRS yield (Figure 4-9). *S. vulgare* however showed a higher tolerance for high substrate concentration since it recorded TRS yields greater than 16.5% DM at substrate concentrations as high as 12% w/v regardless of the reaction time (Figure 4-8). Generally, the TRS yields from the enzymatic hydrolysis of all three seaweeds were very high (> 20% DM) and should be considered strongly as the hydrolysis method of choice for seaweeds. The substrate loading however should receive careful consideration during enzymatic hydrolysis.

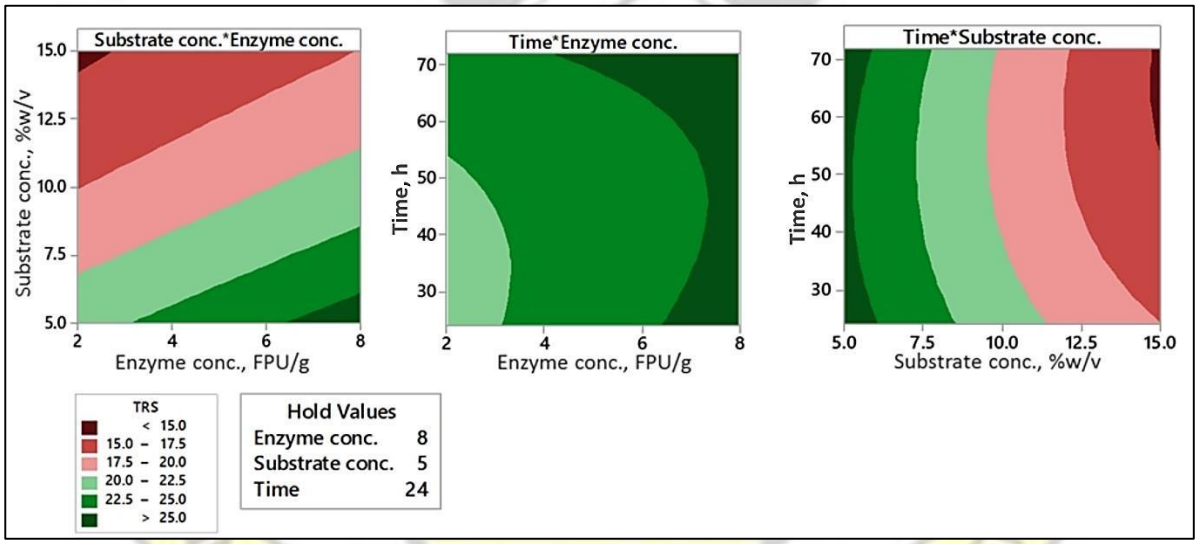

Figure 4-7. Contour plots of effects of various enzymatic hydrolysis parameters on TRS yield for U. fasciata

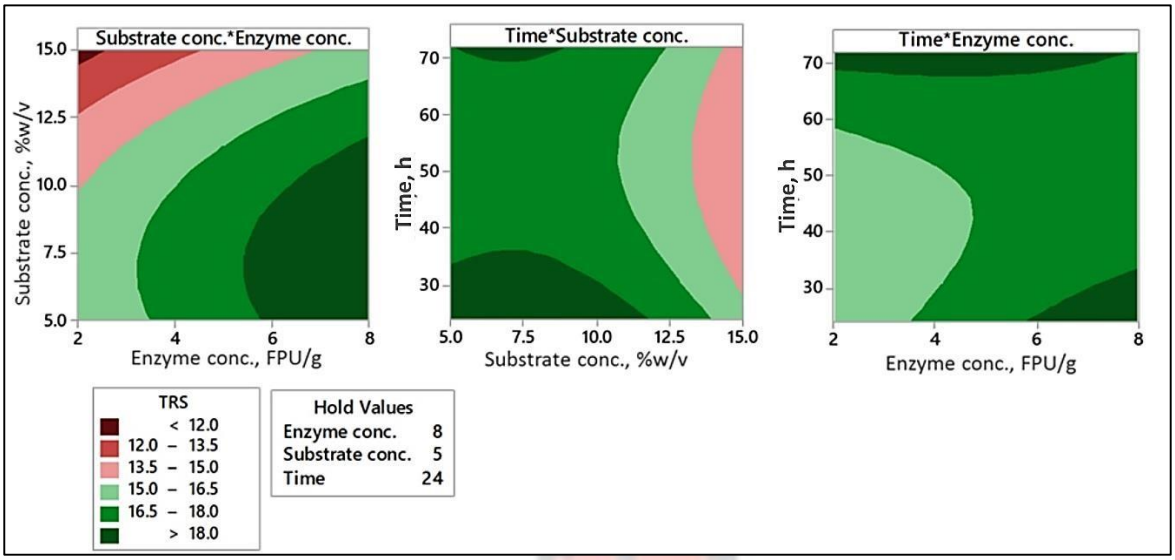

Figure 4-8. Contour plots of the effects of various enzymatic hydrolysis parameters on TRS yield for S. vulgare

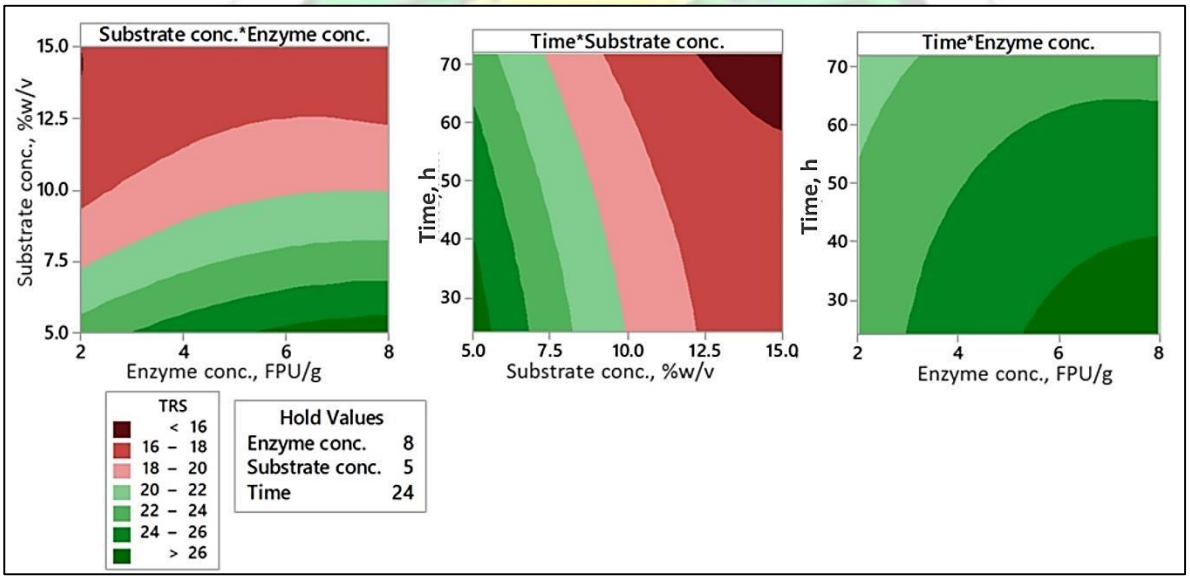

Figure 4-9. Contour plots of the effects of various enzymatic parameters on TRS yield for H. dentata

4.5 Fermentation of seaweeds to ethanol

Reducing sugars obtained after hydrolysis were converted to ethanol in a fermentation process catalysed by yeast as fermenting organism. Four commercial strains of *S. cerevisiae* and a yeast isolate, *A. angophorae* were examined in this study since the organism selection forms one of the most critical factors to an efficient fermentation process (Lee and Lee, 2012). The growth rate of the yeast strains was first assessed followed by an analysis of their selectivity to specific monomeric sugars. The yeast strains were then applied to the three seaweeds using both the SHF and SSF ethanol production pathways.

4.5.1 Yeast growth analysis

Typically, an ethanol producer would want yeast strains with a faster growth rate to not only improve yield but also shorten the fermentation time and maximise productivity in each production year. In this study the growth rate of the five selected yeasts strains were examined using a yeast extract-glucose-ammonium sulphate medium. Their optical densities were then analysed to obtain the yeast cell concentrations at harvest.

Y1 (*S. cerevisiae* SI18) had the highest growth rate which was significantly faster than all the other yeast strains used in the study at 2.4 mg/hr (Table 4-7). Y2 (*S. cerevisiae* CT18) and Y3 (*S. cerevisiae* FT18) had a similar growth rate while Y4 (*S. cerevisiae* CT18) had the slowest growth rate at 0.43 mg/hr which was significantly different from all the others (Table 2-7). Y5 (*A. angophorae*), the yeast isolate, grows significantly faster than Y4 but slower than Y1, Y2 and Y3.

The variation in the growth rates imply that even though Y1, Y2, Y3 and Y4 were all strains of *S. cerevisiae* they were clearly distinct strains with differences in their growth characteristics. They could therefore ferment substrates differently with different yields of ethanol and its by-products. The earliest indication from the growth rates are that a higher yield may be obtained from Y1, Y2 and Y3 over Y4 and Y5.

Table 4-7. Yeast growth yield after culturing

Yeast strain	Dry cell weight (g dcw/l)	Cell growth rate (mg/h)[1]
Y1	4.905 ±0.041	2.405 ±0.041[b]
Y2	4.356 ±0.017	1.856 ±0.017[a]
Y3	4.308 ±0.028	1.808 ±0.028[a]
Y4	2.933 ±0.021	0.433 ±0.021[c]
Y5	3.998 ±0.023	1.498 ±0.023[d]

[1] *Means of the same column with different letters are significantly different; Tukey's HSD, p<0.05)*

4.5.2 **Sugar selectivity analysis of the selected Yeast strains**

The five selected yeast strains were further examined for their response to a pure substrate (SUB-A) and a mixed substrate (SUB-B) as found in seaweeds. The pure substrate was used as a measure of their true theoretical maximum ethanol yield under the fermentation conditions used in this study. The mixed substrate was also used as an artificial control substrate to mimic the various types of monomeric sugars found in seaweed. The ethanol and its by-product yields were evaluated as well as the consumption efficiencies of the various monomeric sugars by the selected yeast strains.

4.5.2.1 Ethanol yield from the selected Yeast strains on SUB-A and SUB-B

There was no significant difference between the ethanol yields from Y1, Y2, Y3 and Y4 for SUB-A which was between 25 and 30% DM (Figure 4-10). Y5 was however significantly lower than the others with an ethanol yield of 0.95% DM. There was also no significant difference between the ethanol yields from Y1, Y2, Y3 and Y4 for SUBB which was between 4.8 and 5.2% DM, respectively. Y5 was however different from the others with an ethanol yield of 0.75% DM. The consistently lower yield from Y5 indicates a probably longer fermentation time preceded by several stages of preculturing and adaptation may be required. The stoichiometric theoretical maximum ethanol yield of 51.2% that can be obtained for the fermentation of glucose by yeast was not attained by any of the yeast strains screened on SUB-A (glucose). This is as result of the formation of the by-products succinic acid, acetic acid and glycerol from the same substrate causing a corresponding decline in ethanol yield (Figure 4-11).

The total initial monomeric sugar concentration in SUB-A and SUB-B were the same at 15g/l during the fermentation process. However, the minimum ethanol yield

reported was 25.96% DM for SUB-A which was much higher than the maximum ethanol yield of 5.32% DM reported for SUB-B. This reiterates the assertion that most yeast strains are indeed selective towards various sugars with much preference towards hexose sugars (Van Maris *et al.*, 2006).

From Figure 4-11, glycerol was the highest by-product released in SUB-A (6.1- 8.2% DM) along with succinic acid by all yeast strains used except Y5. Acetic acid was released by only Y1 and Y4 in very low concentrations (< 0.55% DM). Since glycerol is also a valuable product in the food, pharmaceutical and cosmetic industries, its recovery as a co-product may be considered if deemed economically and technically feasible to extract. By-products from Y5 were not detectable in SUB-A.

In SUB-B, succinic acid which performs a series of cellular functions such as ATP formation, was the dominant by-product released by all the yeast strains except Y1 (Figure 4-12). Glycerol was again released by all the yeast strains except Y5. Acetic acid was absent from all yeast strains except Y1 but in a very low concentration (0.06% DM). The considerable amount of succinic acid and glycerol released when SUB-B was used needs to be examined in closer detail since succinic acid recorded a yield of 7.58% DM which was higher than the ethanol yield 4.99% DM. Parameters such as pH may require particular control with the aid of buffers.

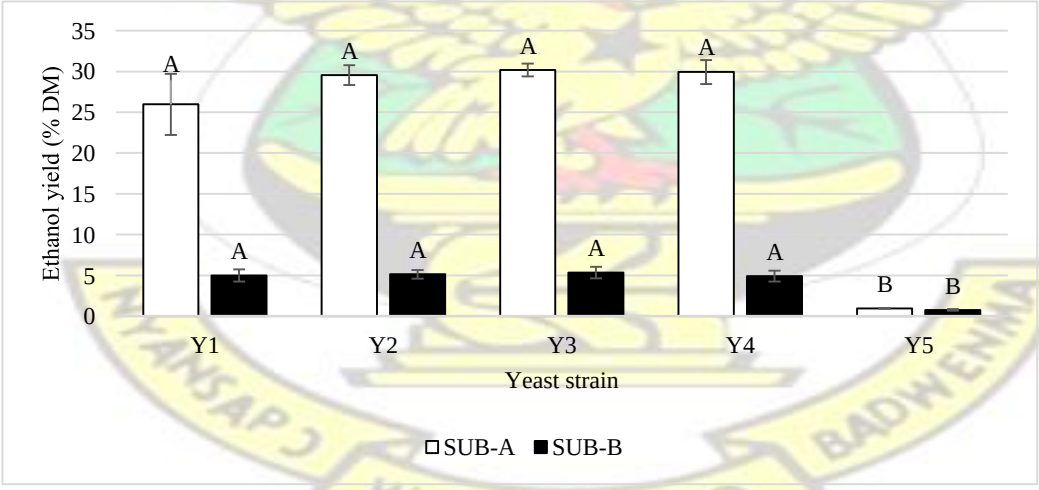

Figure 4-10. Ethanol yields from the SUB-A (pure substrate) and SUB-B (mixed substrate)

(Means of the same bar colour with different letters are significantly different; Tukey's HSD, $p<0.05$)

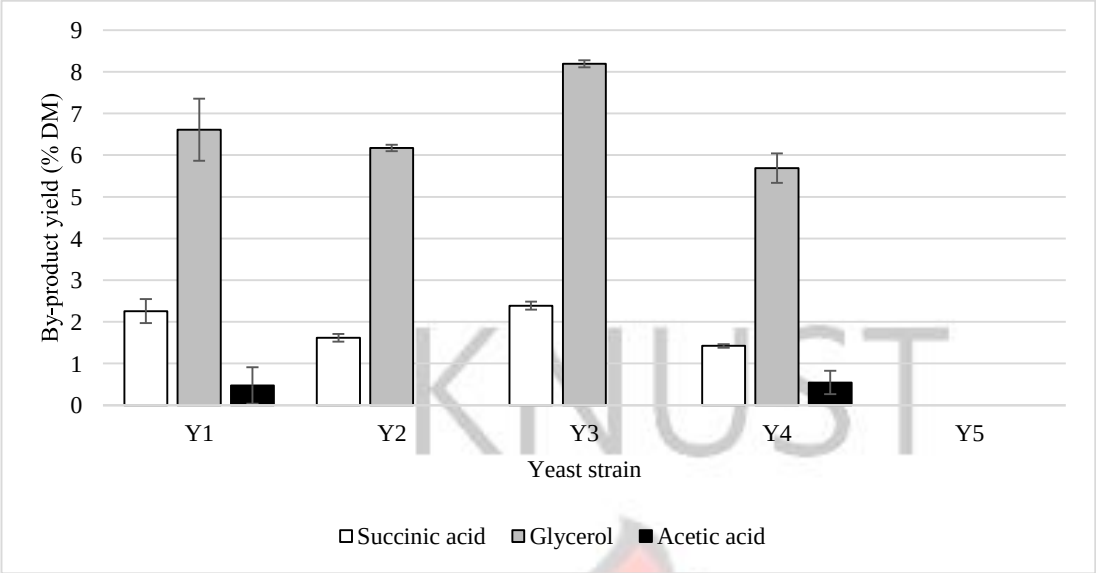

Figure 4-11. By-product yields from SUB-A (pure substrate)

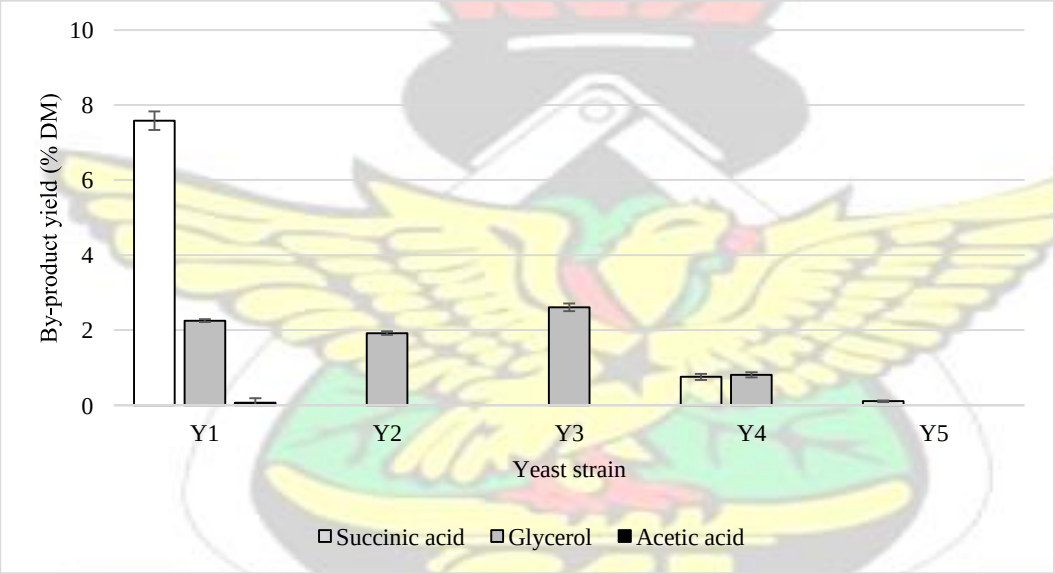

Figure 4-12. By-product yields from SUB-B (mixed substrate)

4.5.2.2 Monomeric sugar consumption by the selected Yeast strains

Glucose in SUB-B was consumed completely by all the yeast strains except Y5 as seen in Figure 4-13. Various fractions of the other sugars in SUB-B were used up but were under 60% consumption. Arabinose and xylose, both pentoses, were sparingly consumed with a maximum of 38% by Y1 for xylose and 23.5% by Y1 for arabinose (Figure 4-13). Rhamnose and galactose were quantified together due to their co-elution

during the HPLC analysis of the products. Mannitol, a sugar alcohol, found predominantly in brown seaweeds was not consumed by Y1 and Y2. The yeast strains Y2 and Y3 showed the highest selectivity for all monomeric sugars. Y1 and Y3 showed the highest overall consumption for the monomeric sugars.

The selectivity of the yeast strains for hexose such as glucose, galactose and rhamnose was quite evident in the consumption efficiencies recorded. This indicates that if the hexose fraction of seaweeds to which the yeast will be applied is higher the corresponding ethanol yields will be higher. If they are however lower, low yields of ethanol would be expected as seen in Figure 2-11.

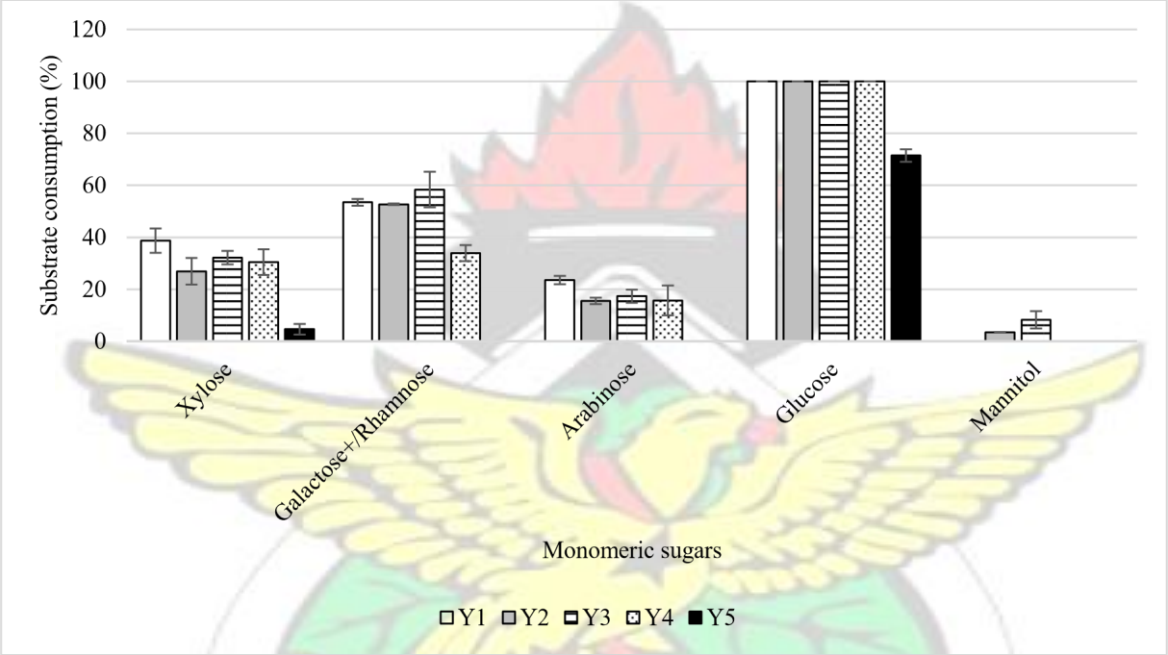

Figure 4-13. Consumption of various monomeric sugars by yeast strains in SUB-B

4.5.3 **SHF processing of seaweeds**

The SHF pathway was used to examine the ethanol yield from the three seaweeds by the five yeast strains. Since the sugar selectivity analysis discussed in the previous section did not clearly distinguish them in terms of performance all five yeast strains were again examined. All three seaweeds were pre-treated with dilute acid and enzymatically hydrolysed with commercial cellulases before fermentation. The liquid hydrolysate was separated from the solid residue and used as the fermentation medium to produce ethanol. The ethanol and its by-product yields from the selected yeast strains on the three seaweeds via the SHF pathway were evaluated.

4.5.3.1 Ethanol yield from the seaweeds via the SHF pathway

For *U. fasciata,* there was no significant difference between ethanol yields from all 5 yeast strains used in the study as seen in Figure 4-14. The yields ranged between 4.45 and 5.06% DM (Figure 4-14, Table 4-8). The ethanol yields from *U. fasciata* also showed that there was no defined correlation between the growth rate reported in section 4.5.1 for the various yeast strains and the ethanol yield obtained from seaweeds. This indicates that any of the five selected yeast strains could be efficiently used for fermenting green seaweeds represented in this study by the *U. fasciata* species. Trivedi *et al.* (2013) recorded a much higher ethanol yield of 45 g/100g TRS for *U. fasciata* as compared to the 13.5 g/100g TRS obtained in this study (Table 4-8). This large difference could be attributed to either a more efficient yeast strain selection or a higher hexose concentration in their TRS than in this study.

For *S. vulgare,* ethanol yields were found to be similar between Y1, Y4 and Y5 with a maximum of 3.53% DM (Figure 4-14). However, the yields from Y2 and Y3 were significantly lower at a maximum of 1.88%. This implies that unlike *U. fasciata*, the yeast strains capable of efficiently fermenting *S. vulgare* were well distinguished. These were two strains of *S. cerevisiae* and the isolate *A. angophorae.* Borines *et al.* (2013) recorded a marginally higher ethanol yield of 17 g/100g TRS from *Sargassum* sp. as compared to the maximum of 12.04 g/100g TRS obtained in this study for the *S. vulgare* species (Table 4-8). This disparity could be accounted for by variations in carbohydrate composition.

For *H. dentata,* similar ethanol yields were recorded for Y1, Y2, Y3 and Y4 with a maximum of 2.44% DM (Figure 4-14). Y4 and Y5 were similar with yields of 1.52 and 1.16% DM but Y5 was significantly lower than Y1, Y2 and Y3. This indicates that *H. dentata* also has a broad acceptance for most of the yeast strains applied.

Comparatively, Y1, Y2 and Y4 produced high yields in all three seaweeds than Y3 and Y5 which were favourable in some seaweeds over others. Also, ethanol yields from *U. fasciata* were significantly higher than both *S. vulgare* and *H. dentata* with a maximum yield of 5.06% DM except for *S. vulgare* fermented with Y1 and Y4 which were comparable to the yield of *U. fasciata* fermented with Y2. *H. dentata* recorded the lowest yield of 1.16% DM between species. This was particularly interesting since *H. dentata* gave a higher total reducing sugar yield (28.33% DM) than *U. fasciata* (26.52% DM) and *S. vulgare* (28.33% DM) after hydrolysis. This indicates even though more reducing sugars were recovered from *H. dentata, U. fasciata* had a larger

fraction that were fermentable. This observation is highlighted clearly in their conversion efficiencies which were highest for Y1 applied to *U. fasciata* (26.48%) and lowest for Y5 for *H. dentata* (5.52%) (Table 4-8).

Generally, the ethanol yields from the three seaweeds, agree favourably with ethanol yields obtained from the artificial substrate (SUB-B in Figure 4-10) used in examining the selectivity of the yeasts strains. The diversity of the monomeric sugars found in the seaweeds indeed could have an adverse effect on ethanol yield due to the variation in the consumption efficiencies of the yeast strains for each type of monomeric sugar. The selection of yeast strains is often done with a target substrate in mind, but this becomes increasingly challenging when faced with a broad diversity of monomeric sugars as found in this study. Since the screening of several yeasts strains on the three types of seaweeds is novel there are no known studies from available literature for it to be compared. A much more detailed study may be required in the future in order to overcome effectively the challenge of yeast selection relative to seaweed type.

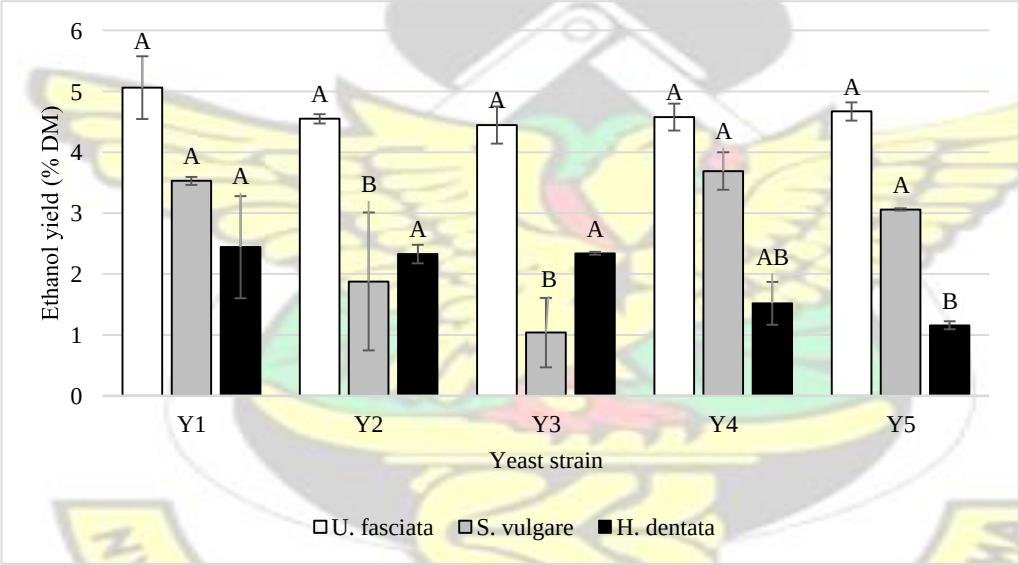

Figure 4-14. Ethanol yields from the selected seaweeds via the SHF pathway

(Means of the same bar colour with different letters are significantly different; Tukey's HSD, $p<0.05$.)

Table 4-8. Summary of seaweed ethanol yields from SHF with the selected yeast strains

Yeast strain	Seaweed species	Ethanol yield (g/100g TRS)[1]	Conversion efficiency (%)

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

Y1	*U. fasciata*	13.505 ±1.369[a]	26.480 ±2.684
	S. vulgare	11.518 ±0.213[bc]	22.583 ±0.418
	H. dentata	5.927 ±2.032[bde]	11.622 ±3.986
Y2	*U. fasciata*	12.138 ±0.202[ac]	23.801 ±0.396
	S. vulgare	6.125 ±3.698[d]	12.010 ±7.250
	H. dentata	5.656 ±0.369[bdg]	11.090 ±0.724
Y3	*U.* fasciata	11.865 ±0.813[ac]	23.264 ±1.594
	S. vulgare	3.388 ±1.864[f]	6.644 ±3.654
	H. dentata	5.682 ±0.055[bdfg]	11.141 ±0.109
Y4	*U. fasciata*	12.210 ±0.588[ac]	23.942 ±1.152
	S. vulgare	12.044 ±0.999[ce]	23.615 ±1.960
	H. dentata	3.686 ±0.860[df]	7.228 ±1.687
Y5	*U. fasciata*	12.461 ±0.401[ac]	24.434 ±0.786
	S. vulgare	9.986 ±0.065[beg]	19.581 ±0.127
	H. dentata	2.813 ±0.156[df]	5.515 ±0.305

4.5.3.2 By-products yield from the seaweeds via the SHF pathway

Succinic acid was the by-product released in largest quantities in both *U. fasciata* and *S. vulgare* from all the yeasts strains used (between 0.89 and 1.99% DM) (Figure 4-15 and 4-16). Glycerol was however the largest by product released from the fermentation of *H. dentata* from all the yeast strains except Y5 where succinic acid was higher (Figure 4-17). Generally, Y5 did not release any glycerol as a by-product. The absence of glycerol indicates that in Y5 (*A. angophorae*) the reduction of dihydroxyacetone phosphate to glycerol phosphate for NADH control during glycolysis (as part of ethanol formation) those not occur. It therefore achieves its redox balance in an entirely different pathway. The production of the organic acids, succinic and acetic acid are often intermediates of the citric acid cycle during fermentation.

Interestingly, all the by-products released during the fermentation of the seaweeds have distinct commercial uses. Succinic acid is used as a food additive and as a precursor to produce various polymers and resins. Acetic acid is used as food additive, for medical

applications and as a solvent. Glycerol is used predominantly as additives in food, pharmaceutical and commercial industries. Since these by-products are valuable, their recovery especially in commercial scale ethanol industries could be considered if technically and economically viable.

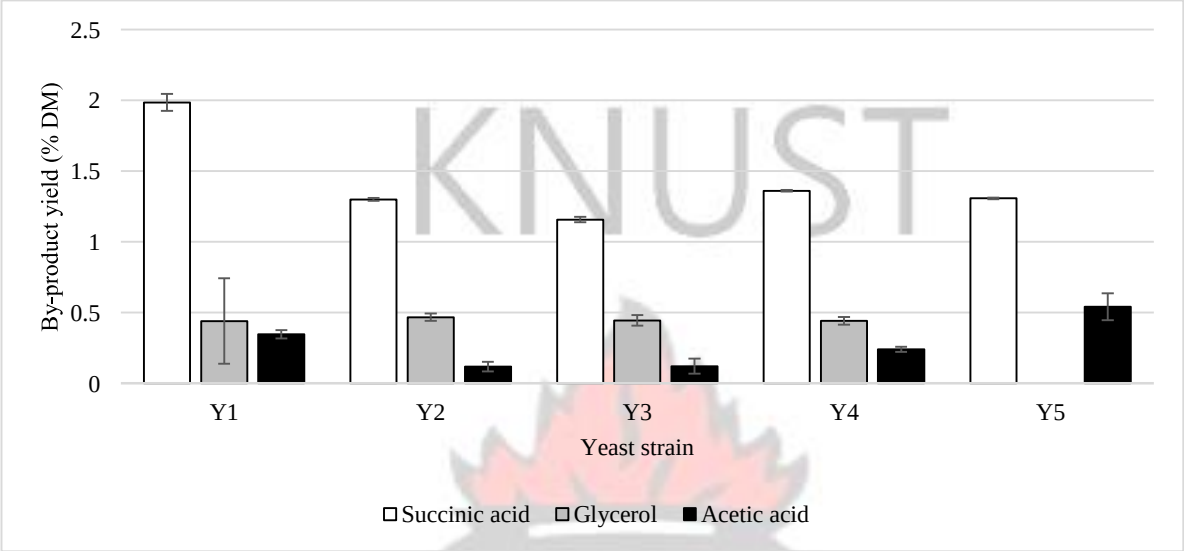

Figure 4-15. By-product yields from U. fasciata via the SHF pathway

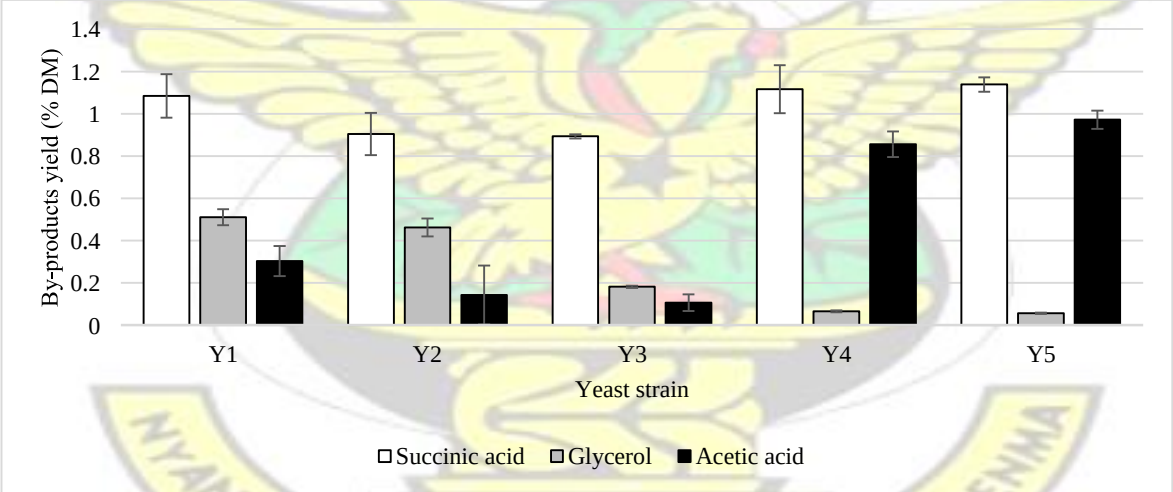

Figure 4-16. By-product yields from S. vulgare via the SHF pathway

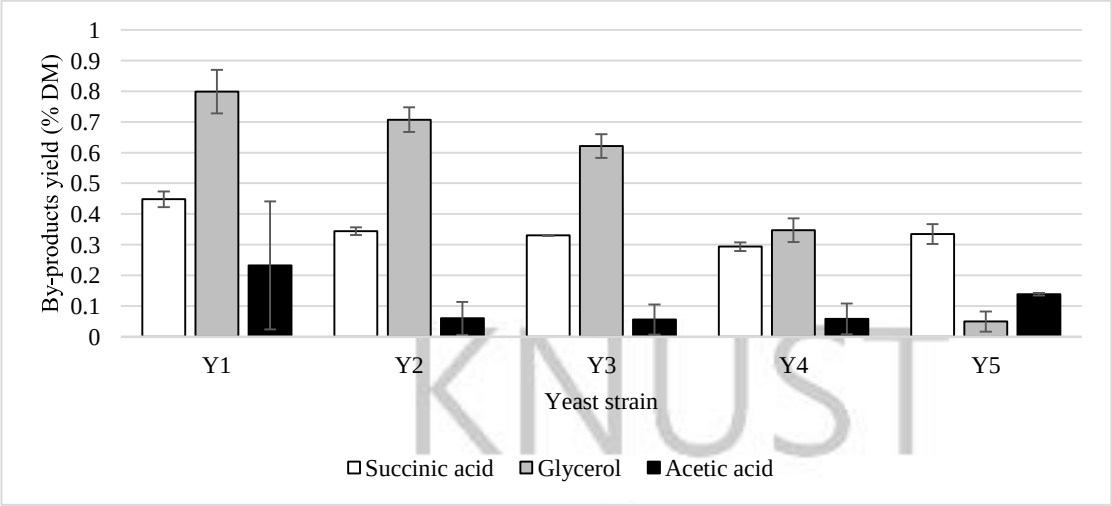

Figure 4-17. By-product yields from H. dentata via the SHF pathway

4.5.4 **SSF processing of seaweeds**

The SSF pathway was also used in this study because it is considered cost and time saving as compared to the SHF pathway. It is also considered by some reports as more efficient than the SHF (Kim *et al.*, 2015). The SSF pathway was applied to the three seaweeds along with the five yeast strains as fermenting organisms. The ethanol and its by-product yield from the selected yeast strains on the three seaweeds via the SSF pathway were evaluated.

4.5.4.1 Ethanol yield from the seaweeds via the SSF pathway

Ethanol yields from *U. fasciata* for Y1, Y3, Y4 and Y5 ranged between 3.3 and 3.8% DM with no significant difference as seen in Figure 4-18. There was also no significant difference between the yields from Y3 and Y2 even though the yields from Y2 differed significantly from Y1, Y4 and Y5. All 5 yeast strains were therefore considered effective in their application to *U. fasciata.*

For *S. vulgare*, ethanol yields from Y1, Y2 and Y3 were between 2.9 and 3.8% DM with no significant difference (Figure 4-18). The ethanol yields for Y2 and Y4 were also comparable with no significant difference. The highest yield recorded was 3.61% DM by both Y3 and Y5 strains. All 5 yeast strains were also considerably effective in their application to the *S. vulgare* seaweed.

The ethanol yields for the *H. dentata* seaweed were between 2.2 and 3% for Y1, Y3, Y4 and Y5 (Figure 4-18). These were comparable with no significant difference. Y2 (1.82% DM) differed significantly from Y3 which gave the highest ethanol yield of

2.95% DM but was not significantly different from Y1, Y4 and Y5. All 5 yeast strains again demonstrated similar application efficiencies with marginal differences on *H. dentata* seaweed.

Generally, between seaweed species high yields with no significant difference was recorded for *U. fasciata* and *S. vulgare* from Y1, Y3 and Y5. These yields ranged between 2.7 and 3.8% DM (Table 4-9). Y2 recorded the least yields generally between species with as low as 1.82% DM from *H. dentata*. The conversion efficiency between species were generally higher for species fermented with Y1, Y3 and Y5. This ranged between 7 and 12% DM.

Comparatively, the ethanol yields from the SHF were generally higher than the SSF pathway but yields were more dependent on seaweed species and yeast strain. Different species with different yeast strains responded differently to each pathway. *U. fasciata* had a maximum ethanol yield of 5.06% DM via SHF and 3.77% DM via SSF all with Y1 as fermenting organism. *S. vulgare* had a maximum ethanol yield of 3.69% DM via SHF and 3.61% DM via SSF with Y4 and Y5, respectively as fermenting organisms. *H. dentata* had a maximum ethanol yield of 2.44% DM via SHF and 2.73% DM via SSF with Y1 and Y5, respectively as fermenting organisms. This implies that in the production of ethanol from seaweeds, selection of the yeast requires a more careful consideration than the choice between pathways.

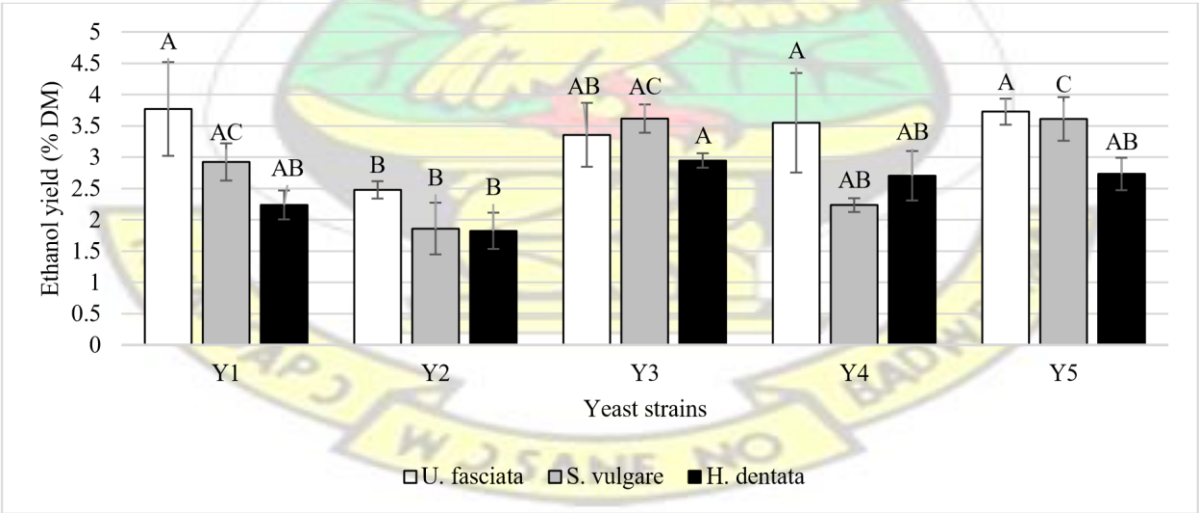

Figure 4-18. Ethanol yields from the selected seaweeds via the SSF pathway

(Means of the same bar with different letters are significantly different; Tukey's HSD, $p<0.05$)

Table 4-9. Summary of seaweed ethanol yields from SSF with the selected yeast strains

Yeast strain	Seaweed species	Ethanol yield (g/100g TRS)[1]	Conversion efficiency (%)
Y1	*U. fasciata*	6.041 ±1.234^{a}	11.846 ±2.419
	S. vulgare	4.515 ±0.471^{ab}	8.852 ±0.924
	H. dentata	3.607 ±0.359^{bc}	7.072 ±0.704
Y2	*U. fasciata*	3.959 ±0.212^{bcd}	7.763 ±0.415
	S. vulgare	2.864 ±0.640^{b}	5.616 ±1.256
	H. dentata	2.942 ±0.466^{b}	5.769 ±0.913
Y3	*U. fasciata*	5.424 ±0.852^{c}	10.635 ±1.670
	S. vulgare	5.572 ±0.323^{ad}	10.925 ±0.634
	H. dentata	4.806 ±0.194^{a}	9.423 ±0.380
Y4	*U. fasciata*	5.677 ±1.264^{ad}	11.132 ±2.478
	S. vulgare	3.441 ±0.169^{bc}	6.748 ±0.332
	H. dentata	4.370 ±0.626^{ab}	8.568 ±1.228
Y5	*U. fasciata*	6.006 ±0.312^{a}	11.776 ±0.611
	S. vulgare	3.714 ±3.240^{a}	7.282 ±6.354
	H. dentata	4.441 ±0.457^{ab}	8.709 ±0.896

4.5.4.2 By-product yield from the seaweeds via the SSF pathway

The by-product acetic acid was recorded in large concentrations in all three seaweeds fermented via the SSF pathway. This ranged between 1.9 and 5.3% DM (Figure 4-19, 4-20 and 4-21). The high acetic acid yield according to Woo *et al.* (2014), is caused by a cellular response by the yeast strains to the high temperatures (37 °C) used during the SSF process. They noted that acetic acid production, an intermediate product from the tricarboxylic acid cycle (which supports cell respiration), was stimulated substantially as compared to the other metabolites such as glycerol, lactic acid and succinic acid. This in turn has an adverse effect on sugar uptake leading to a decline in the ethanol production rate. This is further evident in the lower ethanol recovery from SSF as compared to SSF in the broths from all three seaweeds especially *H.*

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

dentata (Table 4-9).

Traces of lactic acid were also found as by-products in the fermentation broth for *U. fasciata* (Figure 4-19). This was however absent in the broth for both *S. vulgare* and *H. dentata* fermented with Y2, Y3 and Y5 (Figure 4-20 and 4-21). Interestingly, lactic acid was particularly absent during the use of the SHF pathway for ethanol production (Figure 4-15, 4-16 and 4-17). Its presence in the SSF pathway could be attributed to increased stress on yeast cellular activity due to the change in temperature between pathways which caused a significant production of metabolites such as acetic acid. The production and interactive effect of the by-products during fermentation of seaweeds to ethanol has been extensively ignored by available literature. However, its impact on the conversion efficiency during fermentation is well pronounced. Its consideration in this study is therefore novel.

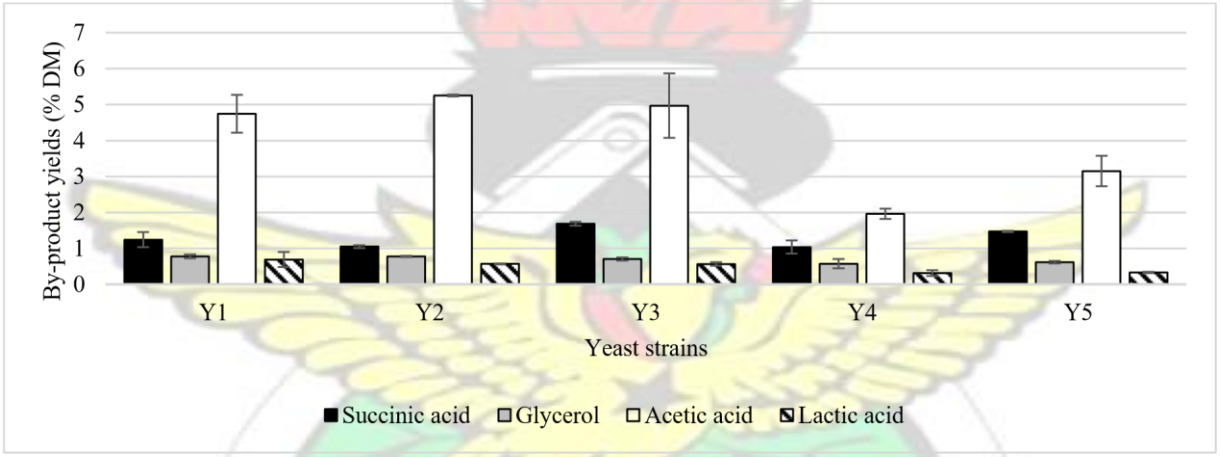

Figure 4-19. By-product yields from U. fasciata via the SSF pathway

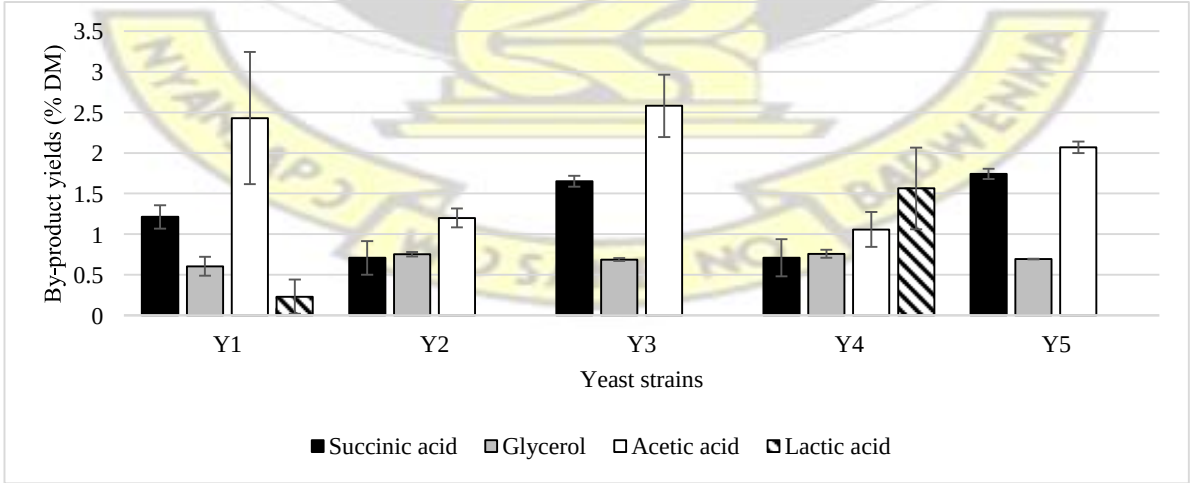

Figure 4-20. By-product yields from S. vulgare via the SSF pathway

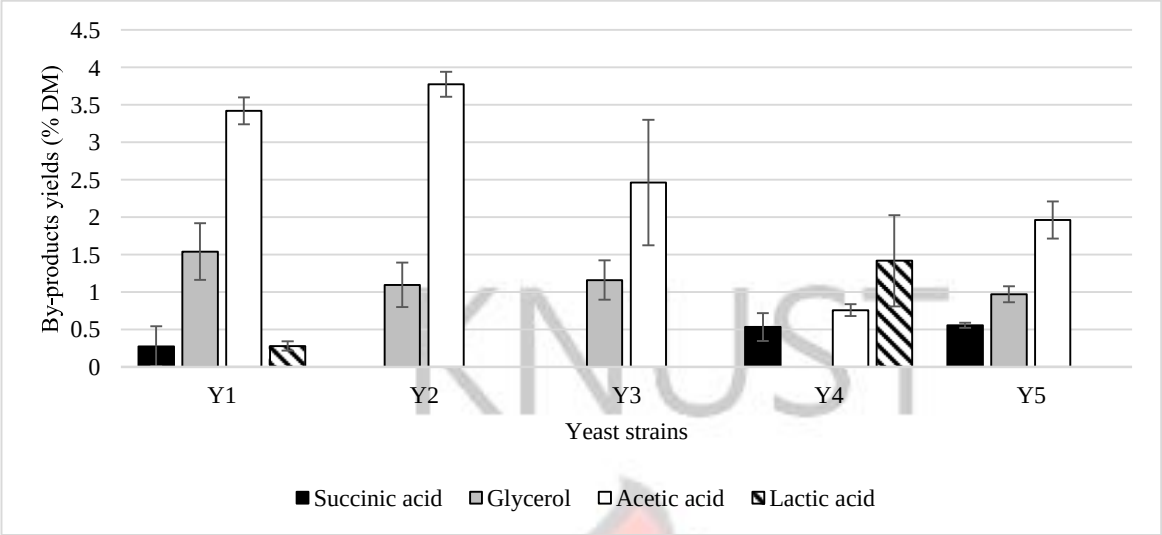

Figure 4-21. By-product yields from H. dentata via the SSF pathway

4.6 Oxidation of seaweed bioethanol production residue in MFCs

The concept of technology integration used in this study to maximise seaweed substrate usage relies also on the application of the microbial fuel cell technology to seaweeds. The solid seaweed residue obtained after the separation of the liquid hydrolysate via centrifuging and decantation was used as the sole source of substrate in pre-inoculated MFCs. The composition of the solid residue was first analysed to examine its suitability for use in MFCs as substrate. Four cells were inoculated before the seaweed residue was introduced in three fed-batch cycles. During each cycle the voltage was monitored with time after which the current, power and substrate consumption were evaluated.

4.6.1 Seaweed residue composition

Four key components were examined in the seaweed bioethanol production residue obtained. They included the total solids, moisture content, volatile solids and ash content. The residue was generally high in moisture for all the three seaweeds. This ranged between 79 and 85% and was significantly different for all three seaweed residues (Table 4-10). The residues were also very high in volatile solids with no significant difference between species. The volatile solids ranged between 83.6 and 83.8%. The high VS (which refers to the biodegradable organic fraction of the

biomass) implies that the seaweed residue would be favourable for use as substrate in the MFCs since the electrogenic bacteria in MFC biofilms have a preference for oxidation of organic substrates to release electric current (Du *et al*., 2007).

Table 4-10. Composition of seaweed bioethanol production residue

Component	Seaweed species		
	U. fasciata	*S. vulgare*	*H. dentata*
Total solids (% biomass)[1]	15.822 ±0.199[a]	13.864 ±0.299[b]	20.109 ±0.318[c]
Moisture content (% biomass)	84.178 ±0.199	86.136 ±0.299	79.891 ±0.318
Volatile solids (% DM)[1]	83.667 ±1.566[a]	83.607 ±1.510[a]	83.837 ±1.217[a]
Ash content (% DM)	16.333 ±1.566	16.393 ±1.510	16.163 ±1.217

4.6.2 **MFC performance during inoculation**

Four microbial fuel cell reactors were inoculated with cow dung solution along with sodium acetate as primary substrate. This was done to facilitate the development of biofilms on the surface of the activated carbon granules used as electrodes in the MFCs. It is within the biofilm that electrogenic bacteria oxidise substrate to release electrons, protons and carbon dioxide. The inoculation process in the MFCs was monitored using voltage time profiles as seen in Figure 4-22. The power generation profile in Figure 4-22 shows that the four MFCs had a brief lag phase of almost two days before an exponential rise to the individual peak voltages within 4-5 days. This was followed by a slow and steady voltage decline phase before termination was done on the 11th day.

All four replicate reactors reached their peak voltage within the 6th to 7th day of inoculation. The time taken to reach the peak voltage is referred to as the length of start-up (Table 4-11). The peak voltage values which ranged between 0.43 to 0.51 V indicates that indeed an active electrogenic biofilm has been formed successfully from the cow dung solution used. These voltages were comparable to the typical working voltage of 0.5 V for MFCs (Kim *et al.,* 2011b). The power densities from the four MFCs which ranged between 0.625 and 0.872 W/m^3 even though replicates were significantly different. This indicated a possible bias may occur in assigning specific

[1] *Means of the same row with different letters are significantly different (Tukey's HSD, $p<0.05$)*

reactors to specific seaweed species. Nonetheless the reactors were randomly assigned to the three seaweeds along with sodium acetate as control.

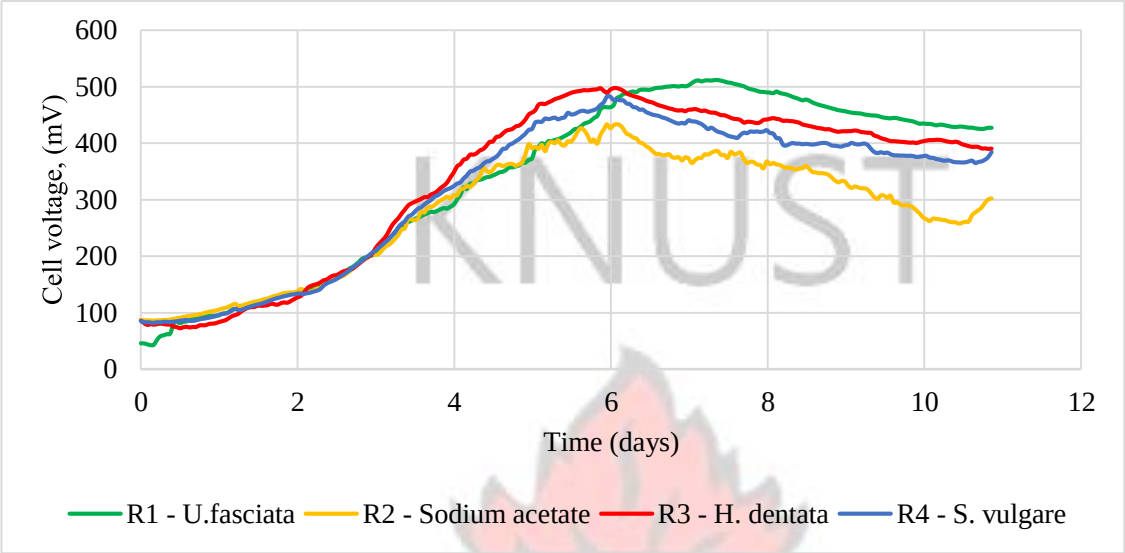

Figure 4-22. Power generation profile of the MFCs during inoculation

Table 4-11. Summary of power generation during inoculation of the MFCs

Cell	Inoculum used	Start-up time (days)	Maximum voltage under load (V)	Power density (W/m_3)[1]
R1	Cow dung	7.3	0.511 ±0.000	0.872 ± 0.002^{a}
R2	Cow dung	6	0.433 ±0.001	0.625 ± 0.003^{b}
R3	Cow dung	6	0.497 ±0.001	0.823 ± 0.003^{c}
R4	Cow dung	6	0.481 ±0.003	0.771 ± 0.010^{d}

4.6.3 **Power generation in seaweed-fed MFCs**

The three seaweed residues from the bioethanol process were fed into the anode compartments of three separate MFCs in three replicate fed-batch cycles. The cycle time for each batch was 5 days. The power generated within each cycle was monitored by collecting voltage-time data throughout the entire cycle duration. Power generation

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

from the MFCs were measured as current and power densities relative to the anode volume.

From Figure 4-23 (fed-batch cycles are separated by black vertical grid lines), the three post-inoculation cycles were steady with minimal voltage fluctuations. Voltage decline was clearly visible in cycles 2 and 3 of all reactors. The voltage decline was slow and steady which was expected since substrate was being consumed during electric current generation. The power densities between the three seaweed residues were between 0.46 and 0.50 W/m^3 with no significant difference between values from each species. They were all however significantly different from the sodium acetate used as a control. The sodium acetate served as the baseline for evaluating the performance of the seaweed fed-MFCs because it is considered the most efficient substrate in MFC research (Sun *et al.,* 2015). The maximum voltages and current densities for the three residues used were between 0.36 to 0.39 V and 1.22 to 1.29 A/m^3, respectively (Table 4-12). These were also similar with no significant difference but varied significantly from the control.

Velasquez-Orta *et al.* (2009) are one of a few known researchers to have directly applied seaweed biomass as substrates in MFCs. They fed powdered *U. lactuca* species to a single chambered microbial fuel cell over a period of 7 days to obtain a maximum power density of 215 W/m^3 and a substrate removal efficiency of 73%. This was much higher than that obtained in this study for the *U. fasciata* species (Table 4-12) which is from the same taxonomic family as the *U. lactuca* species. The difference in power densities could be as a result of the difference in anode volume which was 25 ml in theirs as against 300 ml in this study, used in normalizing the power produced by the reactors. Another notable difference is the use of the whole biomass by VelasquezOrta *et al.* (2009) whiles only seaweed residue was used in this study which forms a significant limitation in the comparison of results due to large substrate compositional variations. The difference in substrate removal efficiencies could also be because of the differences in cycle time which was 7 days for Velasquez-Orta *et al.* (2009) and 5 days in this study.

These results indicate that seaweed residues can indeed be oxidised to electric current but with a lower voltage threshold of 0.39 V from the typical 0.5 V as seen in the control. This limitation could be attributed to the rather longer degradation pathways required for the synthesis of lipids, proteins, fibre and residual sugars which form the

bulk of the residual organics in the seaweed residue. Sodium acetate however is readily degradable by the electrogenic bacteria. The use of seaweed residues as substrates in MFCs can therefore be considered efficient.

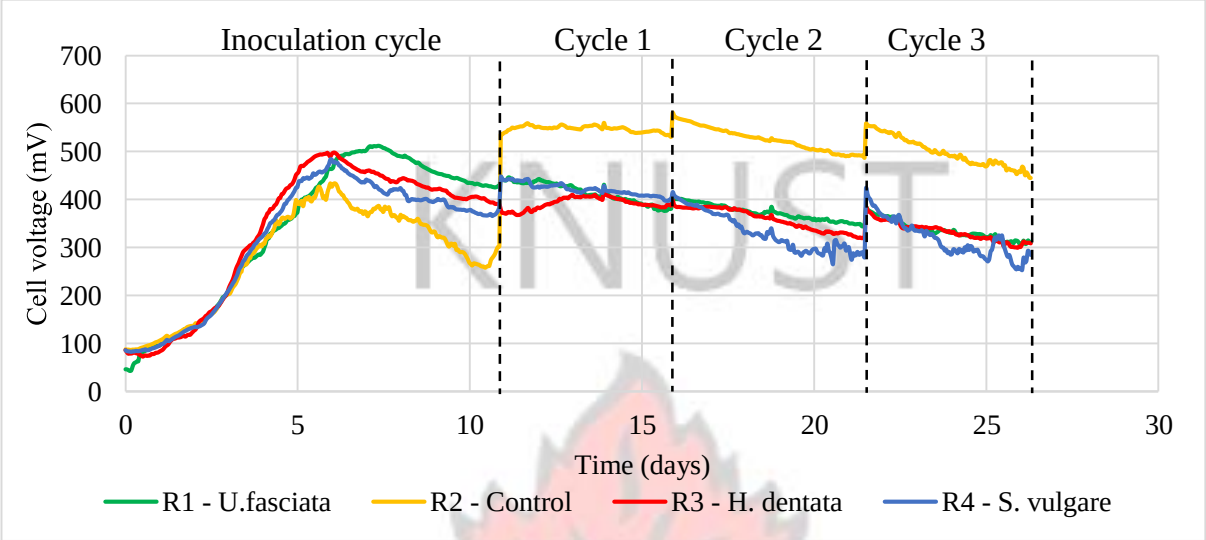

Figure 4-23. Electricity generation profile of the seaweed-fed MFCs

Table 4-12. Summary of power generation performance of the seaweed-MFCs

Substrate	Maximum voltage under load (V)	Current density (A/m^3)	Power density (W/m_3)[1]
U. fasciata residue	0.385 ±0.053	1.285 ±0.178	0.502 ±0.139[a]
H. dentata residue	0.376 ±0.037	1.254 ±0.122	0.475 ±0.091[a]
S. vulgare residue	0.367 ±0.065	1.223 ±0.218	0.458 ±0.168[a]
Sodium acetate	0.534 ±0.018	1.783 ±0.061	0.954 ±0.066[b]

4.6.4 **Internal resistances in seaweed-fed MFCs**

The internal resistance which often limits the power output of any bio-electrochemical system was analysed to examine the extent to which it hampers the performance of the MFCs. Some known locations of cell impedance or resistance include the electrolytes and the electrode material. The overall internal resistance also referred to as ohmic resistance in this study was measured via Electrochemical Impedance Spectroscopy (EIS) using a Potentiostat.

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

The visual output from the EIS analysis in this study was in the form of a Nyquist plot as shown in Figure 4-24. In the plot, the y-axis represents the imaginary part of the impedance while the x-axis represents the real part of impedance from which the actual cell impedance is measured. The cell impedance is read as the minimum plotted point that either directly intersects with the x-axis or can be traced to the x-axis.

The overall internal resistance measured via EIS was significantly different between species and were also different from the control. They ranged between 18.50 to 80.79 Ω (Table 4-13). The Acetate-fed MFC had the least internal resistance while the *S. vulgare*, *U. fasciata* and *H. dentata* in ascending order had higher internal resistances. The disparity between ohmic resistances was found to have a strong correlation (Correlation coefficient = 0.932) with the conductivity of the anode electrolyte for each MFC including the control. The higher the anolyte conductivity the lower the ohmic resistance (Table 4-13).

The relatively higher power density from the Acetate-fed MFC can be attributed to its high anolyte conductivity and lower internal resistance. This trend however may have a limitation since the ohmic resistance between the reactors with the seaweed residues were different (62.66-80.79 Ω) but their power densities were similar (0.46-0.50 W/m^3). In fact, *H. dentata* which had the highest ohmic resistance (80.79 Ω) recorded a higher power density of 0.48 W/m^3 than 0.46 W/m^3 from *S. vulgare* even though *S. vulgare* had the least ohmic resistance between species. The extent to which the internal resistances influences power density especially that which is caused by the electrolyte is therefore limited. Even so, adjustments to the ionic strength of anolytes can be made with the aid of buffers to improve electron transfer and ultimately improved power densities for seaweed-fed MFCs.

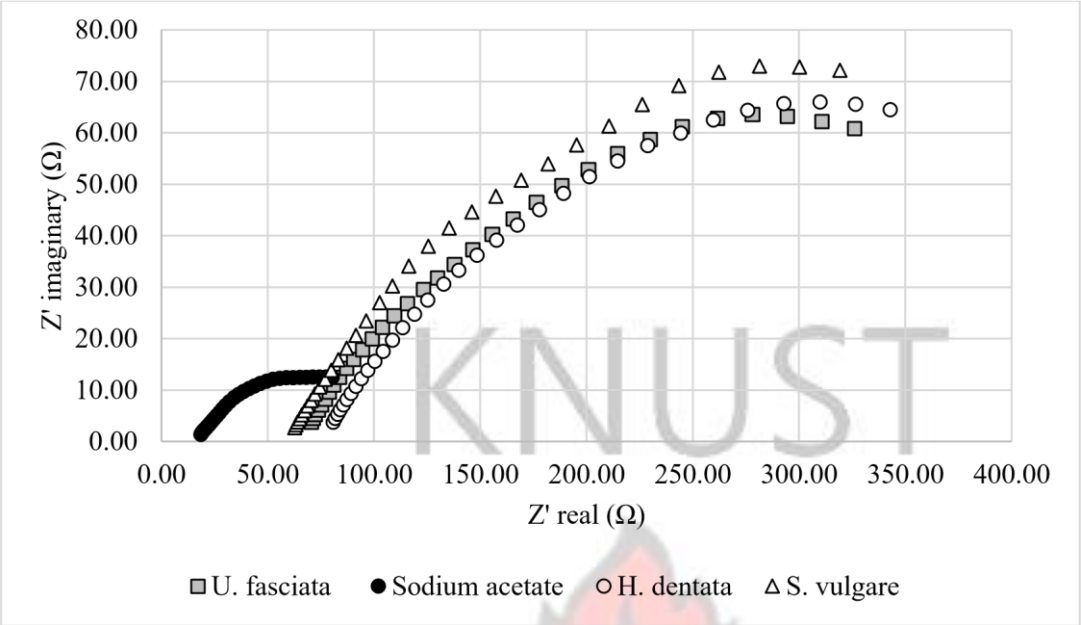

Figure 4-24. Nyquist plots of overall MFC impedance from seaweed fed-MFCs

Table 4-13. Summary of EIS analysis on seaweed-fed MFCs

Substrate	R_{Ohm} (Ω)[1]	Anode Conductivity (mS/cm)	Cathode Conductivity (mS/cm)	OCV during EIS (mV)
U. fasciata residue	63.32 ±0.00[a]	1.336 ±0.185	27.617 ±7.821	0.598 ±0.016
H. dentata residue	80.79 ±0.00[c]	0.973 ±0.065	27.617 ±7.821	0.579 ±0.011
S. vulgare residue	62.66 ±0.00[d]	1.767 ±0.324	27.617 ±7.821	0.563 ±0.044
Sodium acetate	18.50 ±0.00[b]	10.690 ±3.872	27.617 ±7.821	0.619 ±0.037

4.6.5 **Substrate consumption in seaweed-fed MFCs**

In the operation of MFCs, substrates deplete with time since they are the principal components that are converted to electric current. Substrate depletion in MFCs is measured as the change in Chemical Oxygen Demand (COD) over the cycle time. The

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

organic substrate removal within the 5-day cycle time for all three seaweeds and the control were quite similar with no significant difference as seen in Table 4-14. This ranged from 29.96 to 46.02%. These substrate removal efficiencies within such a short operating time can be considered appreciably high as compared to conventional biological pathways such as anaerobic digestion which can span as high as 60 days.

The coulombic efficiency (CE) which refers to the fraction of the substrate that was converted to electric current within the cycle time was however low, ranging from 3.2 to 6.1%. There was no significant difference between all three species and the control. The low CEs obtained could be attributed to the competing biological processes that occur simultaneously in typical anaerobic systems operating at room temperature with mixed microbial cultures. This implies that the substrate could have been used up by other non-electrogenic microbes that co-exists with the electrogenes. The CE is however expected to increase over longer MFC cycle times as reported in Offei *et al.* (2016).

Table 4-14. Summary of substrate consumption in seaweed-fed MFCs

Substrate	Initial COD (g/l)	Final COD (g/l)	Substrate removal efficiency (%)[1]	Coulombic efficiency (%)[1]
U. fasciata residue	5.190 ±0.467	2.910 ±0.171	39.932 ±5.655[a]	3.809 ±1.494[a]
H. dentata residue	2.305 ±0.488	1.337 ±0.139	31.639 ±14.667[a]	6.108 ±0.308[a]
S. vulgare residue	5.880 ±0.000	2.773 ±0.145	46.022 ±9.634[a]	3.277 ±1.915[a]
Sodium acetate	3.920 ±0.000	5.755 ±0.007	29.964 ±2.717[a]	4.320 ±0.600[a]

4.6.6 **Compositional analysis of seaweed-fed MFC effluents**

There have been several studies suggesting the potential use of seaweeds as fertilizers (Dhargalkar and Pereira, 2005). This application is of particular interest in this study since it promotes the biorefinery approach to biomass usage in adding value to every component in the biomass. The effluent from the MFCs were therefore analysed in this

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

study for total nitrogen, phosphorus and ammonia which are key components found in most soil amendment products.

From Table 4-15, the nitrogen levels in the *U. fasciata* effluents were the most appreciable at 21%. This was significantly higher than that from *H. dentata* and *S. vulgare* which were less than 9%. Total phosphorus was low for all three seaweed effluents ranging between 0.43 to 0.65%. Total ammonia was high for *U. fasciata* at 25.62%. *H. dentata* and *S. vulgare* were also quite appreciable at 10.33 and 10.56%, respectively but significantly different from that of *U. fasciata*. This indicates that effluents from *U. fasciata* have the highest potential use for soil amendment. Even though considerable fractions of nitrogen and ammonia were found in all the seaweed effluents, they can only be used as complimentary additives to conventional composts and fertilizers. The potential use of the seaweed-fed MFC effluents in soil amendment will contribute significantly to waste minimization in seaweed biomass processing to biofuels.

Table 4-15. Composition of seaweed-fed MFC effluent for soil amendment

Effluent	Total nitrogen (%)[1]	Total phosphorus (%)[1]	Total ammonia (%)[1]
U. fasciata	20.996 ±0.375[a]	0.649 ±0.112[a]	25.615 ±0.457[a]
H. dentata	8.653 ±0.134[b]	0.479 ±0.027[b]	10.557 ±0.163[b]
S. vulgare	8.467 ±0.686[b]	0.430 ±0.000[b]	10.331 ±0.837[b]

4.7 Seaweed utilization assessment

Maximising the use of the seaweed through an integrated processing pathway forms the core purpose of this study. The overall substrate utilization from the two major processes used in this study were therefore assessed. The utilization threshold for the bioethanol production process was marked as the percentage of sugars recovered to

[1] *Means of the same column with different letters are significantly different (Tukey's HSD, $p<0.05$)*

produce the ethanol while that of the bioelectricity process was marked as the unused substrate remaining after the 5-day MFC operation cycle time.

For the bioethanol production process, *H. dentata* was the most utilized even though its overall ethanol yield was lower. Substrate utilization was 31.64% as against 21.07 and 27.90% for *S. vulgare* and *U. fasciata*, respectively (Table 4-16). This was because more reducing sugars were released by *H. dentata* during hydrolysis than the others. *U. fasciata* was conversely the most utilized seaweed in the bioelectricity production process in the MFCs at 47.67% which was significantly higher than that of *S. vulgare* and *H. dentata* (41.47 and 29.88%, respectively).

Overall substrate utilization was significantly highest for *U. fasciata* at 75.5% while *S. vulgare* and *H. dentata* had similar outputs between 62 and 63% (Table 4-16). The overall waste generated from the integrated processing of the seaweeds was between 24 and 38.5%. This is considerably lower as compared to a potential 69 to 79% that would have been generated from the production of bioethanol alone. The waste generated could be further reduced if the cycle time of the MFCs is extended. The residual substrate quantified as waste also has a good potential use in soil amendment due to its considerable ammonia concentrations.

Yahmed *et al.* (2016) also applied the biorefinery approach to the conversion of the green seaweed, *C. linum* to bioethanol and biomethane. Their substrate utilization was 6.9% for bioethanol and 62.9% for biomethane with a waste generation of 30.2%. Their substrate utilization was comparable to that obtained in this study also for a green seaweed species but via a different biorefinery pathway. However, a shorter processing time of 8 days was used in this study as against the 32 days in their study. The biorefinery pathway chosen in this study proved to be the most efficient with respect to time. The results from Yahmed *et al.* (2016) and this study both demonstrate that the biorefinery approach to seaweed biomass conversion does indeed add more value to the substrate and effectively reduces in waste generation.

Table 4-16. Overall material balance for the biorefinery approach to seaweed use

Component (% g seaweed)	Seaweed species		
	U. fasciata	*S. vulgare*	*H. dentata*
Substrate use for Bioethanol[1]	27.902 ±0.885[a]	21.065 ±0.429[b]	31.639 ±1.120[c]

Substrate use for Bioelectricity[1]	47.671 ±0.585[a]	41.473 ±0.225[b]	29.879 ±0.489[c]
Overall substrate utilization[1]	75.574 ±0.300[a]	62.539 ±0.204[b]	61.518 ±0.631[b]
Waste generation[1]	24.426 ±0.300[a]	37.461 ±0.204[b]	38.482 ±0.631[b]

[1] *Means of the same row with different letters are significantly different (Tukey's HSD, $p<0.05$)*

4.8 Life cycle assessment of bioenergy from seaweeds

The cultivation of seaweeds for commercial scale production of biofuels is currently non-existent. This is largely because the sustainability of biofuels from seaweeds is largely unproven. Research in seaweeds has focused mainly on the recovery of the biofuels; bioethanol and biogas (includes biomethane) with more recent exploits into bioelectricity via MFCs. The conclusions from these studies consistently indicate that biofuel production from seaweeds is indeed promising.

This study examined the sustainability of the integrated pathways to bioenergy recovery from seaweeds with emphasis on processing. The processing pathways examined were bioethanol only, bioethanol-bioelectricity co-production and bioethanol-biogas co-production from seaweeds. The sustainability metric selected was the Energy Return on Investment (EROI). This metric was selected because it has a massive impact on all three pillars of sustainability i.e. economic, social and environmental perspectives. This study further examines the individual processes that contribute largest to energy use in the selected pathways.

4.8.1 EROI of bioenergy process systems

EROI is described generally as the ratio of the energy output of a system to the energy input to the same system (Murphy and Hall, 2010). In the context of this study, the energy output is defined as the lower heating value of the energy carriers or products while the energy input is the total cumulative non-renewable fossil energy demand. The primary system boundary and inventory for the EROI analysis were defined based

on 1 ha of seaweed cultivation area with a functional energy unit of 1MJ as the lower heating value of the energy carriers produced.

It is necessary that the products from any energy production process generates a net gain in energy, which corresponds to an EROI value above 1. However, for the product to be considered sustainable a minimum EROI of 3 is required (Hall *et al.*, 2009). The higher the EROI value the more sustainable the product or process and the lower the depletion of finite fossil energy resources.

The EROI values as shown in Figure 4-25 are for *Scenario 1*, *Scenario 2* and *Scenario 3* which refers to bioethanol production only, integrated bioethanol-bioelectricity production (as described in this study) and integrated bioethanol-biogas, respectively (as described by Aitken *et al.*, 2014). *Ref. Scenario* is a comparative reference scenario with an EROI value for bioethanol production from maize obtained by Murphy and Hall (2010). The two red gridlines in Figure 4-25 refers to the net energy gain baseline (lower red gridline) and the sustainable energy baseline (upper red gridline).

From the EROI analysis, *Scenario 2* was the most sustainable with an EROI value of 4.22 which is well above the target of 3 needed for the process to be considered sustainable (Figure 4-25). This was largely caused by the reduction in energy use due to the absence of mechanical moving parts for the bioelectricity production stage in the integrated process. The direct conversion of substrate to electricity with no intermediate processing could also be a contributing factor to the high the EROI value. *Scenario 3* could also become sustainable if the energy demands in stirring the reactants is reduced and energy recovery efficiencies for the combustion of biogas to energy increased.

Scenario 1 yielded an EROI value of 1.63 which was lower than all other scenarios. This value indicates that indeed a net gain in energy will be obtained if seaweeds are used to produce bioethanol alone, however, a co-product is required to make the production process sustainable. For the reference scenario, maize which was the raw material used for bioethanol production was higher than *Scenario 1* largely because of the higher carbohydrates fraction in the maize which leads to a higher ethanol yield. It also indicates that the commercial production of ethanol from maize indeed has a net gain but cannot be considered sustainable in the long term. There is the need for a coproduct also in this processing pathway.

Generally, high EROI values (> 2) were observed for scenarios 2 and 3 which were both applications of the biorefinery approach to biomass conversion. The use of multiple technologies to maximise the use of biomass to obtain more valuable products is indeed beneficial. The EROI values from the scenarios described indicate that sustainable biofuel production from seaweeds can only be achieved if an integrated biorefinery approach is used.

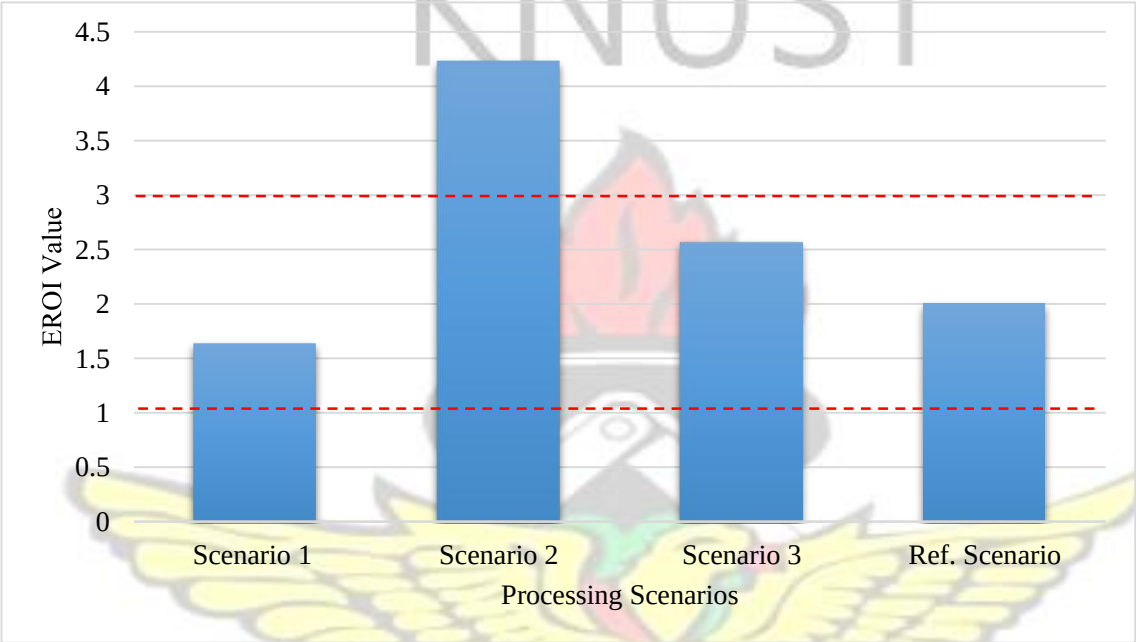

Figure 4-25. EROI values from the various bioenergy process scenarios

4.8.2 **Process contributions to Energy Consumption**

The energy contribution of specific processes to the process scenarios described in the EROI analysis were examined further to identify those that are most energy intensive. Figure 4-26 shows that the hydrolysis, fermentation and distillation processes consume the most energy in scenarios 1 and 2 (up to 75 GJ). The stirring and pumping processes consume up to 95 GJ of energy for the anaerobic digestion process used in Scenario 3. This makes it the most energy consuming process between all three scenarios.

Electricity and heat are clearly the energy forms most required in all the three scenarios particularly for ethanol production and anaerobic digestion. *Scenario 3* consumes the most energy (161.21 GJ) and gives off the most energy (411.94 GJ) obtained from 29,108 m^3 of biogas. *Scenario 1* consumes the least (66.30 GJ) and gives off the least energy as well (108.07 GJ) from 3.64 tons of ethanol (Table 4-17). The energy consumed in the various processes indicate that indeed to maximise and sustainably produce biofuels and bioenergy careful optimization towards energy input reduction is very much needed.

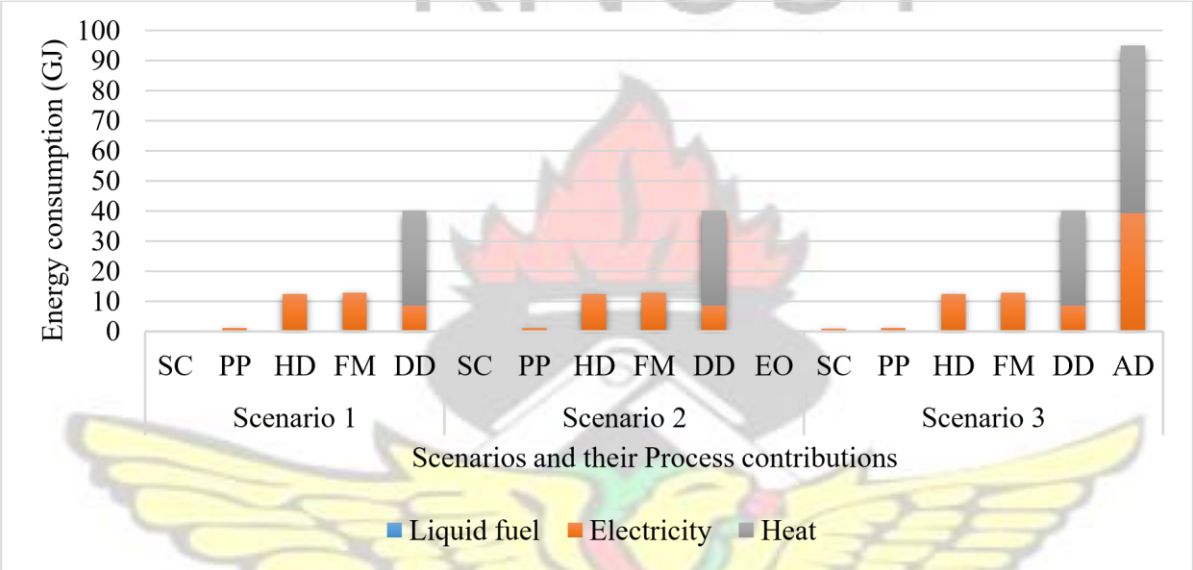

Figure 4-26. Process contribution to energy consumption per hectare of seaweed

(Note: SC: Seaweed cultivation, PP: Pre-processing, HD: Hydrolysis, FM: Fermentation, DD: Distillation and dehydration, EO: Electrogenic oxidation, AD: Anaerobic digestion)

Table 4-17. Energy process balance for the various processing scenarios

Scenario	Energy use	Energy value (GJ)
Scenario 1 (Bioethanol Production)	Total Energy Consumption □ For processing 729.90 tons of freshly harvested seaweed to ethanol	66.303
	Total Energy Production □ From 3.64 tons of 99.7% purity ethanol	108.065

Scenario 2 (Bioethanol and Bioelectricity Production)	Total Energy Consumption • For processing 729.90 tons of freshly harvested seaweed to ethanol • From processing 77.3 tons of seaweed residue to electricity	66.338
	Total Energy Production • From 3.64 tons of 99.7% purity ethanol • From 29.9kW MFCs	279.870
Scenario 3 (Bioethanol and Biogas Production)	Total Energy Consumption • For processing 729.90 tons of freshly harvested seaweed to ethanol • From processing 1495.81 tons of seaweed stillage to biogas	161.207
	Total Energy Production • From 3.64 tons of 99.7% purity ethanol • From 29708.14m3 of biogas	411.935

CHAPTER 6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The introduction and development of Ghanaian seaweeds as substrates for bioethanol production is considered very relevant in this study. This is important in avoiding the dire food security issues that could occur from the continued use of edible biomass such as maize, cassava and sugarcane in commercial bioethanol production. An efficient technology development is also vital in recommending bioethanol as a sustainable alternative fuel in Ghana. The production of bioethanol alone from the seaweeds is known to generate large amounts of organic rich residue (55-80%) which leads to substrate underutilization and massive waste generation.

This study therefore sought to assess the potential of integrating bioethanol and bioelectricity production technologies as an efficient means of maximising seaweed biomass utilization. This study specifically sought to examine the optimal bioethanol production yield from seaweed biomass through the variation of its processing conditions; determine the bioelectricity production potential of seaweed residue after bioethanol production using microbial fuel cells; and to use a life cycle assessment to investigate the sustainability of producing both bioethanol and bioelectricity from

seaweed in an integrated approach. The succeeding subsections outline the conclusions drawn from the results of the study.

6.1.1 Optimal bioethanol production from Ghanaian seaweeds

The typical bioethanol production process involves pretreatment, hydrolysis, fermentation and ethanol recovery. The optimisation process applied in this study emphasized on pretreatment screening, hydrolysis optimisation, and ethanol production pathway selection. The study established that the dilute acid pretreatment was the most efficient in treating all the three selected seaweeds before hydrolysis. The *U. fasciata* seaweed however, can be hydrolysed efficiently without any form of pretreatment.

The study also noted that the dilute acid hydrolysis even though considered cost effective, may not be the best form of hydrolysis for seaweeds since its optimal condition could only release up to 52.6% of the sugars found in the three seaweeds. Yet, its alternative, enzymatic hydrolysis released up to 86.5% of reducing sugars in the seaweeds. The optimal enzymatic hydrolysis process was influenced most by the substrate concentration used for all three seaweeds examined. *S. vulgare* could tolerate substrate concentrations of up to 12% unlike the others which were limited to 10%.

It was observed in the study that the choice of ethanol production pathway (i.e. SHF or SSF) was dependent on the type of seaweed species and the yeast strain used. The SHF pathway was preferred by *U. fasciata* and *H. dentata* whiles the SSF pathway was best suited to the *S. vulgare* species. The differences in ethanol yields between pathways were however marginal.

The study found the optimal ethanol yields for the Ghanaian seaweeds, *U. fasciata, H. dentata* and *S. vulgare* to be 5.06, 2.44 and 3.69% DM. This was obtained via the SHF pathway through enzymatic hydrolysis with a cellulase dosage of 8 FPU/g DM and fermentation with *S. cerevisiae* SI17, C8T17 or PT17 yeast strains. The ethanol yields from this study were lower as compared to known yields from other studies and from conventional biomass for bioethanol production.

6.1.2 Bioelectricity potential of seaweed residue from bioethanol production

The seaweed residues obtained after bioethanol was produced were introduced as substrate in microbial fuel cells for direct conversion to electricity. The study found

the residues from seaweed bioethanol production to be efficient substrates for use in microbial fuel cells since they yielded power densities of up to 0.50 W/m^3 which were comparable to sodium acetate by up to 52.62%. Power densities from seaweed fed MFCs can be improved if measures could be taken to reduce their ohmic resistance which impedes the flow of current from the solution.

Substantial substrate removal efficiencies of up to 46.02% were achieved in a short operating time of 5 days for the seaweed-fed MFCs however coulombic efficiencies were quite poor with a maximum of 6.1%. The study further revealed that effluent from *U. fasciata*-fed MFCs can be considered for use in soil amendment due to their appreciable levels of nitrogen and ammonia. The integrated approach to biomass utilization which formed the core aim of the study was considered successful since waste generation was reduced to as low as 24.43% from a potential 69 to 79% from seaweed bioethanol production alone.

6.1.3 **Sustainability of integrated bioenergy production from seaweeds**

The production of both bioethanol and bioelectricity from Ghanaian seaweeds were found to be sustainable based on the EROI value of 4.2 obtained through the Life Cycle Assessment of the technologies. It was also noted that the production of bioethanol alone from seaweeds would not be sustainable commercially despite a net gain in energy from its LCA. The most energy intensive process in the integrated biorefinery approach to seaweed biomass conversion was the distillation and dehydration stage of bioethanol production requiring up to 40 GJ of energy per hectare of seaweed biomass processed. From the LCA, the use of MFCs in bioelectricity production was less energy intensive due to the absence of mechanical moving parts. This gave it a significant advantage over conversional bioprocesses such as anaerobic digestion.

6.1.4 **General research contributions from study findings**

The novel integration of the bioethanol and bioelectricity technologies to maximise the use of seaweeds was successfully applied. This application of the biorefinery approach was not only efficient but was also found to be sustainable. The study also developed a strong mathematical model that can describe and predict the conditions required for efficient enzymatic hydrolysis of *U. fasciata* seaweed. It also developed other useful models for the description of the enzymatic hydrolysis conditions for *S. vulgare* and

H. dentata seaweeds. The relationship between the three types of seaweeds and some selected strains of yeasts were established through yeast screening.
This would be of particular interest to commercial scale ethanol producers.

6.2 Recommendations for further studies

Based on the findings from this study the following recommendations are made for future research activities:

- The application of the integrated bioethanol and bioelectricity technology is recommended for use on conventional biomass used in commercial bioethanol production to examine the flexibility of the technology.
- Generally, by-product recoveries were high especially for the SSF pathway to bioethanol production. Further examination of process conditions may be required to determine the possible causes in order to minimize the formation of the by-products and possibly maximise ethanol production.
- This study also recommends the screening of more yeast strains to identify strains with a higher pentose conversion. Genetic modification or microbial adaption of yeast strains with a combined potential for hexose and pentose conversion to ethanol is also recommended.

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APPENDIX

A1. Acid and Enzymatic hydrolysis experimental design matrices

A1.1. Experimental design matrix for the dilute acid hydrolysis of seaweeds

Treatment number	Process conditions		
	Time, min	Temperature, °C	Acid concentration, M
1	15	100	0.1
2	15	100	0.2
3	15	100	0.3
4	15	120	0.1
5	15	120	0.2
6	15	120	0.3
7	15	130	0.1
8	15	130	0.2
9	15	130	0.3
10	30	100	0.1
11	30	100	0.2
12	30	100	0.3
13	30	120	0.1
14	30	120	0.2
15	30	120	0.3
16	30	130	0.1
17	30	130	0.2
18	30	130	0.3
19	60	100	0.1
20	60	100	0.2
21	60	100	0.3
22	60	120	0.1

23	60	120	0.2
24	60	120	0.3
25	60	130	0.1
26	60	130	0.2
27	60	130	0.3

A1.2. Experimental design matrix for the enzymatic hydrolysis of seaweeds

Treatment number	Process conditions		
	Time, hour	Substrate concentration, % w/v dry biomass	Enzyme concentration, FPU/g dry biomass
1	24	5	2
2	24	5	8
3	24	15	2
4	24	15	8
5	72	5	2
6	72	5	8
7	72	15	2
8	72	15	8
9	48	10	2
10	48	10	8
11	48	5	5
12	48	15	5
13	24	10	5
14	72	10	5
15	48	10	5
16	48	10	5
17	48	10	5
18	48	10	5
19	48	10	5
20	48	10	5

A2. Yeast culturing

A2.1. Optical density of yeast cultures used in this study

Yeast ID	Optical density at 600nm
Y1	12.335
Y2	10.92
Y3	10.82
Y4	7.295
Y5	10.035

A2.2. One-way ANOVA on Yeast growth rate

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	4.258	4	1.065	F (4, 5) = 1426	P < 0.0001
Residual (within columns)	0.003732	5	0.000746		
Total	4.262	9			

A3. Statistical Analysis for Seaweed composition

A3.1. One-way ANOVA on seaweed compositional analysis (Total solids)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.009978	2	0.004989	F (2, 6) = 270.7	P < 0.0001
Residual (within columns)	0.000111	6	1.84E-05		
Total	0.01009	8			

A3.2. Tukey's HSD test on seaweed compositional analysis (Total solids)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	-0.03173	-0.04249 to -0.02098	Yes	***
U. fasciata vs. H. dentata	-0.08093	-0.09169 to -0.07018	Yes	****
S. vulgare vs. H. dentata	-0.0492	-0.05995 to -	Yes	****

			0.03845		

A3.3. One-way ANOVA on Seaweed compositional analysis (Moisture content)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.01056	2	0.005281	F (2, 6) = 312.6	P < 0.0001
Residual (within columns)	0.000101	6	1.69E-05		
Total	0.01066	8			

A3.4. Tukey's HSD test on Seaweed compositional analysis (Moisture content)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	0.03083	0.02054 to 0.04113	Yes	***
U. fasciata vs. H. dentata	0.083	0.07270 to 0.09330	Yes	****
S. vulgare vs. H. dentata	0.05217	0.04187 to 0.06246	Yes	****

A3.5. One-way ANOVA on Seaweed compositional analysis (Total carbohydrates)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1.23	2	0.6151	F (2, 6) = 0.4282	P = 0.6702
Residual (within columns)	8.62	6	1.437		
Total	9.851	8			

A3.6. Two-way ANOVA on Seaweed monomeric sugar analysis

ANOVA table	SS	DF	MS	F (DFn, DFd)
Interaction	449.8	14	32.13	F (14, 48) = 58.36
Row Factor	1293	7	184.8	F (7, 48) = 335.6
Column Factor	4.013	2	2.007	F (2, 48) = 3.645
Residual	26.43	48	0.5506	

A3.7. Tukey's HSD test on Seaweed monomeric sugar analysis

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Fucose				
U. fasciata vs. S. vulgare	-3.813	-5.278 to -2.348	Yes	****
U. fasciata vs. H. dentata	0.2061	-1.259 to 1.671	No	ns
S. vulgare vs. H. dentata	4.019	2.554 to 5.484	Yes	****
Arabinose				
U. fasciata vs. S. vulgare	-0.7073	-2.173 to 0.7579	No	ns
U. fasciata vs. H. dentata	-0.6337	-2.099 to 0.8315	No	ns
S. vulgare vs. H. dentata	0.07356	-1.392 to 1.539	No	ns
Rhamnose				
U. fasciata vs. S. vulgare	5.379	3.914 to 6.844	Yes	****
U. fasciata vs. H. dentata	5.379	3.914 to 6.844	Yes	****
S. vulgare vs. H. dentata	0	-1.465 to 1.465	No	ns
Galactose				
U. fasciata vs. S. vulgare	-1.996	-3.462 to -0.5311	Yes	**
U. fasciata vs. H. dentata	-12.21	-13.67 to -10.74	Yes	****
S. vulgare vs. H. dentata	-10.21	-11.68 to -8.746	Yes	****
Glucose				
U. fasciata vs. S. vulgare	-0.1707	-1.636 to 1.295	No	ns
U. fasciata vs. H. dentata	3.148	1.683 to 4.613	Yes	****
S. vulgare vs. H. dentata	3.319	1.853 to 4.784	Yes	****
Xylose				
U. fasciata vs. S. vulgare	5.709	4.244 to 7.174	Yes	****
U. fasciata vs. H. dentata	6.644	5.179 to 8.110	Yes	****
S. vulgare vs. H. dentata	0.9355	-0.5298 to 2.401	No	ns
Mannose				
U. fasciata vs. S. vulgare	-0.3933	-1.859 to 1.072	No	ns
U. fasciata vs. H. dentata	-2.211	-3.676 to -0.7453	Yes	**
S. vulgare vs. H. dentata	-1.817	-3.282 to -0.3520	Yes	*
Galacturonic acid				
U. fasciata vs. S. vulgare	0.2413	-1.224 to 1.707	No	ns
U. fasciata vs. H. dentata	0.2133	-1.252 to 1.679	No	ns
S. vulgare vs. H. dentata	-0.02799	-1.493 to 1.437	No	ns

A4. Statistical Analysis for Seaweed Pretreatment screening

A4.1. Two-way ANOVA on Pretreatment Screening

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	286.3	7	40.9	F (7, 31) = 12.33	P < 0.0001
Row Factor	1862	7	266	F (7, 31) = 80.18	P < 0.0001
Column Factor	620.1	1	620.1	F (1, 31) = 186.9	P < 0.0001
Residual	102.9	31	3.318		

A4.2. Tukey's HSD test on *U. fasciata* Pretreatment Screening

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
T1 vs. T2	-14.13	-18.96 to -9.303	Yes	****
T1 vs. T3	-4.483	-9.311 to 0.3447	No	ns
T1 vs. T4	-6.434	-11.26 to -1.606	Yes	**
T1 vs. T5	-11.83	-16.66 to -7.000	Yes	****
T1 vs. T6	-17.91	-22.74 to -13.09	Yes	****
T1 vs. T7	0.4262	-4.402 to 5.254	No	ns
T1 vs. T8	-18.69	-23.52 to -13.86	Yes	****
T2 vs. T3	9.648	4.820 to 14.48	Yes	****
T2 vs. T4	7.697	2.869 to 12.52	Yes	***
T2 vs. T5	2.303	-2.525 to 7.131	No	ns
T2 vs. T6	-3.783	-8.611 to 1.045	No	ns
T2 vs. T7	14.56	9.729 to 19.39	Yes	****
T2 vs. T8	-4.556	-9.384 to 0.2719	No	ns
T3 vs. T4	-1.951	-6.779 to 2.877	No	ns
T3 vs. T5	-7.345	-12.17 to -2.517	Yes	***
T3 vs. T6	-13.43	-18.26 to -8.603	Yes	****
T3 vs. T7	4.91	0.08151 to 9.738	Yes	*
T3 vs. T8	-14.2	-19.03 to -9.376	Yes	****
T4 vs. T5	-5.394	-10.22 to -0.5657	Yes	*

T4 vs. T6	-11.48	-16.31 to -6.652	Yes	****
T4 vs. T7	6.86	2.032 to 11.69	Yes	**
T4 vs. T8	-12.25	-17.08 to -7.425	Yes	****
T5 vs. T6	-6.087	-10.91 to -1.259	Yes	**
T5 vs. T7	12.25	7.426 to 17.08	Yes	****
T5 vs. T8	-6.859	-11.69 to -2.031	Yes	**
T6 vs. T7	18.34	13.51 to 23.17	Yes	****
T6 vs. T8	-0.7727	-5.601 to 4.055	No	ns
T7 vs. T8	-19.11	-23.94 to -14.29	Yes	****

A4.3. Tukey's HSD test on *S. vulgare* Pretreatment Screening

Tukey's multiple comparisons Diff.	Mean t?	95% CI of diff.	Significan	testSummary
T1 vs. T2	-1.129	-5.957 to 3.699	No	ns
T1 vs. T3	0.1288	-4.699 to 4.957	No	ns
T1 vs. T4	-0.7672	-6.165 to 4.631	No	ns
T1 vs. T5	-0.3385	-5.166 to 4.490	No	ns
T1 vs. T6	-11.97	-16.80 to -7.142	Yes	****
T1 vs. T7	-1.252	-6.080 to 3.576	No	ns
T1 vs. T8	-17.39	-22.22 to -12.57	Yes	****
T2 vs. T3	1.258	-3.570 to 6.086	No	ns
T2 vs. T4	0.3622	-5.036 to 5.760	No	ns
T2 vs. T5	0.791	-4.037 to 5.619	No	ns
T2 vs. T6	-10.84	-15.67 to -6.012	Yes	****
T2 vs. T7	-0.1225	-4.950 to 4.706	No	ns
T2 vs. T8	-16.27	-21.09 to -11.44	Yes	****
T3 vs. T4	-0.896	-6.294 to 4.502	No	ns
T3 vs. T5	-0.4672	-5.295 to 4.361	No	ns
T3 vs. T6	-12.1	-16.93 to -7.271	Yes	****
T3 vs. T7	-1.381	-6.209 to 3.447	No	ns
T3 vs. T8	-17.52	-22.35 to -12.70	Yes	****
T4 vs. T5	0.4288	-4.969 to 5.827	No	ns
T4 vs. T6	-11.2	-16.60 to -5.805	Yes	****
T4 vs. T7	-0.4847	-5.883 to 4.913	No	ns

T4 vs. T8	-16.63	-22.03 to -11.23	Yes	****
T5 vs. T6	-11.63	-16.46 to -6.803	Yes	****
T5 vs. T7	-0.9135	-5.741 to 3.915	No	ns
T5 vs. T8	-17.06	-21.88 to -12.23	Yes	****
T6 vs. T7	10.72	5.890 to 15.55	Yes	****
T6 vs. T8	-5.425	-10.25 to -0.5967	Yes	*
T7 vs. T8	-16.14	-20.97 to -11.31	Yes	****

A5. Statistical Analysis for Seaweed hydrolysis

A5.1. Two-way ANOVA on Dilute Acid hydrolysis

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1931	52	37.14	F (52, 162) = 24.99	P < 0.0001
Row Factor	2649	26	101.9	F (26, 162) = 68.54	P < 0.0001
Column Factor	1708	2	854.1	F (2, 162) = 574.7	P < 0.0001
Residual	240.8	162	1.486		

A5.2. Two-way ANOVA on Enzymatic hydrolysis

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	273.8	28	9.78	F (28, 90) = 4.637	P < 0.0001
Row Factor	1236	14	88.26	F (14, 90) = 41.85	P < 0.0001
Column Factor	371.9	2	186	F (2, 90) = 88.17	P < 0.0001
Residual	189.8	90	2.109		

A6. Statistical Analysis for Sugar selectivity

A6.1. Two-way ANOVA on SUB-A for sugar selectivity analysis

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	42.98	4	10.74	F (4, 10) = 130.2	P < 0.0001
Residual (within columns)	0.8254	10	0.08254		
Total	43.8	14			

A6.2. Tukey's HSD test on SUB-A for sugar selectivity analysis

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Y1 vs. Y2	-0.5356	-1.308 to 0.2364	No	ns
Y1 vs. Y3	-0.6338	-1.406 to 0.1382	No	ns
Y1 vs. Y4	-0.5949	-1.367 to 0.1771	No	ns
Y1 vs. Y5	3.752	2.980 to 4.524	Yes	****
Y2 vs. Y3	-0.09821	-0.8702 to 0.6738	No	ns
Y2 vs. Y4	-0.05927	-0.8313 to 0.7127	No	ns
Y2 vs. Y5	4.287	3.515 to 5.059	Yes	****
Y3 vs. Y4	0.03894	-0.7331 to 0.8109	No	ns
Y3 vs. Y5	4.385	3.613 to 5.157	Yes	****
Y4 vs. Y5	4.346	3.574 to 5.118	Yes	****

A6.3. Two-way ANOVA on SUB-B for sugar selectivity analysis

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1.018	4	0.2546	F (4, 10) = 32.23	P < 0.0001
Residual (within columns)	0.07898	10	0.007898		
Total	1.097	14			

A6.4. Tukey's HSD test on SUB-B for sugar selectivity analysis

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Y1 vs. Y2	-0.02043	-0.2592 to 0.2184	No	ns
Y1 vs. Y3	-0.05005	-0.2889 to 0.1888	No	ns
Y1 vs. Y4	0.01445	-0.2244 to 0.2533	No	ns

Y1 vs. Y5	0.6351	0.3963 to 0.8739	Yes	****
Y2 vs. Y3	-0.02962	-0.2684 to 0.2092	No	ns
Y2 vs. Y4	0.03488	-0.2039 to 0.2737	No	ns
Y2 vs. Y5	0.6555	0.4167 to 0.8943	Yes	****
Y3 vs. Y4	0.06449	-0.1743 to 0.3033	No	ns
Y3 vs. Y5	0.6851	0.4463 to 0.9240	Yes	****
Y4 vs. Y5	0.6206	0.3818 to 0.8595	Yes	****

A7. Statistical Analysis for Seaweed fermentation

A7.1. Two-way ANOVA on SHF ethanol production from seaweeds

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	14.53	8	1.816	F (8, 30) = 9.088	P < 0.0001
Row Factor	5.868	4	1.467	F (4, 30) = 7.343	P = 0.0003
Column Factor	59.28	2	29.64	F (2, 30) = 148.4	P < 0.0001
Residual	5.994	30	0.1998		

A7.2. Two-way ANOVA on SSF ethanol production from seaweeds

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3.371	8	0.4214	F (8, 29) = 2.685	P = 0.0243
Row Factor	9.634	4	2.408	F (4, 29) = 15.34	P < 0.0001
Column Factor	5.985	2	2.993	F (2, 29) = 19.07	P < 0.0001
Residual	4.552	29	0.157		

A7.3. Tukey's multiple comparisons on SHF ethanol production from seaweeds

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Gsw				
Y1 vs. Y2	0.5121	-0.5465 to 1.571	No	ns
Y1 vs. Y3	0.6147	-0.4439 to 1.673	No	ns

Y1 vs. Y4	0.4852	-0.5734 to 1.544	No	ns
Y1 vs. Y5	0.3911	-0.6675 to 1.450	No	ns
Y2 vs. Y3	0.1026	-0.9560 to 1.161	No	ns
Y2 vs. Y4	-0.02695	-1.086 to 1.032	No	ns
Y2 vs. Y5	-0.1211	-1.180 to 0.9375	No	ns
Y3 vs. Y4	-0.1295	-1.188 to 0.9291	No	ns
Y3 vs. Y5	-0.2236	-1.282 to 0.8350	No	ns
Y4 vs. Y5	-0.09413	-1.153 to 0.9645	No	ns
Bsw				
Y1 vs. Y2	1.653	0.5941 to 2.711	Yes	***
Y1 vs. Y3	2.492	1.433 to 3.550	Yes	****
Y1 vs. Y4	-0.1613	-1.220 to 0.8973	No	ns
Y1 vs. Y5	0.4694	-0.5892 to 1.528	No	ns
Y2 vs. Y3	0.8388	-0.2198 to 1.897	No	ns
Y2 vs. Y4	-1.814	-2.873 to -0.7554	Yes	***
Y2 vs. Y5	-1.183	-2.242 to -0.1248	Yes	*
Y3 vs. Y4	-2.653	-3.711 to -1.594	Yes	****
Y3 vs. Y5	-2.022	-3.081 to -0.9636	Yes	****
Y4 vs. Y5	0.6307	-0.4280 to 1.689	No	ns
Rsw				
Y1 vs. Y2	0.1118	-0.9468 to 1.170	No	ns
Y1 vs. Y3	0.1011	-0.9575 to 1.160	No	ns
Y1 vs. Y4	0.9234	-0.1352 to 1.982	No	ns
Y1 vs. Y5	1.283	0.2247 to 2.342	Yes	*
Y2 vs. Y3	-0.01067	-1.069 to 1.048	No	ns
Y2 vs. Y4	0.8116	-0.2470 to 1.870	No	ns

Y2 vs. Y5	1.171	0.1129 to 2.230	Yes	*
Y3 vs. Y4	0.8222	-0.2364 to 1.881	No	ns
Y3 vs. Y5	1.182	0.1236 to 2.241	Yes	*
Y4 vs. Y5	0.3599	-0.6987 to 1.419	No	ns

A7.4. Tukey's multiple comparisons on SSF ethanol production from seaweeds

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Gsw				
Y1 vs. Y2	1.292	0.3521 to 2.233	Yes	**
Y1 vs. Y3	0.4142	-0.5261 to 1.355	No	ns
Y1 vs. Y4	0.2216	-0.7187 to 1.162	No	ns
Y1 vs. Y5	0.04372	-0.8966 to 0.9840	No	ns
Y2 vs. Y3	-0.8782	-1.818 to 0.06212	No	ns
Y2 vs. Y4	-1.071	-2.011 to -0.1305	Yes	*
Y2 vs. Y5	-1.249	-2.189 to -0.3084	Yes	**
Y3 vs. Y4	-0.1926	-1.133 to 0.7477	No	ns
Y3 vs. Y5	-0.3705	-1.311 to 0.5698	No	ns
Y4 vs. Y5	-0.1779	-1.118 to 0.7624	No	ns
Bsw				
Y1 vs. Y2	1.063	0.1229 to 2.003	Yes	*
Y1 vs. Y3	-0.692	-1.632 to 0.2483	No	ns
Y1 vs. Y4	0.6865	-0.2538 to 1.627	No	ns
Y1 vs. Y5	-0.6878	-1.739 to 0.3634	No	ns
Y2 vs. Y3	-1.755	-2.695 to -0.8148	Yes	****

Y2 vs. Y4	-0.3767	-1.317 to 0.5636	No	ns
Y2 vs. Y5	-1.751	-2.802 to -0.6997	Yes	***
Y3 vs. Y4	1.378	0.4381 to 2.319	Yes	**
Y3 vs. Y5	0.004149	-1.047 to 1.055	No	ns
Y4 vs. Y5	-1.374	-2.426 to -0.3230	Yes	**
Rsw				
Y1 vs. Y2	0.4147	-0.5256 to 1.355	No	ns
Y1 vs. Y3	-0.7088	-1.649 to 0.2315	No	ns
Y1 vs. Y4	-0.4666	-1.407 to 0.4737	No	ns
Y1 vs. Y5	-0.4951	-1.435 to 0.4452	No	ns
Y2 vs. Y3	-1.123	-2.064 to -0.1832	Yes	*
Y2 vs. Y4	-0.8813	-1.822 to 0.05901	No	ns
Y2 vs. Y5	-0.9098	-1.850 to 0.03049	No	ns
Y3 vs. Y4	0.2422	-0.6981 to 1.182	No	ns
Y3 vs. Y5	0.2137	-0.7266 to 1.154	No	ns
Y4 vs. Y5	-0.02852	-0.9688 to 0.9118	No	ns

A8. Statistical Analysis for seaweed bioethanol residue composition

A8.1. One-way ANOVA on Composition of Seaweed bioethanol residue (Total solids)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	61.23	2	30.61	F (2, 6) = 399.2	P < 0.0001
Residual (within columns)	0.4602	6	0.07669		
Total	61.69	8			

A8.2. Tukey's HSD test on Composition of Seaweed bioethanol residue (Total solids)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	1.958	1.265 to 2.652	Yes	***
U. fasciata vs. H. dentata	-4.287	-4.981 to -3.594	Yes	****
S. vulgare vs. H. dentata	-6.246	-6.939 to -5.552	Yes	****

A8.3. One-way ANOVA on Composition of Seaweed bioethanol residue (Volatile solids)

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.08525	2	0.04263	F (2, 6) = 0.02058	P = 0.9797
Residual (within columns)	12.42	6	2.071		
Total	12.51	8			

A8.4. Tukey's HSD test on Composition of Seaweed bioethanol residue (Volatile solids)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	0.06035	-3.545 to 3.665	No	ns
U. fasciata vs. H. dentata	-0.1696	-3.775 to 3.436	No	ns
S. vulgare vs. H. dentata	-0.2299	-3.835 to 3.375	No	ns

A9. Statistical Analysis for MFC operations

A9.1. One-way ANOVA on MFC inoculation power densities

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	10265	3	3421	F (3, 8) = 984.6	P < 0.0001
Residual (within columns)	278	8	34.75		
Total	10293	11			

A9.2. Tukey's HSD test on MFC inoculation power densities

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
R1 vs. R2	246.9	231.5 to 262.3	Yes	****
R1 vs. R3	48.64	33.23 to 64.06	Yes	****
R1 vs. R4	100.7	85.25 to 116.1	Yes	****
R2 vs. R3	-198.3	-213.7 to -182.9	Yes	****
R2 vs. R4	-146.3	-161.7 to -130.8	Yes	****
R3 vs. R4	52.02	36.60 to 67.43	Yes	****

A9.3. One-way ANOVA on MFC operating power densities

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	512275	3	170758	F (3, 8) = 11.33	P = 0.0030
Residual (within columns)	120602	8	15075		
Total	632877	11			

A9.4. Tukey's HSD test on MFC operating power densities

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
R1 vs. R2	-452.5	-773.6 to -131.5	Yes	**
R1 vs. R3	26.66	-294.4 to 347.7	No	ns
R1 vs. R4	43.17	-277.9 to 364.2	No	ns
R2 vs. R3	479.2	158.2 to 800.2	Yes	**
R2 vs. R4	495.7	174.7 to 816.8	Yes	**
R3 vs. R4	16.51	-304.5 to 337.5	No	ns

A9.5. One-way ANOVA on the Ohmic resistance from the MFCs used

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	6360	3		2120 F (3, 8) = 2.103e+006	P < 0.0001
Residual (within columns)	0.008067	8	0.001008		
Total	6360	11			

A9.6. Tukey's HSD test on the Ohmic resistance from the MFCs used

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
R1 vs. R2	44.82	44.74 to 44.90	Yes	****
R1 vs. R3	-17.51	-17.59 to -17.42	Yes	****
R1 vs. R4	0.66	0.5770 to 0.7430	Yes	****
R2 vs. R3	-62.33	-62.41 to -62.24	Yes	****
R2 vs. R4	-44.16	-44.24 to -44.08	Yes	****
R3 vs. R4	18.17	18.08 to 18.25	Yes	****

A9.7. One-way ANOVA on MFC substrate removal efficiencies

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	336.4	3	112.1	F (3, 4) = 1.291	P = 0.3919
Residual (within columns)	347.3	4	86.82		
Total	683.7	7			

A9.8. Tukey's HSD test on the Ohmic resistance from the MFCs used

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
G vs. SA	9.968	-27.96 to 47.90	No	ns
U. fasciata vs. Sodium Acetate				

U. fasciata vs. S. vulgare	8.293	-29.64 to 46.23	No	ns
U. fasciata vs. H. dentata	-6.090	-44.02 to 31.84	No	ns
Sodium Acetate *vs. S. vulgare*	-1.675	-39.61 to 36.26	No	ns
Sodium Acetate *vs. H. dentata*	-16.06	-53.99 to 21.87	No	ns
S. vulgare vs. H. dentata	-14.38	-52.32 to 23.55	No	ns

A9.9. One-way ANOVA on MFC coulombic efficiencies

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	9.065	3	3.022	F (3, 4) = 1.902	P = 0.2706
Residual (within columns)	6.353	4	1.588		
Total	15.42	7			

A9.10. Tukey's HSD test on MFC coulombic efficiencies

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
G vs. SA	-0.5116	-5.642 to 4.619	No	ns
U. fasciata vs. S. vulgare	-2.299	-7.430 to 2.831	No	ns
U. fasciata vs. H. dentata	0.5315	-4.599 to 5.662	No	ns
SA vs. B	-1.788	-6.918 to 3.343	No	ns
SA vs. R	1.043	-4.087 to 6.174	No	ns
S. vulgare vs. H. dentata	2.831	-2.300 to 7.961	No	ns

A10. Statistical Analysis for MFC effluent analysis

Table. One-way ANOVA on MFC effluent total nitrogen

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	309.3	2	154.7	F (2, 6) = 737.2	P < 0.0001
Residual (within columns)	1.259	6	0.2098		
Total	310.6	8			

A10.1. Tukey's HSD test on MFC effluent total nitrogen

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	12.34	11.20 to 13.49	Yes	****
U. fasciata vs. H. dentata	12.53	11.38 to 13.68	Yes	****
S. vulgare vs. H. dentata	0.1855	-0.9620 to 1.333	No	ns

A10.2. One-way ANOVA on MFC effluent total phosphorus

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value

Treatment (between columns)	0.07959	2	0.03980	F (2, 6) = 8.932	P = 0.0159
Residual (within columns)	0.02673	6	0.004455		
Total	0.1063	8			

A10.3. Tukey's HSD test on MFC effluent total phosphorus

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	0.1703	0.003122 to 0.3376	Yes	*
U. fasciata vs. H. dentata	0.2195	0.05224 to 0.3867	Yes	*
S. vulgare vs. H. dentata	0.04912	-0.1181 to 0.2163	No	ns

A10.4. One-way ANOVA on MFC effluent total ammonia

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	460.4	2	230.2	F (2, 6) = 737.2	P < 0.0001
Residual (within columns)	1.874	6	0.3123		
Total	462.3	8			

A10.5. Tukey's HSD test on MF
tot al ammonia

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	15.06	13.66 to 16.46	Yes	****
U. fasciata vs. H. dentata	15.28	13.88 to 16.68	Yes	****
S. vulgare vs. H. dentata	0.2263	-1.174 to 1.626	No	ns

A11. Statistical Analysis for seaweed substrate utilization

A11.1. One-way ANOVA on Substrate utilization for bioethanol

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	172.5	2	86.23	F (2, 6) = 116.4	P < 0.0001
Residual (within columns)	4.445	6	0.7408		
Total	176.9	8			

A11.2. Tukey's HSD test on Substrate utilization for bioethanol

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	6.836	4.680 to 8.992	Yes	***

U. fasciata vs. H. dentata	-3.736	-5.893 to -1.580	Yes	**
S. vulgare vs. H. dentata	-10.57	-12.73 to -8.416	Yes	****

A11.3. One-way ANOVA on Substrate utilization for bioelectricity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	489.4	2	244.7	F (2, 6) = 1160	P < 0.0001
Residual (within columns)	1.266	6	0.2110		
Total	490.7	8			

A11.4. Tukey's HSD test on Substrate utilization for bioelectricity

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	6.198	5.048 to 7.349	Yes	****
U. fasciata vs. H. dentata	17.79	16.64 to 18.94	Yes	****
S. vulgare vs. H. dentata	11.59	10.44 to 12.74	Yes	****

A11.5. One-way ANOVA on waste generation

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	368.5	2	184.3	F (2, 6) = 1045	P < 0.0001
Residual (within columns)	1.058	6	0.1763		
Total	369.6	8			

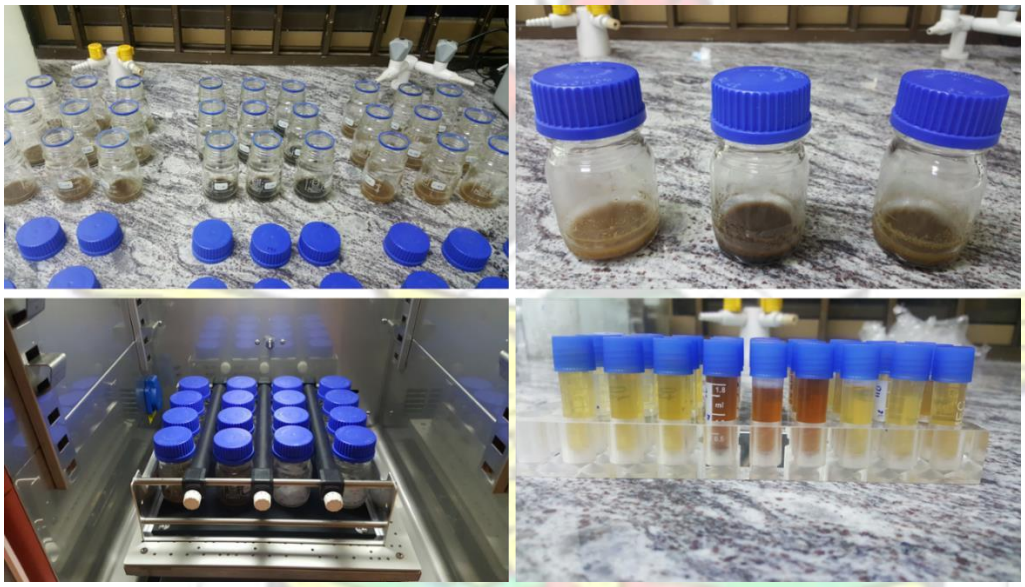
A11.6. Tukey's HSD test on waste generation

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
U. fasciata vs. S. vulgare	-13.03	-14.09 to 11.98	Yes	****
U. fasciata vs. H. dentata	-14.06	-15.11 to -13.00	Yes	****
S. vulgare vs. H. dentata	-1.022	-2.074 to 0.03030	No	ns

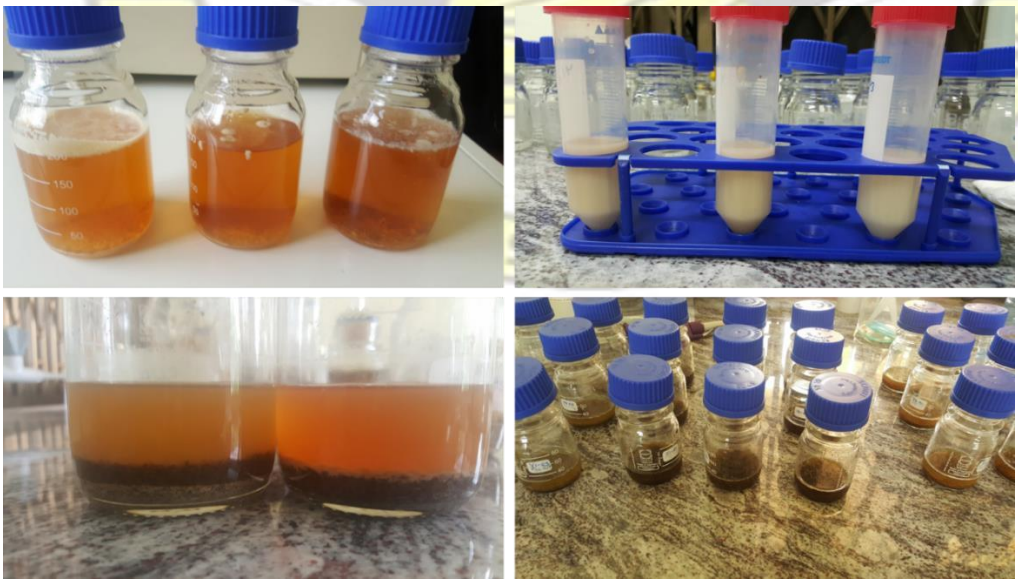
A12. Pictures of Seaweed bioethanol and bioelectricity production processes

A12.1. Mumford beach where *U. fasciata* and *H. dentata* seaweeds were harvested

A12.2. Drying of freshly harvested seaweeds



A12.3. Pretreatment and hydrolysis of seaweeds



A12.4. Yeast culturing and seaweed fermentation

A12.5. Set-up of the seaweed-fed microbial fuel cells

A13. Life cycle inventories for seaweed bioenergy production

A13.1. Full LCA inventory for seaweed cultivation

Process	Value	Unit
Preparation for cultivation		
Wet weight of biomass thalli to be planted	18000	kg
Shed area (for one hectare of cultivation)	20	m_2
Lighting density	14	W/m_2
Number of days required to prepare one hectare	10	days
Number of working hours per day for Preparation	8	h
Number of preparation times per year (Once every 4 years)	0.25	times/y
Energy from lighting	5.6	kWh
Cultivation		

Number of days it takes a Diver to plant 1 hectare	4	days
Diesel consumption per day (Using a Skiff/ Barge)	30	L
Diesel consumption for preparation	120	L
Diesel consumption per year (cultivation is once every 4 years)	30	L
Calorific value of diesel	38.6	MJ/m^3
Energy from diesel	1.158	MJ
Harvesting		
Number of days it takes to harvest 1 hectare	3	days
Diesel consumption per day (Using a Skiff/ Barge)	30	L
Total diesel consumption for harvesting	90	L
Seaweed harvest yield per hectare per year	40.55	kg/ha/y
Number of harvests per year	1	
Wet-weight of seaweeds harvested	72990	kg
	0	
Moisture content of wet seaweed harvested	0.9	
Energy from diesel	3.474	MJ

A13.2. Full LCA inventory for seaweed pre-processing after harvesting

Process	**Value**	**Unit**
Drying		
Assumed distance from drying beds to Plant gate	1	km
Moisture content of dried seaweeds	0.15	
Mass of seaweed dried	85870.588	kg
Estimated fuel consumption of a 40t truck	0.015	kg/t.km
Number trips per day	3	
Mass of diesel consumed in transporting dried SWs to Plant	3.8642	kg
Calorific value of diesel	45.5	MJ/kg
Energy from diesel	175.820	MJ
Grinding		t/h
Throughput of Grinding attritor (Q-100 from Union process)	32.7	
Average Power requirement of the Grinder	93.2	kW
Energy consumption of the Grinder	244.744	kWh
Conveyor belt Specifications (Grinder to Hydrolyser)		
Assumed distance between Grinder and Hydrolyser	2	m
Belt width	500	mm

Angle of surcharge (on a flat roller)	5	o
Fixed coefficient of resistance	2.1	
Passive coefficient of resistance	1	
Coefficient of friction for internal rotating parts	0.016	
Belt weight per linear meter	3.45	kg/m
Weight of lower rotating parts	1.2	kg/m
Weight of upper rotating parts	3.09	kg/m
Weight of conveyed material	3.5	kg/m
Height change	3	m
Tangential force of the Conveyor belt	11.269	N
Belt velocity	1	m/s
Efficiency of reduction gear	0.86	
Belt driving Power	0.131	kW
Load volume of the conveyor for the belt size	12.6	m^3/h
Bulk density of dried seaweed	1000	kg/m^3
Energy consumption of the conveyor	0.893	kWh

A13.3. Full LCA inventory for Processing Scenario 1 (Bioethanol production only)

Process	Value	Unit
Hydrolysis (Assuming no pretreatment is required)		
Volume of dried seaweed to be used (bulk volume)	85.871	m3
Volume of enzyme to be added (Cellulase assuming 8FPU/g dry biomass from 25FPU/ml stock)	23.357	m3
Total volume of slurry for Hydrolysis	1500.417	m3
Volume reactor (80% reaction volume)	1800.500	m3
Number of days required to process feed	60	days
Volume of reactor (To Process feed in 60 days)	30.008	m3
Volume of slurry to be processed per day	25.007	m3
Height to diameter ratio	2	
Diameter of reactor	2.673	m
Height of reactor	5.347	m
Hydrolyser Agitator Specifications		
Diameter of agitator	0.891	m
Rotational speed of the agitator	150	rpm
Power Number for narrow blade high efficiency impeller	0.27	
Density of slurry (density of water)	1000	kg/m^3
Power of the agitator	2.370	kW
Reaction time for hydrolysis	24	h

Energy consumption of agitator	56.884	kWh
Energy consumption of agitator *Per hectare of biomass harvest	3413.061	kWh

Hydrolysate Pumping (from Hydrolyser to Fermenter)

Flow rate	0.069	m^3 /s
Specific gravity	9.81	m/s2
Head	6.347	m
Power consumption for fluid pumping	5362.083	W
Pumping time	0.101	h
Energy consumption by Pump	0.541	kWh
Energy consumption by Pump *Per hectare	32.436	kWh

Fermentation

Energy consumption of agitator (Assuming the same as hydrolyser)	56.884	kWh
Energy consumption of agitator *Per hectare of biomass harvest	3413.061	kWh

Fermentation broth Pumping (from Fermenter to Distillation column)

Flow rate	0.069	m^3/s
Head (Assuming the same reactor dimensions for Hydrolyser)	6.347	m
Power consumption for fluid pumping	5362.083	W
Pumping time	0.101	h
Energy consumption by Pump	2.899	kWh
Energy consumption by Pump *Per hectare	173.923	kWh

Distillation and Dehydration

The system is assumed to composed of Vapour compression steam stripping- unit with Heat exchange, Vapour compression distillation unit and a Molecular sieve adsorption unit		
Electricity needed for Distilling and Dehydrating	32.33	kWh using (per ton dry Seaweed)
Heat needed for Distilling and Dehydrating using (per	0.43	GJ ton dry Seaweed)
Energy consumption by Compression (60days/ Full usage)	2359.767	kWh hectare

Total heat energy required for Distillation &Dehydration (60days/ Full hectare usage)	31.386	GJ

Product Pumping (from Condenser to Storage)

Mass of Ethanol produced (Assuming 99.7% recovery) per Hectare (60 days)		kg
Density of ethanol (at 20 °C)	789	kg/m^3
Volume of ethanol produced	4.612	m_3
Flow rate	0.069	m^3/s
Head	2	m
Power consumption for fluid pumping	1689.7725	W
Pumping time	0.019	h
Energy consumption by Pump	0.031	kWh

Bottoms Pumping (from Distillation bottoms to End-unit)

Volume of bottoms (Stillage/ Vinasse)	1495.805	m_3
Flow rate	0.069	m^3 /s
Head (Assuming the same reactor dimensions for Hydrolyser)	4	m
Power consumption for fluid pumping	3379.545	W
Pumping time	6.030	h
Energy consumption by Pump	5.661	kWh
	9649.177	
Total electrical energy required for bioethanol production		kWh
Total heat energy required for bioethanol production	31.386	GJ
Calorific value of Ethanol	29.7	MJ/kg
Energy in the ethanol produced	108064.98	MJ
	0	
Total energy required for bioethanol production	66.303	GJ

0.05

Ethanol yield (Dry basis) 3638.552 A13.4. Full LCA inventory for Processing Scenario 2 (Bioethanol and Bioelectricity Production)

Process	Value	Unit
Bioethanol Production		
Total energy required for bioethanol production (same as scenario 1)	66.303	GJ
Substrate oxidation *(No energy input required) Mass of hexose sugar in seaweeds (Assuming 10%)	8587.059	kg

Initial mass of substrate to MFCs (Assuming 100% hexose conv. to Ethanol)	77283.529	kg
Substrate removal efficiency	0.99	
Final mass of substrate from MFCs	772.835	kg
Coulombic efficiency	0.14	
Power generation per kg substrate	3.094	W/kg
Power generation from MFCs (Based on Power gen per kg substrate)	33141.372	W
MFC runtime (Assuming 60 days of operation)	1440	h
Total Power from MFCs	47723.576	kWh
Effluent treatment		
Volume of residue from MFCs	1419.294	m3
Flow rate	0.0689	m^3 /s
Head	2	
Power consumption for fluid pumping	1689.773	W
Pumping time	5.722	h
Energy consumption by Pump	9.669	kWh

A13.5. Full LCA inventory for Processing Scenario 3 (Bioethanol and Biogas production)

Process	Value	Unit
Bioethanol Production		
Total energy required for bioethanol production (same as scenario 1)	66.303	GJ
Anaerobic digestion		kW/m^3
Power requirement for mixing $1m^3$ of digester volume	0.008	
Influent volume (per hectare)	1495.805	m3
Retention time	38	day
Mixing time per day	24	h
Energy for mixing	10913.395	kWh
Influent mass (including water)	1495805.189	kg
Operating temperature for digestion	37	ºC
Influent temperature (ambient 28-32 ºC)	28	ºC
Heat capacity of influent	4.129	
Energy required to heat the sludge	15439.324	kWh
Initial mass of substrate to Digestor	77283.529	kg
Volatile solids content of SW residue	0.83	

Substrate removal efficiency	0.94	
Biogas yield	492.7	L/kg VS
Volume of Biogas produced	29708139.57	L
Calorific value of Biogas with 63% CH_4	22.73	MJ/m^3
Energy generation from Biogas generated (assuming 45% CHP conversion efficiency)	303869.706	MJ

Effluent treatment

Volume of residue from Digestor	1423.159	m3
Flow rate	0.069	m^3/s
Head (Assuming the same reactor dimensions for Hydrolyser)	2	m
Power consumption for fluid pumping	1689.773	W
Pumping time	5.738	h
Energy consumption by Pump	9.695	kWh