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Biogas Production from *Ecklonia Maxima* Using an Anaerobic Digestion Process: Investigation, Analysis and Optimisation

by

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DECLARATION

I, Ashleen Marshall, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

15 November 2023

Signed **Date**

ABSTRACT

South Africa is in the grip of an energy crisis. The SA government is unable to provide a consistent supply of energy, implementing loadshedding as a coping measure, while alternate energy sources are being investigated and electrical power units upgraded. Worldwide, there is significant research conducted on the use of land-based crops as feedstocks for biofuel production. The use of these crops are often limited by the food vs. fuel dilemma. As a result, waste products and algal products are being considered as alternative sources for feedstocks for energy production.

The research presented investigates the use of brown macroalgae, Ecklonia Maxima, which is widely available in South Africa, as a potential feedstock for biogas production. The process utilised is anaerobic digestion (AD), which is able to utilise a wet feedstock, therefore removing the additional drying costs required from other processes. Four inocula were investigated at both mesophilic (□C) and thermophilic (□C) temperatures. These included two synthetic inocula as well as an organic inoculum and a starter fluid from the wastewater treatment plant. Results show that at mesophilic temperatures, the synthetic inoculum, BioGanic, shows the highest biomethane yield (40.5 ml/g VS). The highest overall yield when comparing the mesophilic and thermophilic yields was obtained at thermophilic temperatures.

The BioGanic/Ecklonia Maxima system was subjected to a series of pre-treatment processes to investigate if the biomethane yield could be improved upon. Mechanical, chemical and microwave pre-treatment was applied at both mesophilic and thermophilic temperatures. Size reduction of the Ecklonia Maxima was applied first. Results show that biomethane yield increased in the mesophilic range from 31.5 ml/g VS using raw seaweed to 126 ml/g VS. Biomethane yield decreased in the thermophilic range from 222.6 ml/g VS using raw seaweed to 110.6 ml/g VS using mechanically pre-treated seaweed. The increase in yield in the mesophilic range using pre-treated seaweed was less than the yield obtained for the thermophilic range of the raw seaweed.

Chemical pre-treatment using 0.15M HCl showed improved biomethane yield (60.3 ml/g VS) when compared to raw seaweed at mesophilic temperatures. The thermophilic range recorded a decrease in biomethane yield when compared to the raw seaweed. Biomethane yield was 70.3 ml/g VS using 0.3M HCl at thermophilic conditions. Alkaline pre-treatment using NaOH at two different concentrations gave mixed results. The biomethane yield was slightly lower than the acid pre-treatment at 59.8 ml/g VS, and slightly higher for the thermophilic range at 72.5 ml/g VS when compared to the acid pre-treated seaweed.

Microwave pre-treatment of Ecklonia Maxima saw similar results for both the mesophilic and thermophilic ranges at biomethane yields of 57.8 ml/g VS and 56 ml/g VS, respectively.

Kinetic modelling indicated that the first order modified Gompertz equation was a good fit for the raw seaweed and pre-treated seaweed data, which allowed for the determination of the maximum methane yield for the batch system. It also allows for the prediction of the performance of the seaweed in biomethane production.

A techno-economic study with two case studies proved that seaweed biomaterial could produce biomethane, which could then be converted to electricity using a CHP unit. The levelised cost of electricity of the seaweed was determined at R5.65/kWh. Replacing the existing feedstock with seaweed was more expensive. The cost of the seaweed feedstock is the over-riding factor in determining system feasibility.

Keywords: Seaweed; *Ecklonia Maxima,* biomethane, biogas, anaerobic digestion.

DEDICATION

For my brother Paul looking down on me, who always encouraged me to chase my passions.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

AD	Anaerobic digestion
ANOVA	One way analysis of variance
BESC	Break-even electricity selling costs
BI	Biodegradability Index
CAPEX	Capital expenses
CHNS	Carbon - Hydrogen - Nitrogen - Sulphur analysis
CHP	Combined heat and power engine
COD	Chemical oxygen demand
EDS	Energy dispersive spectroscopy
EIOR	Energy return on investment
HHV	Higher heating value
HRT	Hydraulic retention time
IRR	Internal rate of return
LCOE	Localised cost of electricity
LHV	Lower heating value
NPV	Net present value
OPEX	Operational expenses
ORC	Organic Rankine Cycle
ROI	Return on investment
SBA	Specific biological activity
SEM	Scanning electron microscope photographs
SHF	Separate hydrolysis and fermentation
SRT	Solids retention time
SSF	Simultaneous saccharification and fermentation
TVS	Total volatile solids
VAT	Value Added Tax
VOLR	Volumetric organic loading rate

CHAPTER ONE: INTRODUCTION

1.1 Background

Carbon dioxide emissions from burning fossil fuels contribute to the global warming problem. The emission rate increased 16-fold between 1900 and 2008 and approximately 1.5 times between 1990 and 2008 alone (Soleymani & Rosentrater, 2017). The usage of alternate energy, using biofuels, is on the increase in an attempt to curb carbon dioxide emissions (Aizawa et al., 2007). Other factors influencing the need for an alternative renewable and sustainable energy source include climate change due to global warming, increasing crude oil prices, energy security, and limiting fossil fuel resources (Ghadiryanfar et al., 2016).

There is significant attention on the production of biofuels as an alternate fuel source. They are divided into three broad categories: first-generation, second-generation and third-generation. First-generation biofuels are the most common, including ethanol, biodiesel, and pure plant oil. The feedstocks for these biofuels include sugarcane, corn, soybean, potato, wheat, and sugar beet. Second-generation biofuels are also known as advanced biofuels. The feedstocks include lignocellulose biomasses and agricultural wastes that do not directly compete with human food resources. Third-generation biofuels include biofuels, which do not directly compete with land-based foods or crops as they are grown in water (Ghadiryanfar et al., 2016). The feedstocks for these biofuels include macroalgae and microalgae (Sudhakar, et al., 2018).

There has been considerable research into the use of land-based crops for energy. They are, however, limited by the increasing demands for food products, making less land available for the cultivation of energy crops. Using macroalgae as biomass for energy systems can significantly contribute to the worldwide supply of cleaner energy (Dave et al., 2013) while reducing the food vs. fuel argument (Soleymani & Rosentrater, 2017).

Algae are a diverse range of aquatic plants consisting of unicellular and multicellular forms. It contains chlorophyll but no natural stems and roots. Algae grow in brackish or salt water and do not compete with land-based crops for cultivation. This means algae do not require land or freshwater for food production (Soleymani & Rosentrater, 2017). Biofuels derived from algae are therefore known as third-generation biofuels as they do not directly compete with land-based fruits or crops as they grow in water (Ghadiryanfar et al., 2016). *Ecklonia Maxima* seaweed, found abundantly along the West Coast of the Western Cape in South Africa, could offer this alternative.

Ecklonia Maxima is a brown seaweed lacking lignin and contains low amounts of cellulose, making it more straightforward to convert microbially to biofuels than terrestrial plants (Adams et al., 2011). *Ecklonia Maxima* also has the advantage of higher growth rates than most terrestrial plants, which makes them a more readily available resource for biofuel production with minimal environmental impact (Ross et al., 2008).

One process for producing biofuels from algae is anaerobic digestion (AD). Anaerobic digestion is a biological process that occurs due to microorganisms degrading organic matter in the absence of oxygen to form biogas. The primary products are methane and carbon dioxide. The biogas can be used to run combined heat and power (CHP) engines or compressed and used as transport.

There are currently no existing economically viable commercial systems producing biofuel from seaweed. The typical research focus has been on using microalgae, which are small, microscopic aquatic photosynthetic plants, instead of macroalgae, which are large aquatic photosynthetic plants, in the anaerobic digestion process (Milledge et al., 2014).

The current study aims to investigate the use of *Ecklonia Maxima* in biogas production and the optimisation and cost of up-scaling the process to a commercial system, considering energy input and the cost of biogas production. A suitable South African Inoculum will be identified and optimised for maximum biogas production.

1.2 Problem statement

Current energy-producing methods strain natural resources and negatively affect the environment due to high carbon emissions, which contribute to global warming. Most 1st and 2nd generation biofuels use feedstock from land-based food sources, exacerbating the fuel vs. food argument. The cost of producing these biofuels is further hampered due to the need for additional water for growing in an already water-scarce country.

The latest energy sector reports for South Africa indicate that only 11% of energy produced is from renewable resources, while 65% is still produced using coal. With the continual load-shedding, implemented for the first time in 2007 and set to continue for the foreseeable future, as well as the dependence of the SA energy sector on coal, a sustainable renewable energy source is required to ensure a stable energy supply.

Seaweed, or macroalgae, is a water-grown biomass that can be grown without added nutrients, pesticides, or using agricultural land. Seaweed can improve the water quality in which it is grown as the seaweed can oxygenate the water while utilising the ammonia excreted by surrounding fish. A very fast growth rate is expected as seaweed has a higher photosynthesis efficiency than land plants (Ghadiryanfar et al., 2016).

Alternative energy sources are actively sought, which will not compete with terrestrial plants and resources and have a net positive carbon intensity effect on the environment. Using *Ecklonia Maxima* to produce biogas could be a viable option.

1.3 Research question

Can *Ecklonia Maxima* be utilised as an alternate and viable bio-resource for biogas production in South Africa?

1.4 Hypothesis

Ecklonia Maxima can produce biogas as an alternate energy source via the anaerobic digestion process.

1.5 Research aim

The study aims to investigate and optimise the anaerobic digestion process for producing biogas from *Ecklonia Maxima* for possible commercial use.

1.6 Research objectives

The objectives are to:

i. Identify a suitable inoculum for the anaerobic digestion process.
ii. Optimise the production of biogas from seaweed with respect to:
    a. The effect of temperature
        i. Mesophilic temperature (37 °C)
        ii. Thermophilic Temperature (52 °C)
    b. Pre-treatment of the seaweed
        i. Mechanical: Biomaterial size distribution through grinding and maceration
        ii. Chemical: acid and base pre-treatment
        iii. Thermal: microwave treatment
iii. Analyse the kinetics of the anaerobic process.
iv. Complete a techno-economic comparison for commercial biogas production using an anaerobic digestion process.

1.7 Significance of the research

Fossil fuel-derived energy is subjected to emission regulations, carbon taxes, and government and environmental subsidies. Energy derived from biomass is not subjected to these additional costs. Macroalgae could have an associated additional charge due to the drying of the biomaterial, which can be overcome if a technology is utilised that uses wet biomass, like anaerobic digestion. Identifying low-cost macroalgae that can be utilised for energy extraction will significantly reduce the overall costs of the anaerobic digestion process, making it more economically viable.

The macroalgae do not compete with terrestrial land crops and will not impact food security. Although some research has been done on utilising seaweed for bio-energy production, there are no large-scale commercial applications of biogas production from seaweed. Research cites the production of bio-hydrogen and methane using thermal gasification, bioethanol production from fermentation, bio-oil production by pyrolysis, and biodiesel production using trans-esterification. The optimisation of the biogas production process, specifically for the use of macroalgae, will increase the academic pool of knowledge as there is very little research available on this particular process combination using South African biomaterials. It may also produce an alternative biofuel source, which could be utilised as a complementary energy source.

A pilot plant will indicate the feasibility of industrial application and allow for a costing of the process to be completed, which, along with the optimisation parameters, will give a good indication of the upscale value of the designed process while considering the production process's carbon footprint. To propose a successful upscale, the system must be modelled appropriately, increasing the biosorption biofuel research niche's knowledge base. Should the *Ecklonia Maxima* biomass prove to be a viable source for biogas production and be utilised by either industry or government, growth and harvesting opportunities for communities along the West Coast of South Africa could significantly increase, stimulating the economy in those areas.

1.8 Limitations and assumptions

The research is limited to the following:

i. The macroalgae to be investigated is *Ecklonia Maxima*, a brown seaweed indigenous to the West Coast of the Western Cape in South Africa. The rationale for using seaweed is that it is readily available, has a short growth cycle, and is relatively cost-effective as it will be purchased, dried, and milled from a local supplier. Seaweed will, therefore, not be harvested specifically for this research application.

ii. Cultivation methods for the seaweed are excluded from the investigation. The economic evaluation considers only the cost of purchasing dried, milled seaweed, not the seaweed's aquaculture.

iii. The investigation is limited to the temperature range of 30 °C to 60 °C. Based on the literature, the optimum temperature for the mesophilic culture is 34 °C to 37 °C, and the optimum temperature for thermophilic culture is 54 °C to 57 °C. The mesophilic temperature for this study will be 37 ^{o}C, and the thermophilic temperature will be 52 ^{o}C. Psychrophilic temperatures, i.e., operating below 20 °C, will not be considered.

iv. Three inocula will be investigated for the optimisation process. These inocula are selected based on what is available and cost-effective in the surrounding areas of Cape Town and what is easily transportable. A discussion will be held on selecting these inocula and their performance in the anaerobic digestion process.

v. The selection of the best inoculum is based solely on the performance of the inoculum in the formation of biogas.

vi. The techno-economic study will be a paper-based study based on available literature on the AD processes in South Africa and the results of the investigation performed for the anaerobic production of biogas from *Ecklonia Maxima*. Transport and processing costs for the harvesting of the seaweed will not be considered.

1.9 Chapter overview

Chapter One: Introduction

This chapter gives a brief overview of the background to the research problem, along with a discussion on the aim and objectives of the study, listing limitations and assumptions made to delineate the research. The novelty of the research conducted is discussed, and details of the significance of the research to academia and external stakeholders, including industry, government, and society, are outlined.

Chapter Two: Literature review

An in-depth analysis is done of what is currently known on the subject of biogas production using anaerobic digestion and other processes, primarily concentrating on literature produced by experts in the field over the last 15 years. Particular emphasis is placed on the use of algae for biogas production. Also included is an overview of gaps in the research and possible research areas that need to be developed and investigated further.

Chapter Three: Methodology

This chapter gives an overview of the anaerobic digestion process and the equipment and methods utilised to investigate each research objective. An overview of the analytical methods utilised, laboratory analysis performed, as well as the data manipulation and calculations performed for each objective is given.

Chapter Four: Results and discussion

This Chapter gives an overview of the results and a discussion of the first three objectives.

- **Objective 1: Identification of a suitable inoculum –** The process followed to identify a suitable inoculum is discussed. The experimental results obtained are listed, analysed, and discussed. A determination is made on the way forward for subsequent experimentation utilising the best-performing inoculum, considering the assumptions and limitations stated above.
- **Objective 2: Optimisation of biogas production –** This section investigates aspects of the process to determine whether they have any significant impact on the production of biogas. The first is the effect of temperature on the AD process, where the mesophilic and thermophilic regions are investigated. The effect of pre-treatment of the *Ecklonia Maxima* seaweed utilised in the AD process is investigated. Chemical, thermal, and physical methods are investigated. Analyses are carried out on whether a single or combination of these treatment processes will increase biogas production.
- **Objective 3: Kinetics of the anaerobic process –** Based on the results obtained, the kinetics of the system is determined. The determination is based on the most effective inoculum/seaweed combination. Curve fitting of the experimental data is performed to predict the AD process's performance for biogas production.

Chapter Five: Techno-economic evaluation of the AD process for biogas production

This chapter focuses on the techno-economic results comparing replacing the feedstock with seaweed on two farms.

Chapter Six: Conclusion

This chapter gives an overview of the general conclusions and recommendations of the current study.

Bibliography

A listing of all the resources utilised in the study.

Appendices

Detailed supplemental material is used in the study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Some of our society’s most significant challenges include energy security, increasing oil prices, climate change, and resource depletion. Global environment quality has spurred the search for renewable energy research. Fossil fuels are associated with toxic compounds released during combustion, including oxides of nitrogen and sulphur, which eventually lead to the much bigger problem of acid rain (Chynoweth et al., 2001). To mitigate these challenges, alternate renewable energy sources are actively being sourced. Renewable energy is generated from natural resources like agriculture, forestry, and aquatic sources. Technologies include solar power, wind power, hydroelectricity, and biomass and biofuels for transportation fuel.

Biomass is a renewable organic matter and can include biological matter derived from living or recently living organisms and waste. It is a plant matter formed by photosynthetic solar energy capture, stored as chemical energy (Gunaseelan, 1997). Seaweeds, also known as macroalgae, which are water-grown biomass, can be grown without added nutrients or pesticides and without using agricultural land. Seaweed can improve the water quality in which it is grown as the seaweed can oxygenate the water while utilising the ammonia excreted by surrounding fish. A very fast growth rate is expected as seaweed has a higher photosynthesis efficiency than land plants (Ghadiryanfar et al., 2016).

Documented advantages of using seaweed as an alternate energy source include:

- The biomass does not require agricultural land for cultivation (Obata et al., 2020).
- Many species grow in brackish or salt water, so there is no competition with agricultural products for freshwater (Milledge et al., 2014; Obata et al., 2020).
- The potential biomass yield per unit area is often higher than in terrestrial plants, with a seaweed yield of approximately 13.1 kg dry weight/m^2.yr and sugar cane yield of approximately 10 kg dry weight/m^2.yr (Rajkumar et al., 2013; Leu & Boussiba, 2014).
- Seaweed has a fast seed-to-harvest growth rate, with up to eight harvests per year, yielding a 1 to 2 m plant height (Dave et al., 2013).
- Macroalgae are rich in carbohydrates and are therefore suited for the production of biogas (Dave et al., 2013).
- Macroalgae have a low lignin content and are well-suited for biogas production using anaerobic digestion (Dave et al., 2013; Obata et al., 2020).
- Energy products are more sustainable for algae biomass than bio-energy resources based on land-based crops (Florentinus et al., 2014).

Since bioenergy is a renewable energy source that can be stored, it can be utilised as needed (Obata et al., 2020). The major disadvantage is that the production costs for energy from algae biomass are higher than that of energy from fossil fuels and higher than most conventional land-based bio-energy productions (Florentinus et al., 2014; Chynoweth et al., 2001). Fossil fuel-derived energy, however, is subjected to emission regulations, carbon taxes and subsidies. Considering all these additional costs, biomass energy could be cost-competitive (Chynoweth et al., 2001).

Energy derived from biomass can be in the form of heat, steam, electricity, hydrogen, ethanol, methanol, and methane. Biogas can replace fossil fuels in power and heat generation or as gaseous vehicle fuel. Biomethane can also be used as a replacement for natural gas and as a feedstock for producing chemicals. (Weiland, 2010). The derived energy is dependent on the conversion process used as well as the following factors (Chynoweth et al., 2001):

- The need for direct heating or steam generation
- The conversion efficiencies of the biomass utilised
- Costs of hardware and equipment for the conversion process
- Scale-up costs
- The impact on the environment
- Waste processes are required

Ultimately, selecting a conversion technology for biomass depends on the form of energy required (McKendry, 2002).

2.2 Seaweed as a biomaterial

Aquatic and photosynthetic plant-like organisms are known as algae. Algae are the primary producers of the ocean. The ocean, in turn, sequesters approximately 60 times more CO_2 than the atmosphere. According to Sudhakar et al. (2018), the oceans and seas cover 72% of the earth’s surface, which is significant in carbon dioxide sequestration. Tiny unicellular algae that are not visible to the naked eye are called microalgae. Macroalgae are more significant algae, which can be seen with the naked eye. Seaweed is referred to as a macroalgae and is a benthic organism as it lives on the seabed. A typical seaweed structure is given in Figure 2.1.

The seaweed plant consists of the following components:

- *Thallus*: total structure which can photosynthesise.
- *Laminaria (Blades):* The main photosynthetic leaf-like structure that absorbs sunlight.
- *Floats*: Gas-filled bladders are found between the blade and the stipe, which keep the blades near the water surface where more sunlight is available for photosynthesis.

- *Stipe*: Stem-like structure, which can be long and tough to provide support; it is also used to carry sugars from the blades to the rest of the plant.
- *Holdfast*: Specialised root-like structure that holds the plant to the bottom surface of the ocean or rocks but does not aid in gathering nutrients.
- *Frond*: combined part of the blade and stipe.

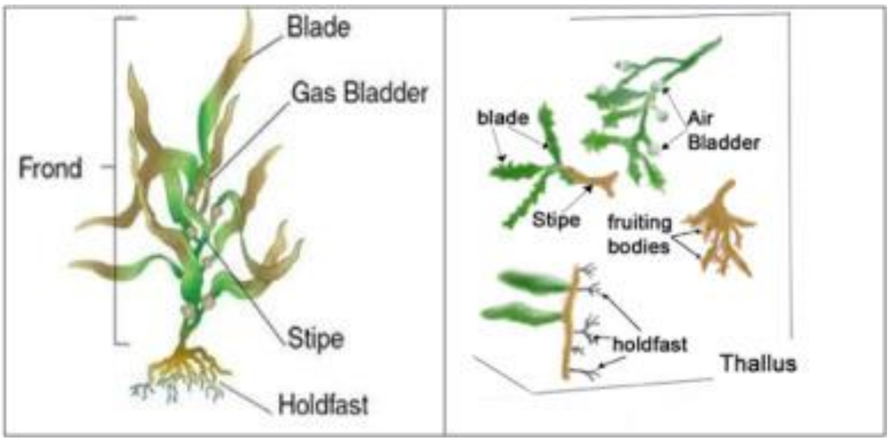

Figure 2.1: Structure of seaweed (Sudhakar et al., 2018)

Seaweeds lack the complex structure of plants. They contain high water content (90% fresh wt.), carbohydrates, protein, and lipids. On a dry weight basis, the composition of seaweed is 25–50% carbohydrates, 7–15% protein and 1–5% lipids. Approximately 9,000 species of seaweed form a stable, multi-layered series of vegetation capable of capturing photons from sunlight (Sudhakar et al*.,* 2018). Seaweeds are broadly characterised as either red, brown, or green according to their photosynthetic pigments. Approximately 10,000 to 12,500 species of macroalgae have been taxonomically classified. Red algae account for approximately 6,000 species and are the most abundant. This is followed by green algae, accounting for 4,500 species, predominantly found in freshwater habitats. Brown algae are the largest and most complex macroalgae, accounting for 2,000 named species (Thompson et al*.,* 2019).

Ecklonia Maxima is a brown seaweed of the order *Laminariales* and the family *Lesseniaceae*. They grow in the cold waters of the southern oceans along the coast of South Africa up to 10 meters tall (Anderson et al., 2016). On a dry weight basis, the composition of brown seaweed is 30–50% carbohydrates, 3–15% protein, and 1–3% lipids, with a mineral content of 7–38% (Sudhakar et al*.,* 2018). *Ecklonia Maxima* are large, floating kelp which form extensive forests on the ocean floor down to 10 m. They have been recorded to grow as large as 17 m by Rothman et al. (2017). It is one of the most prominent members of four *Laminariales*, which grow along the coast of South Africa from Cape Columbine for approximately 1,600 km. In South Africa, *Ecklonia Maxima* is exploited commercially, mainly as a feed for abalone. The juice is also extracted and used as a growth stimulant in the agricultural industry (Rothman et al., 2017).

Brown seaweed consists of water, ash, proteins, and small quantities of lipids and saccharides. Laminarin and mannitol are easily fermentable sugars and fermentation of the alginate is possible with fermenting organisms (Philippsen et al., 2014). The morphology of the *Ecklonia Maxima* does not change along the South African coastline despite significant changes in wind speed and wave height (Rothman et al., 2017). The seaweed can either be whole-harvested, killing the plant, or frond-harvested, keeping the plant alive. There are advantages and disadvantages to harvesting the whole plant or only the fronds. These should be weighed when the environmental impact study is completed (South Africa: Department of Agriculture and Forestry, 2012).

Table 2.1: Advantages and disadvantages of harvesting techniques

Whole plant harvesting	Harvesting the fronds
All fronds and stripe obtained	70% of the fronds are obtained
Kelp plants are killed and will have to be replaced	Kelp plants are trimmed and can regrow
Kelp bed takes two years to recover	Fronds regrow within six months
Harvesting is done by a diver from a boat	No diver is needed for harvesting; done by boat
Yields of fronds are lower in the long term	Yields of frond are higher in the long term

Based on Table 2.1 above, harvesting the fronds alone will have less impact on the oceans and be more sustainable. However, the financial impact that either method may have needs to be considered. The dried seaweed has a rough surface, as shown in the scanning electron microscope (SEM) photographs below in Figure 2.2:

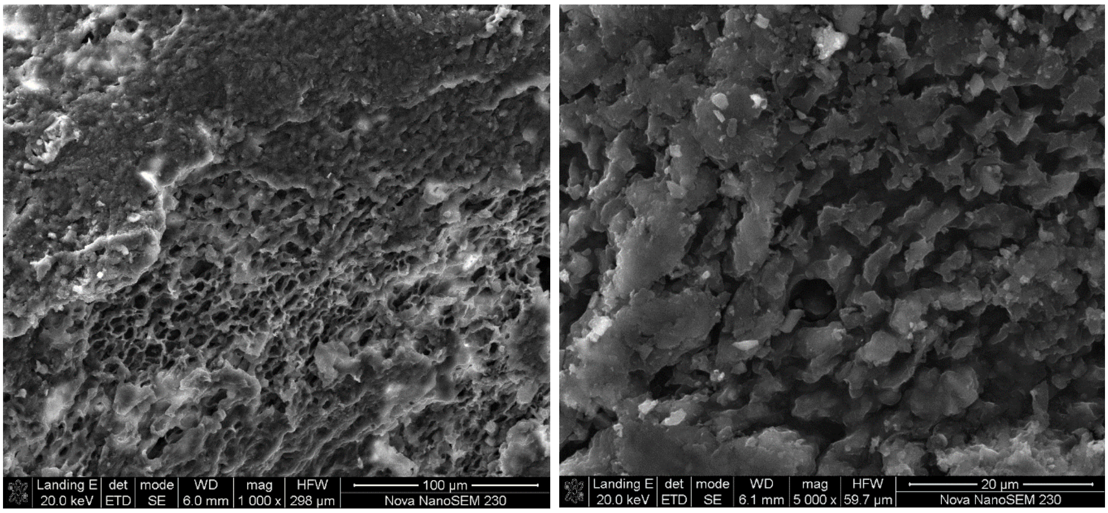

Figure 2.2: Raw *Ecklonia Maxima* SEM images

The chemical composition of macroalgae is significantly different from that of terrestrial plants and varies among macroalgae types. Macroalgae contain carrageenan, mannitol, agaran, laminarin, mannan, ulvan, fucoidan and alginate, which are not present in lignocellulosic or microalgae biomasses (Ghadiryanfar et al., 2016). Gunaseelan (1997) reported that mannitol and alginate were the most biodegradable, and protein and cellulose were the least biodegradable during anaerobic digestion. Table 2.2 shows a comparison of brown macroalgae composition.

Table 2.2: Brown macroalgae composition

		Macroalgae				
Components		*Macrocystis*	*Laminaria*	*L.digitata*	*Ascophyllum nodosum*	[a]*Ecklonia Maxima*
Water		88.2	88	85	67–82	
Dry solids		11.8	12	15	33–18	
Proximate analysis - % dry basis	Proteins	17.3	12	4.63	4.8–9.8	7.8
	Lipids		2	0.53	1.9–4.8	
	Cellulose	5.2	6		3.5–4.6	
	Ash	41.1	26	26.5	18–24	24.3
	Volatile solids	58.9	74			
	Laminarin	0.8	14		1.6–6.6	
	Mannitol	20.2	12		6.8–10.4	4
	Alginate	15.3	23		24–29	13
Elemental composition - % dry basis	C	28	34.6	32.4		
	H	3.92	4.7	4.04		
	O	2.3	31.2	62.3		
	N	1.86	2.4	0.74		4.10
	S	1.09	1	0.52		3.51
	P	0.33	0.35			2.10
	K	0.014	0.0096			5

(Source: Ghadiryanfar et al., 2016)

The lower heating value (LHV) of *L. digitata* is 6.35 MJ/kg, and the higher heating value (HHV) is 17.84 MJ/kg (Dave et al., 2013).

According to Maneein et al. (2018), seaweed harvesting increased by 39% between 2014 and 2016 to 29 million tonne (wet weight). The harvested seaweed was mainly used for human food, animal feed, and the use in the production of hydrocolloids. Seaweed has been used as a food source in China for the last 1,700 years. It can be used for direct human consumption or processed into fertilisers, biofuels, cosmetics, and medicines. The major problem experienced by the seaweed industry includes over-exploitation of marine seaweed and scarcity of high-quality seaweed biomass as the composition changes depending on growth conditions (Sudhakar et al., 2018).

Research has been conducted into producing various types of biofuels from seaweed. These include the production of bio-hydrogen and methane via thermal gasification or anaerobic digestion, bioethanol production by fermentation, bio-oil production by pyrolysis, and biodiesel production by transesterification (Sudhakar et al., 2018).

The large-scale production of biofuel production utilising seaweed depends on the seaweed composition, specifically the percentage of carbohydrates and the ease of carbohydrate conversion. The preferred seaweed would have high carbohydrate content and low ash, water, and cellulose content (Sudhakar et al., 2018). Seaweeds also do not contain significant amounts of lignin, making them easier to convert into biofuel than land-based plants (Ra et al., 2016).

In the revised 2014 report on the Worldwide Potential of Aquatic Biomass, Florentinus et al. (2014) stipulated that seaweed cultivated a maximum of 25 km from the shore, having a water depth of approximately 20 m, showing very rich nutrient availability in a moderate to tropical climate. The average seaweed harvest was 32 tonne per hectare per year, with an energy yield of 6,000 to 7,000 m^3 per hectare per year.

For anaerobic digestion, seaweed with a low polyphenol content is preferred as polyphenols inhibit the growth of the methanogens. It is, therefore, considered the rate-limiting factor in anaerobic digestion (Sudhakar et al., 2018). Methane formation occurs over a narrow pH interval between 6.5 and 8.5, with an optimum pH interval reported by Weiland (2010) as between 7.0 and 8.0.

Seaweed has been used as a food source in China for the last 1700 years. It can be used for direct human consumption or processed into fertilisers, biofuels, cosmetics, and medicines. The major problems experienced by the seaweed industry include over-exploitation of marine seaweed and scarcity of high-quality seaweed biomass, as the composition changes depending on growth conditions (Sudhakar et al., 2018).

2.3 Biofuels

Biofuels are generally classified by source, type, and generation (Figure 2.3). There are primary biofuels and secondary biofuels. Primary biofuels can be used directly in their unprocessed form. These include firewood and agro-residues. Secondary biofuels are processed from biomass. These include charcoal, ethanol, bio-oil, and biodiesel (Sudhakar et al., 2018).

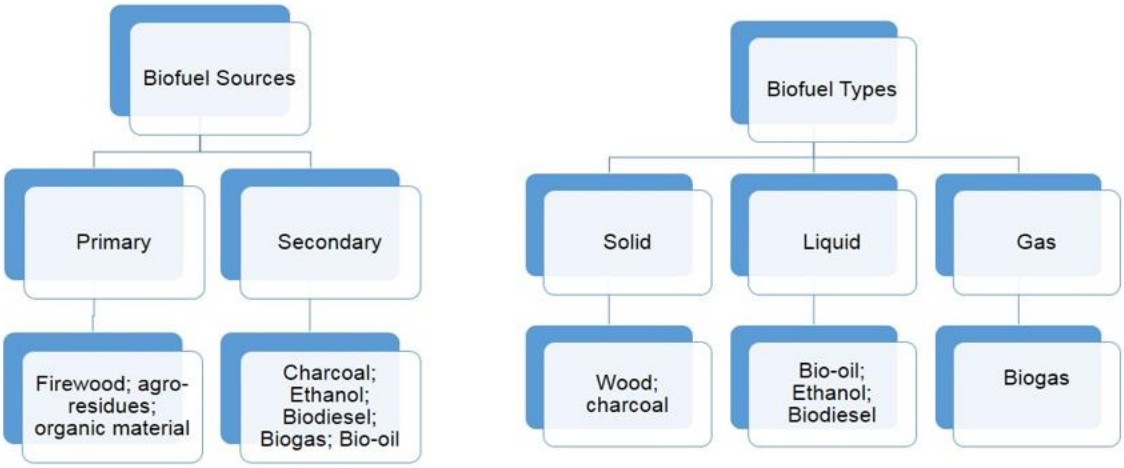

Figure 2.3: Biofuel types and sources (Adapted from Sudhakar et al., 2018)

Biofuels can be classified into first, second, third, and fourth-generation fuels based on the biomass feedstock and the processing technology used (Figure 2.4).

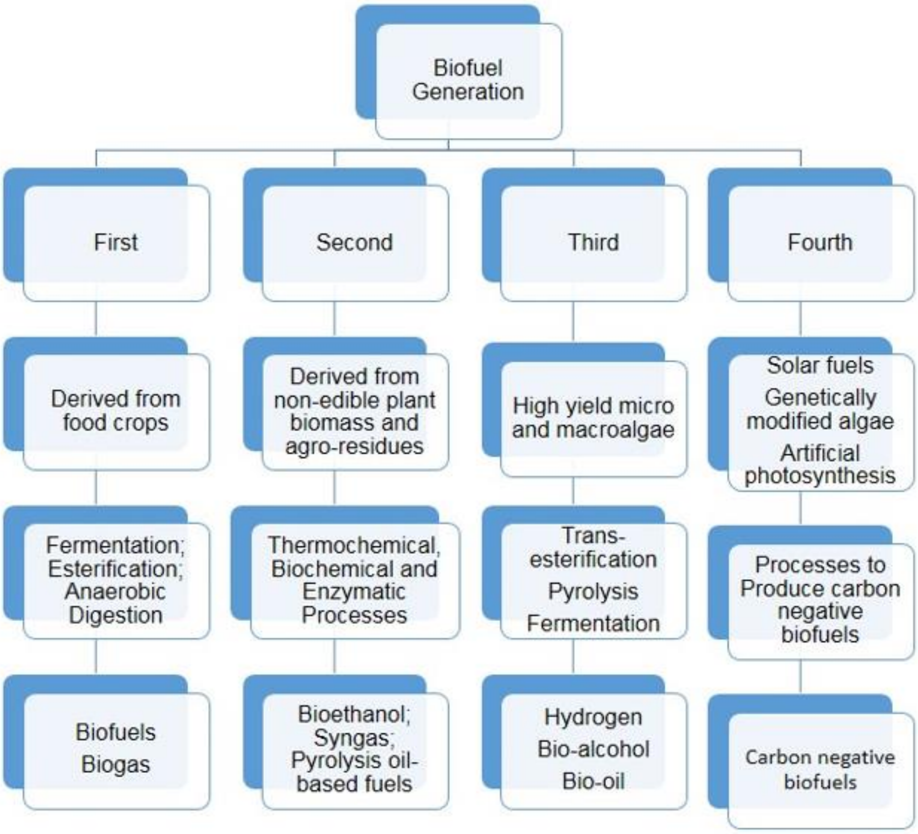

Figure 2.4: Overview of biofuel classification (Adapted from Sudhakar et al., 2018)

First-generation biofuels are derived from food crops such as soybean, corn, maize, and sugar cane. Biofuel production uses well-established processes like fermentation, trans-esterification, and anaerobic digestion (Sudhakar et al., 2018). Vast areas of land are required to produce adequate biofuels to substitute the energy currently obtained from fossil fuels. This could lead to food vs. fuel debates (Carriquiry et al., 2010).

Second-generation biofuels are derived from non-edible plant biomaterials, agro-residues, and non-cellulosic feedstock that grow on marginal land. These include crops like switch grass, straw, Neem (evergreen tree), and Jatropha (flowering plant). Biofuel production uses technologies including thermochemical, biochemical, and enzymatic processes to produce bioethanol, syngas, and pyrolysis oil-based fuels (Sudhakar et al., 2018). Second and subsequent-generation biofuels overcome the food vs. fuel disadvantage. On the other hand, second-generation biofuels have a low net energy yield, added costs of feedstock transportation, and high downstream processing costs, with only a moderate reduction in greenhouse gas. This significantly limits its use (Carriquiry et al., 2010).

Third-generation biofuels are energy sources derived from high-yield microalgae and macroalgae biomass. Hydrogen is produced from algae, and bio-alcohol and bio-oil from macro and microalgae (Sudhakar et al., 2018). Third-generation biofuels are the most promising to combat environmental problems through carbon dioxide sequestering and wastewater management. They do not compete with terrestrial land, have low lignin content, and require less energy input for production (Pfromm et al., 2011). They also have a high biomass yield and are widely available (Sudhakar et al., 2018).

Fourth-generation biofuels are the next generation and produce ultra-clean carbon-negative biofuels. They are expected to be carbon-negative at the raw material and process technology level (Arora et al., 2016). Sources of fourth-generation bioenergy include solar fuels, artificial photosynthesis, genetically modified algae, and flue gases (Sudhakar et al., 2018). Fourth-generation biofuels are aimed to be carbon-negative. They are still at the conceptual or laboratory scale, requiring much development (Sudhakar et al., 2018).

2.4 Processes utilised for energy extraction

Energy extraction from macroalgae can be categorised into two categories, depending on the need for drying before processing. Figure 2.5 details the various processes depending on the category of macroalgae.

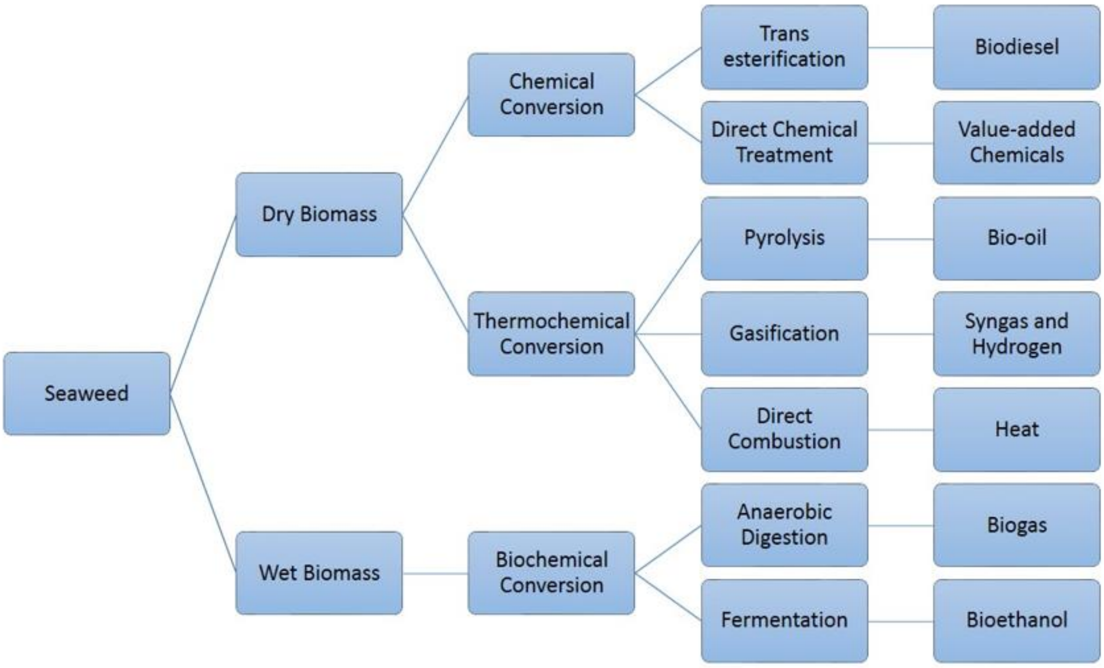

Figure 2.5: Energy extraction processes (Sudhakar et al., 2018)

2.4.1 Dry biomass technologies

Dry biomass can be converted using either chemical conversion or thermochemical conversion, which is done at high temperatures.

2.4.1.1 Direct combustion

Direct combustion of seaweed has been investigated by many researchers (Bruhn et al*.,* 2011; Wang et al*.,* 2009; Yu et al*.,* 2008). Using feedstock with high (more than 2% for wood) ash content is generally not recommended for this process (Sudhakar et al*.,* 2018). Macroalgae ash content can range between 3.5% and 46% depending on the type and harvesting time of the seaweed. The higher ash content results in the biomass having a higher heating value (HHV) that is lower than that of terrestrial energy crops (Milledge et al*.,* 2014), which is reported between 14 and 20 MJ/kg. The minimum and maximum values of the higher heating value of macro-algal biomaterial reported in the literature range between 4.35 MJ/kg and 20.1 MJ/kg (Sudhakar et al*.,* 2018). High ash content can be problematic as the macroalgae have a high alkali index (the amount of alkali metal oxide in the fuel per unit energy (kg alkali/GJ), which can lead to fouling and a reduction of the overall process efficiency. Pre-treatment to remove ash-generating components is therefore needed before the seaweed is combusted.

The combustion efficiency of the seaweed biomass is reported by Sudhakar et al. (2018) to be very low, usually between 15% and 30%. Seaweed has high levels of sulphur and nitrogen, which can hinder the use of this macroalgae as a direct combustion fuel (Milledge et al*.,* 2014). The use of seaweed biomass for direct combustion is therefore not recommended due to the risk of corrosion, fouling, and particulate emissions (Sudhakar et al., 2018).

Fluidised bed boilers were suggested by Yu et al. (2008) to combust materials with a high volatile content, high ash, and low thermal value (HHV). This will require the seaweed particle size to be 50 mm to minimise heat-transfer resistance. This will add to the energy required to process the seaweed being almost doubled due to the grinding of the seaweed. Energy requirements will increase further if the seaweed is wet.

The heat content on a dry mass basis is not a true indication of the net heat available as a correction is needed for the natural content of the biomass, which can reduce the available heat by as much as 20% (Demirbas, 2001).

2.4.1.2 Gasification

Gasification is defined as the conversion of organic matter by the partial oxidation of the matter at high temperatures (800 ^{0}C to 1000 ^{0}C), mainly into combustible gas mixtures called syngas. The calorific value of the gas is 4 to 6 MJ/m^3 and is a mixture of hydrogen (30–40%), Carbon monoxide (20–30%), methane (10–15%), ethylene (1%), and trace amounts of nitrogen, carbon dioxide, and water vapour (IEA and IRENA, 2013). The mixture of gases can then be upgraded to a higher grade of methane by utilising the hydrogen and carbon dioxide produced (Chynoweth, et al., 2001). The gas can be burnt to produce heat or converted to electricity and heat in a combined gas turbine system (IEA and IRENA, 2013). Syngas can produce methanol and hydrogen as fuel for transport and other uses. The cost to produce methanol from methane obtained from biomass is 1.5 – four times higher than that from fossil fuel gas (Sudhakar et al., 2018).

Low temperature (well below 1000 ^{0}C) biological gasification can convert wet and dry feeds. The product gas is a mixture of methane plus carbon dioxide and hydrogen sulphide, similar to anaerobic digestion. The main disadvantage is that conversion is incomplete due to the lower temperature utilised (Chynoweth et al., 2001).

2.4.1.3 Pyrolysis

Pyrolysis is defined as the thermal decomposition of organic components of dry biomass by heating in the absence of air. It is the preferred method for bio-oil extraction in tropical and subtropical regions (Sudhakar et al., 2018). The process can be classified as either slow, fast, or flash. Slow pyrolysis means the process has a long residence time at a low temperature (less than 400 ^{0}C) with low heating rates. Fast pyrolysis means the process has high operating temperatures (above 500 ^{0}C), with short vapour residence times (Milledge et al., 2014). Flash pyrolysis has fast heating rates (more than 1000 ^{0}C per second) and high reaction temperatures between 900 ^{0}C and 1,300 ^{0}C. The advantage of using pyrolysis is that it can produce high volumes of fuel material relative to the volume of biomass feed. The process can also be optimised to favour the production of bio-oil, syngas, or solid char (Milledge et al., 2014). Pyrolysis can be used in the presence of solvents to yield biofuels with specific characteristics. For example, hydrocarbons are produced during the pyrolysis of *Enteromorpha Prolifera* at 300 ^{o}C in the presence of vacuum gas oil. However, in the presence of ethanol, oxygenated products are generated.

Fast pyrolysis yields a greater liquid product and gas yields of between 70% and 80%. Slow pyrolysis, on the other hand, only yields between 16% and 65% gas products. Bio-oil production is more desirable as an end-product than char or syngas, as it has a higher density and is easily transported and stored (Jena & Das, 2011). Fast pyrolysis is recommended for macroalgae with a heating rate of 1–200 ^{0}C.s^{-1}. It does, however, produce a yield of approximately 76% of bio-oil, which is less than the microalgae production noted by Milledge et al. (2014). This could be due to the lipid content of microalgae, which is thought to influence the energy balance of pyrolysis. A higher lipid content generally leads to an improved energy balance using microalgae (Milledge et al., 2014).

2.4.1.4 Direct chemical treatment

Direct chemical treatment is utilised as a pre-treatment step before another technology produces biofuel. Alternately, chemicals are utilised as a secondary treatment step to recover value-added products. The use of chemical treatment will be discussed in the section on pre-treatment methods.

2.4.1.5 Trans-esterification

Trans-esterification is a chemical reaction that converts triglycerides (fats) contained in oils into biodiesel. Research has primarily focused on the use of microalgae in the production of biodiesel using trans-esterification due to the higher lipid content when compared to macroalgae (Milledge et al., 2014). Low yields of biodiesel have been produced using macroalgae. Aresta et al. (2005) reported on *Chaetomorpha linum* macroalgae; Suganya and Renganathan (2012) reported on *Ulva lactuva* macroalgae; Suganya et al. (2013) reported on *Enteromorpha compressa* macroalgae. The biodiesel yield was below 11% of the total dry macroalgal biomass in all three cases. Macroalgae does not appear to be the optimal feedstock for the transesterification process.

The high water content (up to 90% by mass) of the seaweed biomass has a negative impact on the energy balance of applications such as direct combustion, pyrolysis and gasification, or any process that is dependent on dry biomass as a feed source (Maneein et al., 2018).

Therefore, applications utilising wet biomass would be more suited to using seaweed as a feed source, negating the cost and energy required of drying the seaweed before processing.

2.4.2 Wet biomass technologies

Wet biomass can be converted using biochemical conversion processes described in the following sections.

2.4.2.1 Fermentation to bioethanol

Bioethanol production from first-generation sources such as corn and sugarcane is widely produced and used (Yang et al., 2011). There is now considerable interest in producing bioethanol from second-generation sources such as cellulosic biomass (Balat et al., 2008) due to the food vs. fuel dilemma and the droughts occurring across the globe. Despite extensive research and the availability of low-cost lignocellulosic biomasses, there still needs to be large-scale commercial production of fuel bioethanol from lignocellulose material (Balat et al., 2008; Puri et al., 2013). The main problem is the biodegradation of hemicellulose and cellulose, which is inhibited by lignin in many second-generation biofuel biomasses (Gressel, 2008).

Macroalgae, a third-generation biofuel, do generally not contain significant quantities of lignin (provide value or range). However, seaweed contains low amounts of polysaccharides composed of glucose (Table 2.2). The production of ethanol will have to be produced from other carbohydrate components of the seaweed, including sulphated polysaccharides, mannitol, alginate, agar, and carrageenan (Yanagisawa et al., 2013). Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are currently used for producing bioethanol from seaweed (Sudhakar et al., 2018). The seaweed biomass is hydrolysed during SHF and then subjected to bacterial or yeast fermentation in separate units. With SSF, however, the hydrolysis and fermentation are carried out simultaneously in a single process (Sudhakar et al., 2018).

Hydrolysis of the algal biomass is required to release the sugars in polysaccharides for fermentation (Jung et al., 2013). The method of pre-treatment and saccharification prior to fermentation not only influences the amount of sugar available for ethanol production but may also inhibit microbial fermentation with both acidic and heat treatments, producing inhibitor compounds (Jung et al., 2013). Another inhibitor to microbial fermentation could be attributed to the higher content of metals/minerals of the macroalgae when compared to terrestrial biomasses. The inhibitors could be removed by applying lime and activated charcoal treatments, adding considerable costs to the seaweed bioethanol production relative to the more readily fermented substrates such as simple sugars extracted from sugarcane.

Brown algae are suggested as the principal feedstock for bioethanol production because they have high carbohydrate content (25–50% on a dry weight basis) and can be readily mass-cultivated (Jung et al., 2013). A study concluded in 2013 found that the potential ethanol yield corresponded to approximately 90 litres per ton of dried macroalgae, which is low when compared to terrestrial fuel crops such as corn (Yoza & Masutani, 2013), which is reported at 345–394 litres per ton (Eckert et al., 2018). The energy balance for the fermentation and ethanol distillation of seaweed is thought to be similar to that for land crops, with energy inputs being approximately 70% of the calorific value of the ethanol produced (Aizawa et al., 2007). Seaweed's energy return on investment (EROI) has been estimated to be comparable to corn ethanol at 1.78 (Philippsen et al., 2014).

Table 2.3 compares ethanol production for first-generation fuel sources and seaweed. The seaweed has the highest water content, with the lowest carbohydrate content. Therefore, ethanol production is expected to be lower than the terrestrial crops as the water content is so high.

Table 2.3: Ethanol production from major land crops and seaweed

Raw material	**Moisture in raw material**	**Carbohydrates**	**Ethanol production per ton of raw material**	
			kg/tonne	l/tonne
Corn	14.5	70.6	360.8	462.6
Barley	14	76.2	389.5	499.3
Wheat	10	75.2	384.4	492.8
Rice	15.5	73.8	377.2	483.6
Sweet Potato	66.1	31.5	161	206.4
Potato	79.8	17.6	90	115.3
Sugarcane	60	15	76.7	98.3
Seaweed	90	5.8	29.6	38

Extrapolating the data in Table 2.3 and only considering dry biomasses, the kg/tonne of ethanol production can be estimated based on the dry mass of seaweed. Seaweed performs comparably to sugarcane, with seaweed producing 296 kg ethanol/tonne of dry seaweed, and sugarcane producing 192 kg ethanol/tonne of dry sugarcane. The costs for drying the seaweed prior to fermentation have to be taken into consideration.

2.4.2.2 Anaerobic digestion of macroalgae

From the 1970s to the 1990s, there have been various groups assessing the suitability of seaweed anaerobic digestion (Sutherland & Varela, 2014). Tokyo gas showed that 20 m^3 of methane could be produced from one ton of seaweed, capable of powering a 9.8 kW electrical generation plant when blended with natural gas (Huesemann et al., 2010). Seaweeds contain high levels of structural polysaccharides and low levels of lignin, making them attractive feedstocks for the production of liquid biofuels via fermentation as well as biogas production via anaerobic digestion (Ghadiryanfar et al*.,* 2016). There are four stages to the anaerobic digestion process, as described below in Table 2.4 (Milledge & Harvey, 2018).

Table 2.4: Anaerobic digestion stages

Stage 1	Hydrolysis	Proteins and fats are decomposed into monosaccharides, disaccharides, amino acids, and fatty acids.
Stage 2	Acidogenesis	Acidifying bacteria convert hydrolysis products to short-chain organic acids.
Stage 3	Acetogenesis	Acetogenic bacteria produce acetic acid, hydrogen, and carbon dioxide from fermentation products.
Stage 4	Methanogenesis	At the end of the degradation chain, two groups of methanogens produce methane from acetate, hydrogen, and carbon dioxide.

Hydrolysis is the rate-limiting step in AD (Thompson et al*.,* 2019).

Figure 2.6 gives a schematic overview of the anaerobic digestion process. During hydrolysis, cellulose and water are converted to form glucose as a primary product (Anukam et al*.,* 2019) according to the following equation:

$$(C_6H_{10}O_5)_n + n\ H_2O \rightarrow n\ C_6H_{12}O_6 + n\ H_2 \qquad (2.1)$$

In some instances, some hydrolysis products can be used directly by the methanogens (H_2 and CH_3COO), while others have to be converted to acetic acid. In stage 2, fermentation occurs, where the soluble compounds from stage 1 are degraded and converted into CO_2 and H_2 (Anukam et al*.,* 2019). The following reaction sequence summarises the acidogenesis stage:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$$

$$C_6H_{12}O_6 + 2\ H_2 \rightarrow 2\ CH_3CH_2COOH + 2\ H_2O$$

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH \qquad (2.2)$$

In stage 3, the hydrogen-producing acetogens break down the volatile fatty acids into carbon dioxide and hydrogen via the following chemical reaction:

$$CH_3CH_2CH_2COOH + 4H_2O \rightarrow CH_3COOH + 2CO_2 + 6H_2 \qquad (2.3)$$

The homoacetogens reaction is:

$$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O \quad (2.4)$$

In stage 4, The chemical reaction for the methane production from acetate is as follows (acetoclastic methanogenesis):

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.5)$$

Whereas the production of methane from hydrogen and carbon dioxide would be (autotrophic methanogenesis):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2.6)$$

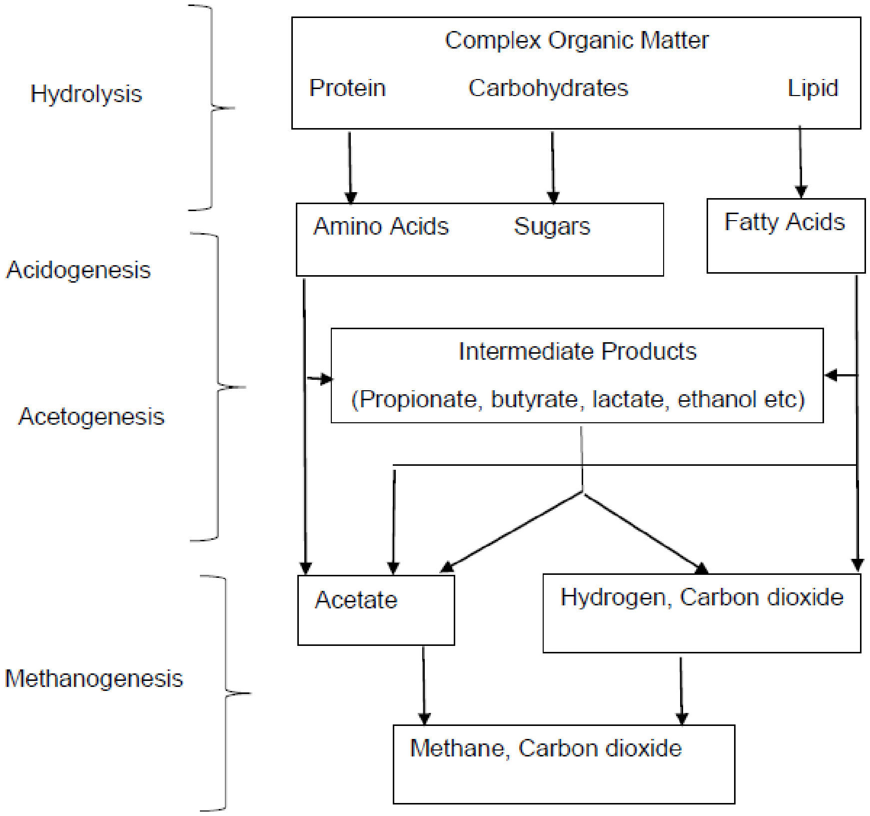

Figure 2.6: Schematic overview of the biochemical process to produce biogas (Sawyer et al., 2019)

Microorganisms used in methane production are sensitive to the chemical composition of the feedstock (Park et al., 2011; Samson & LeDuy, 1983; González-Fernández et al., 2012). The percentages of carbohydrates, proteins and lipids are essential (Park & Li, 2012), with lipids being more critical than either carbohydrates or proteins (Weiland, 2010; Zamalloa et al., 2011). The low lipid content (Table 2.2) of macroalgae restricts oil extraction. Therefore, biofuel from macroalgae will be produced via the conversion of carbohydrates using conversion technologies most tolerant to ash, like anaerobic digestion (Ghadiryanfar et al., 2016). Essential considerations in anaerobic digestion include biomass yield, substrate utilisation rate, hydraulic retention time, solids retention time, start-up time, environmental factors such as pH and temperature, and reactor configuration.

Macroalgae biomass has the potential to produce various biofuels, but there are significant technological hurdles to overcome before it is commercially viable. This is mainly due to the low practical yields of biogas production compared to the maximum calculated yields using the Buswell equation (Milledge & Harvey, 2014). Practical yields are approximated at 50% of the calculated maximum yield (Passos et al*.,* 2015). A sensitivity analysis conducted in 2015 found that increasing the methane yield of the biomass was the most critical factor in improving the process energy balance and, therefore, reducing greenhouse gas emissions, thus necessitating further research into the development of a commercially viable process (Mayfield, 2015).Ghadiryanfar et al. (2016) reported two macroalgae species that have high methane production yields. These are *Ulva* (*Cladophora* and *Chaemomorpha*) and *Macrocystis pyrifera*, with yields of 0.48 and 0.31 m^3 CH_4 per kg volatile solids, respectively. Ethanol production was reported to be comparable to that produced by land-based crops at 29.6 kg per 1 ton of wet seaweed. This is corroborated by Aizawa et al. (2007), who estimated ethanol production at 27 kg per ton of raw wet material (seaweed contains approximately 80–90% water). Research conducted by Gunaseelan in 1997 identified the empirical formula of *Macrocystis pyrifera,* a brown macro alga, as $C_{2.32}H_{3.73}O_{1.48}$. Based on stoichiometry, the theoretical yield for the biomethanation of algae was found to be 0.51 m^3/kg VS. The methane yields were in the range of 0.31–0.34 m^3/kg VS, corresponding to 55% of the theoretical yield value. Methane conversion was found to be higher in the thermophilic range at 55 ^oC than the mesophilic range at 35 ^oC. A longer retention time (increase from 10 to 18 days) in the mesophilic range increased the methane yield by 29% but had no effect in the thermophilic range. According to Hanssen et al. (1987), the percentage of volatile solids of certain brown macroalgae ranges between 3.6 and 6.2%. *L. Hyperborea* has a VS % of between 4.9 and 6.2. *L. Saccharina* has a VS% of between 3.6 and 5.7, and *A. Nodosum* has a VS% of 6.2. Approximately 50% of the total volatile solid content of the seaweed will be converted to biogas.Brown algae generally produce methane in the range of 110 to 410 m^3 per ton of VS of the algae. The theoretical yield for the biomethanation of kelp, a brown macroalga based on stoichiometry, was found to be 0.51 m^3/kg VS, which is 510 m^3 per ton of VS (Gunaseelan, 1997). The extensive range in methane production listed in the table below (Table. 2.5) is mainly due to the type of seaweed and the cultivation methods used, which would affect the composition of the final seaweed frond. The other influencing factor is that some macroalgae are pre-treated or rinsed before the anaerobic digestion process, removing impurities from the seaweed frond.

Table 2.5: Overview of methane production of brown, green, and red seaweeds

Seaweed name	Inoculum used	Process conditions	Methane produced		Reference	Year published
			ml/kg VS	**m^3/tonne TS**		
Brown Seaweed						
Ascophyllum nodosum	None listed	35 ^{0}C; 24 days	110		Ghadiryanfar et al.	2016
Ascophyllum nodosum	None listed	36.5 ^{0}C; 30 days	280		Hanssen et al.	1987
Ascophyllum nodosum	None listed	35 ^{0}C; 24 days	110		Hanssen et al.	1987
Durvillea antarctica	Cow manure	37 ^{0}C; 31 days	179		Vergara-Fernandez et al.	2008
Fucus serratus	Anoxic sediment	37 ^{0}C; 50 days	100		Obata et al.	2020
Fucus serratus	Bovine slurry	35 ^{0}C; 32 days	60		Vanegas et al.	2013
Fucus serratus	Digested sludge	37 ^{0}C; 50 days	103		Obata et al.	2020
Laminaria digitata	Anoxic sediment	37 ^{0}C; 50 days	205		Obata et al.	2020
Laminaria digitata	Bovine slurry	35 ^{0}C; 32 days	163		Vanegas et al.	2013
Laminaria digitata	Digested sludge	37 ^{0}C; 50 days	256		Obata et al.	2020
Laminaria digitata	Digested sludge	35 ^{0}C; 36 days	240		Migliore et al.	2012
Laminaria digitata	Food waste sludge	55 ^{0}C; 50 days		200	Manzano-Agugliaro et al.	2013
Laminaria digitata	Green peas	37 ^{0}C; 220 days	500		Akunna & Hierholtzer	2016
Laminaria digitata	None listed	37 ^{0}C; 15 days	232		Ghadiryanfar et al.	2016
Laminaria digitata	None listed	52 ^{0}C;	200		Alvarado-Morales et al.	2013
Laminaria digitata	None listed	52 ^{0}C; 30 days	133		Ghadiryanfar et al.	2016
Laminaria hyperborea	None listed	35 ^{0}C; 24 days	280		Ghadiryanfar et al.	2016
Laminaria hyperborea	None listed	36.5 ^{0}C; 30 days	430		Hanssen et al.	1987
Laminaria hyperborea	None listed	35 ^{0}C; 24 days	280		Hanssen et al.	1987
Laminaria saccharina	None listed	35 ^{0}C; 24 days	230		Ghadiryanfar et al.	2016
Laminaria saccharina	None listed	37 ^{0}C; 25 days	220		Gunaseelan et al.	1997

Laminaria saccharina	None listed	35 ^{0}C; 24 days	230		Hanssen et al.	1987
Laminaria saccharina	None listed	35 ^{0}C; 24 days	230		Hanssen et al.	1987
Laminaria saccharina lamour	None listed	36.5 ^{0}C; 30 days	460		Hanssen et al.	1987
Macrocystis pyrifera	Cow manure	37 ^{0}C; 31 days	181		Vergara-Fernandez et al.	2008
Macrocystis pyrifera	None listed	35 ^{0}C; 18 days	310		Ghadiryanfar et al.	2016
Macrocystis pyrifera	None listed	35 ^{0}C; 18 days	310		Gunaseelan et al. Chynoweth et al.	1997 2001
Saccharina latissima	Anoxic sediment	37 ^{0}C; 50 days	175		Obata et al.	2020
Saccharina latissima	Bovine slurry	35 ^{0}C; 32 days	245		Vanegas et al.	2013
Saccharina latissima	Bovine slurry	35 ^{0}C; 109 days	565		Vanegas et al.	2013
Saccharina latissima	Cattle manure sludge	53 ^{0}C; 34 days	340		Tabassum et al.	2018
Saccharina latissima	Digested sludge	37 ^{0}C; 50 days	229		Obata et al.	2020
Saccharina latissima	None listed	37 ^{0}C; 119 days	127		Ghadiryanfar et al.	2016
Saccharina latissima	None listed	37 ^{0}C; 119 days	155		Ghadiryanfar et al.	2016
Sacchorhiza polyschides	Bovine slurry	35 ^{0}C; 32 days	175		Vanegas et al.	2013
Sacchorhiza polyschides	Bovine slurry	35 ^{0}C; 109 days	468		Vanegas et al.	2013
Green Seaweed						
Chaetomorpha linum	Cattle manure sludge	53 ^{0}C; 34 days	166		Tabassum et al.	2018
Enteromorpha sp.	Wastewater treatment digested sludge	37 ^{0}C; 82 days	154		Costa et al.	2012
Spirulina Maxima	Sewage sludge	35 ^{0}C; 20 days	310		Samson & LeDuy	1983
Ulva lactuca	Bovine slurry	35 ^{0}C; 32 days	110		Vanegas et al.	2013
Ulva lactuca	Bovine slurry	35 ^{0}C; 109 days	191		Vanegas et al.	2013
Ulva lactuca	Cattle manure sludge	37 ^{0}C; 82 days	196		Costa et al.	2012
Ulva lactuca	Cattle manure sludge	53 ^{0}C; 34 days	152		Tabassum et al.	2018

Ulva lactuca	Food waste sludge	55 ^{0}C; 50 days		150	Manzano-Agugliaro et al.	2013
Ulva lactuca	None - nutrient feed	55 ^{0}C; 42 days	271		Bruhn et al.	2011
Ulva lactuca	None - nutrient feed	37 ^{0}C; 58 days	162		Bruhn et al.	2011
Red Seaweed						
Gracilaria vermiculophylla	Cattle manure sludge	53 ^{0}C; 34 days	132		Tabassum et al.	2018
Gracilaria vermiculophylla	Wastewater treatment digested sludge	37 ^{0}C; 82 days	182		Costa et al.	2012

The potential for using seaweed as an alternate fuel source is good. However, additional factors need to be considered, which could affect the economic feasibility of using seaweed in the anaerobic digestion process. Table 2.5 provides an overview of biogas production using brown, green, and red seaweeds. It details the inoculum applied and the process conditions of the reactors used to produce the biogas. Process conditions range between mesophilic and thermophilic conditions, and the time taken starts at 15 days. The methane produced is based on the amount of volatile solids and not necessarily on the mass of seaweed utilised.

There are very few documented cases of using red seaweed to produce biogas in literature. Based on Table 2.5, optimal operating conditions seem to be at 37 ^{o}C and a retention time of 30 days for the brown seaweeds. *Ascophyllum nodosum* showed an almost doubling of methane production with half a degree increase in temperature and six days longer retention time from 24 to 30 days (Table 2.5). A substantial increase in methane production can also be seen for *Laminaria saccharina* and *Laminaria hyperborean*, with a similar temperature and retention time increase.

For *Laminaria digitata*, however, increasing the temperature and extending the retention time by two weeks did not yield a significant increase in methane yield. This means temperature and retention time are not the only factors to consider for determining optimal methane production (Table 2.5). Additional process conditions need to be considered, including operating pH, inoculum type and preparation, and pre-treatment process for the macroalgae. These factors are investigated in the research conducted in an endeavour to answer the question of optimal process conditions required for maximum biogas production. Generally, the brown seaweed yielded more methane than the green seaweeds, which yielded more methane than the red seaweeds, based on the data in Table 2.5. This can be attributed to the reasonably high ash content of the green seaweed compared to the brown and red seaweeds. Rodrigues et al. (2015) found that green edible seaweed investigated had approximately 36% ash content, compared to 24–31% for red seaweed and 22–28% for brown seaweed. Higher ash contents could result in reduced conversion efficiencies, requiring pre-treatment to increase the biogas yield. Furthermore, Rodrigues et al. (2015) found that red and brown seaweed had a higher percentage of organic matter than green seaweed, indicating a higher volatile solid content and higher biogas formation.

2.4.3 Emerging technologies

2.4.3.1 Nanotechnology

Nanotechnology is an emerging technology. The immobilisation of the cellulose using nano-particles to hydrolyse the seaweed biomass for ethanol production is a new area of bioenergy research, which reduces the consumption of the hydrolysing enzymes required (Sudhakar et al., 2018). Nano-particles are an efficient carrier of biomolecules due to their large surface area and volume ratio.

2.4.3.2 Hydrothermal liquefaction

Hydrothermal liquefaction is currently considered a promising alternative, energy-efficient technology for the conversion of seaweed into biofuels and chemicals (Sudhakar et al., 2018). Liquefaction is a low-temperature, high-pressure process where the biomass is converted into a stable liquid hydrocarbon fuel (bio-oil) in the presence of a catalyst and hydrogen (Demirbas, 2001; McKendry, 2002). During the hydrothermal upgrading, the biomass is converted to partially oxygenated hydrocarbons at high pressure in the presence of a catalyst. The process can be seen as a pressurised aqueous pyrolysis process (Marcilla et al., 2013) and produces bio-oil that is lower in oxygen and moisture content than the bio-oil from pyrolysis (Neveux et al., 2014). This means a more stable product is produced than the pyrolysis process alone (Neveux et al., 2014).

Thermal treatment reviews of biofuel production have concluded that commercial interest in liquefaction is low due to the more complex feed systems and higher costs when compared to pyrolysis and gasification (Demirbas, 2001; McKendry, 2002; Marcilla et al., 2013). Hydrothermal upgrading of the algae does have the advantage of having the conversion take place in a water-containing environment, thus not requiring the drying of the biomass after harvesting (Minowa et al., 1995; Brown et al., 2010). This is to avoid the latent heat of vaporisation at high temperatures and pressure (Sudhakar et al., 2018). However, hydrothermal liquefaction of biomass with a moisture content of more than 90% is thought to have an unfavourable energy balance (Vardon et al., 2012). A cost-effective microwave-assisted hydrothermal liquefaction process is the latest novel process under investigation. It provides a non-enzymatic route for de-polymerising the biomass into sugars, which can be used in the biological production of fuels and chemicals (Sudhakar et al., 2018). Hydrothermal liquefaction has the advantage of using wet biomass, which avoids energy losses due to drying (Neveux et al., 2014).

2.5 Anaerobic digestion

Anaerobic digestion is a biological process performed in an oxygen-free environment. The process allows organic matter and inoculum to react to produce biogas. The primary products are methane and carbon dioxide (Mata-Alvarez et al., 2014).

2.5.1 Important considerations in anaerobic digestion

2.5.1.1 Volumetric organic loading rate

The volumetric organic loading rate (VOLR) is defined as the amount of organic matter fed per unit volume of a digester per day. Anaerobic processes usually have high volumetric organic loading rates, which depend on the kinetics of the degradation and level of the biomass in the bioreactor (Khanal, 2008).

Mathematically, the VOLR is described by the following equation:

$$VOLR = \frac{C_i Q}{V}$$

Where
C_i is the influent substrate biodegradable (COD) concentration (mg/L)
Q is the influent flow rate of the substrate (m^3/day)
V is the bioreactor volume (m^3)

The VOLR is essential in the anaerobic digestion process because it is shown as the quantity of volatile solids fed to the reactor daily. Sun et al. (2017) indicate that biogas production increases with an increase in organic loading rate for macroalgae between 1.37 and 4.12 kg VS/m^3 day.

2.5.1.2 Biomass yield

The biomass yield is a quantitative measure of the cell growth in a system for a given substrate. The yield is determined by ascertaining the ratio of the biomass concentration increase and the substrate concentration decrease. Yield depends on the biodegradable organic matter which is removed and the type of substrate which is metabolised (Khanal, 2008).

$$Yield = \frac{\Delta X}{\Delta S} = \frac{increase\ in\ biomass\ concentration}{decrease\ in\ substrate\ concentration}$$

Substrates with carbohydrates and protein generally have high yield coefficients as acidogenesis and methanogenesis metabolise these constituents to methane. The overall yield is, therefore, the sum of the yield for acidogenesis and methanogenesis (Khanal, 2008).

2.5.1.3 Specific biological activity

The specific biological activity (SBA) is the ability of the biomass to use the substrate. Values range between 0.75 to 1.5 kg COD/kg VSS per day (Khanal, 2008).

$$SBA = \frac{kg\ COD}{kg\ VSS/day} = \frac{kg\ biodegradable\ material}{kg\ volatile\ solids\ per\ day}$$

2.5.1.4 Hydraulic retention time

The hydraulic retention time (HRT) is the time required for the biomass to remain in the reactor in contact with the microbes or inoculum (Khanal, 2008). The HRT allows the user to determine the size of the reactor vessel, where:

$$Reactor\ volume = flow\ rate\ of\ influent \times\ HRT$$

2.5.1.5 Solids retention time

The solids retention time (SRT) is the time required for the reactor's microbial mass to achieve waste stabilisation. It measures the capability of the biological system to achieve a specified biodegradation rate (Khanal, 2008).

2.5.1.6 Start-up time

The initial commissioning period of the anaerobic digestion process is finalised once the biological treatment system is constant, at a continuous substrate feeding for a continuous system. It is affected by the growth rate of the microorganisms and the inoculum used at start-up as it affects the methanogens, which are dependent on environmental factors of the system (Khanal, 2008). The start-up time can be reduced by using a start-up culture from an operating AD system. Generally, the start-up time for a system operating in the mesophilic temperature range (37 ^{o}C) is between two and four months. This timeframe can exceed a year in the thermophilic range (55 ^{o}C) due to the high biomass decay rate (Khanal, 2008). Start-up time is affected by the pH in the reactor vessel, the nutrient availability, operating temperature, and the oxidation-reduction potential, which must all be maintained within the microbe's comfort limits (Khanal, 2008).

2.5.1.7 Microbiology

Anaerobic digestion is a multi-step process where diverse microorganisms degrade organic matter (Khanal, 2008). The inoculum plays a vital role in the start-up of the anaerobic reactor, providing the system with the initial microbial population ultimately causing the organic matter to degrade (Obata et al*.,* 2020; Maneein et al*.,* 2018). The most common practice is to obtain a starter microbial community from an already running AD plant as an inoculum (Wojcieszak et al*.,* 2017). Alternatively, animal slurry waste or sewage sludge can be used as an inoculum source. Other sources include riverbed sludge or nitrogen-rich compost.

Three groups of microorganisms carry out anaerobic biodegradation: hydrolytic, acetogenic and methanogenic (Vergara-Fernandez et al*.,* 2008). The typical inoculum used in the AD process is animal manure, which amounts to 54% of the inoculum used as indicated in publications between 2010 and 2013 (Mata-Alvarez et al*.,* 2014). There are documented disadvantages to using a single inoculum. These include:

- It is typically characterised by a low organic load with a high nitrogen concentration. This could inhibit the formation of methane.
- Municipal solid wastes have relatively high concentrations of heavy metals.
- Agro-industrial wastes and crops are seasonal and might not have sufficient nitrogen.
- Slaughterhouse wastes have high nitrogen concentrations and/or long fatty acid chains, which can inhibit methanogenesis.

Single organic waste inocula have become an essential source of raw material for the anaerobic digestion process, but it is often associated with low biogas production due to these limitations.

Hessami et al. (2019) incubated the inoculum used for 14 days to increase the bacterial population before the AD process. Chynoweth et al. (2001) used mesophilic sludge to react with *L. Digitata* to produce 280 ml/g VS of methane after 30 days. Similarly, Nielsen and Heiske (2011) used thermophilic sludge from cattle manure to degrade *S. Latissima* and produced 340 ml/g VS methane after 34 days. Bruhn et al. (2011) used thermophilic cattle manure to degrade *Ulva lactuca* to produce 271 ml/g VS of methane after 42 days. In newer research, anoxic sediment was utilised by Miura et al. (2014, 2015) to produce methane, as cited by Obata et al. (2020). Organic inocula are, therefore, critical in producing biogas utilising seaweed. What is essential is that the process is optimised for the co-digestion process conditions for optimum biogas production.

2.5.1.8 Environmental factors

The control of environmental factors like pH and temperature will affect the methane yield in the anaerobic digestion process (Khanal, 2008). Hydrolysis is the rate-limiting factor, and the environmental factors affecting it should be closely monitored and controlled. When the seaweed is harvested, is also crucial as it can affect the composition and ash content of the seaweed. The ideal C: N ratio should be greater than 20 for optimal digestion of the seaweed. The ash-to-volatile solids ratio is also a key factor in the digestion of seaweed (Tabassum et al., 2017).

i. Temperature

The AD process is strongly temperature-dependent. There are three optimal temperature ranges for methanogenesis, namely:

The psychrophilic range where bacteria called psychrophiles thrive between 5 ^{o}C and 15 ^{o}C.

The mesophilic range where mesophiles thrive is between 25 ^{o}C and 40 ^{o}C.

The thermophilic range where thermophiles thrive at 55 ^{o}C.

Mesophilic bacteria generally have a higher methane generation rate, whereas thermophilic bacteria produce a more constant rate of methane (Khanal, 2008). According to Khanal (2008), the highest efficiency of organic matter conversion to methane is at 35 ^oC to 40 ^oC for mesophilic operation and 55 ^oC for thermophilic operation. The anaerobic digestion process can, however, operate between 10 ^oC and 45 ^oC without any significant changes in the microbial ecosystem (Khanal, 2008). The thermophilic operation could improve AD performance and was found by Mata-Alvarez et al. (2014) to be slightly higher than in mesophilic conditions. Proper temperature control leads to an improvement in digester performance and presents better stability parameters for the system. The rates for AD usually increase with an increase in temperature up to 60 ^oC. Biological activity doubles every 10 ^oC within the optimal temperature ranges of various microorganisms (Khanal, 2008).

The Arrhenius equation describes the digestion rate temperature dependence of the AD system.

$$r_t = r_{30}(1.11)^{t.30}$$

Where:
t = temperature in ^oC
r_{30} and r_t = digestion rates at temperature t and 30 ^oC

ii. pH

Acidogens are acid-producing bacteria and prefer a pH of 5.5 to 6.5. Methanogens prefer a pH of 7.8 to 8.2. Where both cultures exist in the anaerobic digestion process, the optimal pH range is 6.8 to 7.4. However, the reactor pH should be kept as close as possible to neutral since methanogenesis is the region where methane gas is formed for the anaerobic digestion process (Khanal, 2008).

iii. Nutrients and trace metals

Essential microbial growth and synthesis components are crucial (Khanal, 2008). Trace elements (sometimes called micro-nutrients) include Co, Ni, Cu, Mn, Fe, Za, Se, and Mo. The role of some trace elements like Mo and Fe are understood in the anaerobic digestion process, whereas the role of the others still needs some investigation (Myszograj et al., 2018). In some instances, supplementation of nutrients is required for optimal biogas production. Biodegradability of the waste is dependent on the following optimal ratios:

C: N from 10:1 to 25:1
C:P from 113:1
C: N:P:S from (500-1000): (15-20): 5:3

Substances with a low C: N ratio cause a high ammonium nitrogen concentration increase, inhibiting methane formation. Supplementation with Fe, on the other hand, plays a significant role in nitrogen circulation and keeps the nitrogen in the digestate, allowing it to be used as fertiliser (Myszograj et al., 2018).

2.5.2 Considerations for biogas production improvement

2.5.2.1 Pre-treatment of the biomaterial

Pre-treatment processes are used to improve biogas production by increasing the bio-digestibility of the biomaterial. Pre-treatments generally increase the hydrolysis of polysaccharides to sugars, which could significantly affect the rate of biogas production and the yield of biogas (Maneein et al., 2018). Processes employed include physical, biological, chemical and heat pre-treatments (Hessami et al., 2019). Pre-treatments are used to:

a. Destroy or remove recalcitrant materials.
b. Improve the yield of biogas production.
c. Increase the bioavailability of polysaccharides, proteins, and lipids to enhance the rate and yield of biogas production (Hessami et al., 2019).

Methane production has been documented to increase by 19% and 68% after the breakdown of the biomaterial through various pre-treatment processes. Research has focused on optimising pre-treatment processes to improve methane yield while reducing costs (Maneein et al., 2018).

Pre-treatment processes will vary depending on the chemical composition of the seaweed biomass. Brown seaweeds contain fucoidan, laminaran alginates and cellulose polysaccharides. A pre-treatment method would, therefore, have to be able to hydrolyse these polysaccharides into sugars for maximum methane yield (Maneein et al., 2018). Since seaweed contains much moisture, drying the seaweed is recommended before storage to improve shelf-life and reduce transport costs (Maneein et al., 2018).

The biodegradability index (BI) indicates the efficiency of the pre-treatment process relative to the theoretical yield. This allows for comparing the effectiveness of the various pre-treatment processes used (Tabassum et al., 2017).

$$BI = \frac{methane\ yield\ after\ pre-treatment}{theoretical\ yield\ of\ methane} \times 100$$

Table 2.6 gives an overview of the pre-treatment methods of primarily brown seaweeds and the effect the pre-treatment processes have on the methane yield compared to an untreated sample. An in-depth analysis is given in the sections that follow.

Overall, mechanical pre-treatment and steam explosion positively affect the methane yield compared to other pre-treatment methods. Steam explosion requires less energy than mechanical pre-treatment and has the added advantage of lower chemical use than other pre-treatment methods. The disadvantage is that steam explosion can inhibit fermentation and enzymatic hydrolysis. Overall, steam explosion costs 30% of mechanical pre-treatment and is widely seen as one of the industry’s most cost-effective and environmentally friendly pre-treatment methods.

Table 2.6: Seaweed single pre-treatment technologies

Seaweed	Pre-treatment duration	Type of pre-treatment	Effect on methane yield (-= increase; += decrease)	Reference
Mechanical				
Laminariacea spp.	10 minutes	beating	54%	Tedesco et al., 2014
P. canaliculata	60 minutes	beating	74%	Rodriguez et al., 2018
Ulva lactuca		chopping/milling	55%	Nikolaison et al., 2012
Chaetamorpha linum		maceration	17.50%	Nielsen & Heiske, 2011
Gracillaria vermiculophylla		maceration	11%	Nielsen & Heiske, 2011
S. latissima		maceration	-2%	Nielsen & Heiske, 2011
Ulva lactuca		maceration	167%	Nielsen & Heiske, 2011
Thermal				
F. vesiculosus	24 hours	water at 20 ^{0}C	-19%	Barbot, Falk et al., 2015
F. vesiculosus	24 hours	water at 50 ^{0}C	-21%	Barbot, Falk et al., 2015
F. vesiculosus	24 hours	water at 80 ^{0}C	51%	Barbot, Falk et al., 2015
Biological				
L. digitata	24 hours	enzymes (cellulose)	-2%	Vanegas et al., 2015
Mexican carribbean macroalgae	6 days	White-rot fungi (Trametes hirsuta)	20%	Tapia-Tussel et al., 2018
Mexican carribbean macroalgae	6 days	enzymes	-6%	Tapia-Tussel et al., 2018
Chemical				
L. digitata	1 hour	2.5% citric acid	4%	Vanegas et al., 2015
L. digitata	1 hour	6% citric acid	-330%	Vanegas et al., 2015
Microwave				
Laminaria sp.	30 seconds	microwave @ 50Hz; 560W	-26%	Montingelli et al., 2016
Steam				
S. latissima	10 minutes	steam explosion at 130 ^{0}C	17%	Yazdani et al., 2015

S. latissima	10 minutes	steam explosion at 130 ^{0}C	20%	Maneein et al., 2018
S. latissima	10 minutes	steam explosion at 160 ^{0}C	20%	Yazdani et al., 2015
Chaetamorpha linum	5 minutes	steam explosion at 200 ^{0}C	18%	Maneein et al., 2018
Sargassum sp.	15 minutes	autoclave at 1 bar, 121 ^{0}C	60%	Costa et al., 2015

i. Mechanical pre-treatment

Mechanical or physical pre-treatment is used to reduce the particle size of the biomaterial (Hessami et al*.,* 2019) to improve access of the hydrolysing agents to the polysaccharides in the seaweed (Maneein et al*.,* 2018). It is the simplest form of pre-treatment and includes techniques like beating, milling and sonication, which increases the surface area to volume ratio of the biomass (Maneein et al*.,* 2018) and frees complex sugars for enzyme saccharification (Thompson et al., 2019). Excessive particle size reduction speeds up the hydrolysis and acidogenesis phases of the AD process. However, it can lead to an accumulation of VFAs (volatile fatty acids) and a decreased pH, ultimately hampering the methanogenic activity (Montingelli et al*.,* 2016). Ball milling is the primary treatment method and is commonly the most used (Thompson et al*.,* 2019). Maceration has been employed to improve the methane production of seaweed (Montingelli et al*.,* 2017). The results were conflicting, as a 68% biomethane increase was seen in *Ulva Lactuca* compared to results obtained when using *Ulva Lactuca* that was not pre-treated. In direct opposition to this result, methane production decreased when macerating *Saccharina latissima* biomass. Mechanical pre-treatment promotes the start of digestion and could, at the beginning of the AD process, increase biogas yield. Once digestion is completed, the AD process is affected by other conditions that must be considered.

Nielsen and Heiske (2011) used maceration as a pre-treatment method during the AD of four different seaweed species from Denmark. It was found that the methane yield increased for *Chaetamorpha linum* from 166 ml/g VS to 195 ml/g VS after maceration. A similar increase was noted for *Ulva lactuca*, where the methane yield increased from 152 ml/G VS to 255 ml/g VS, as well as *Gracillaria vermiculophylla*, where the methane yield increased slightly from 132 ml/g VS to 147 ml/g VS. *Saccharina latissima* showed a decline in methane yield from 340 ml/g VS to 333 ml/g VS. Maceration significantly affected the methane yield for *Ulva lactuca,* with only minimal effects on the other seaweeds investigated. This could be due to the complex chemical composition of the seaweed species, which is dependent on growth, geography, and season of harvesting of the seaweed.

Chopping or milling is advantageous with seaweeds having more fibrous cell walls (Maneein et al., 2018). The reduction in the size of the algal fronds before AD has been shown to significantly increase the yield of methane from *Ulva Lactuca* by 55% (Nikolaison et al., 2012) and from Baltic beach-cast seaweed (*Laminaria spp.*) by 53% (Tedesco et al., 2014; Montingelli et al., 2017). There have been mixed results for reducing particle size. According to Maneein et al. (2018), a mixture of smaller and larger particles would benefit the methane yield during AD. Smaller particles might be easier to digest at the beginning of the AD process. However, they would not necessarily increase the overall biogas production as factors such as pH, reactor blockages, and inoculum must be considered.

ii. Chemical pre-treatment

Chemical pre-treatment is used to destroy the cellular structure of the biomaterial. Chemical pre-treatment is often used along with temperature and harsh chemicals, increasing the costs of the process (Hessami et al., 2019). Hydrolysis could be enhanced during both acid and alkali pre-treatments. An alkali addition (e.g., sodium hydroxide) could cause swelling of the seaweed's fibres, increasing the pore size, which will quickly release the sugars from within the cell wall (Maneein et al., 2018). The biomass undergoes a simultaneous solvation and saponification process, ultimately increasing the sugars available for microbial digestion (Thompson et al., 2019).

Acid pre-treatment of seaweed could hydrolyse cellulose, hemicellulose, and carbohydrates to release cell contents. In the study by Hessami et al. (2019), the biomass was mixed with distilled water and 5M HCl using a dilute acid pre-treatment method. The mixture was then boiled at 100 ^{0}C for 1 hour and cooled to room temperature. The pH of the mixture was then adjusted to 7. Dilute-acid pre-treatment is favoured as it is a relatively low energy-intensive process. Materials, equipment, and recycling costs are lower than other pre-treatment processes, such as chemical and enzymatic treatment processes. Acid pre-treatment has been criticised due to the high costs associated with acid recycling and the cost of acid-resistant equipment required for the process (Maneein et al., 2018). Acid pre-treatment is more effective in delignifying the biomass (Thompson et al., 2019).

iii. Thermal pre-treatment

Thermal pre-treatment is used to release sugars and extract polysaccharides from seaweed. Operating temperatures are usually between 50 ^{0}C and 200 ^{0}C to break the hydrogen bonds of the biomass. Low-temperature thermal pre-treatment takes place at temperatures below 110 ^{0}C. High-temperature thermal pre-treatment takes place at temperatures above 110 ^{0}C. Temperatures above 180 ^{0}C promote the formation of inhibitory compounds and are not recommended (Thompson et al., 2019).

Steam explosion is one way to thermally pre-treat a substrate. It involves treating the biomaterial with steam at high temperatures (150–250 ^{0}C) for a few seconds (sometimes up to a few minutes), accompanied by a rapid drop in pressure to reduce the crystallinity of the biomaterial and therefore increase the solubility thereof (Mata-Alvarez et al., 2014). It is widely used to treat lignocellulosic biomaterials (Maneein et al., 2018). Montingelli et al. (2017) describe using steam explosion to increase the methane yield by 20% compared to untreated *Saccharina latissima.* Similarly, Maneein et al. (2018) cited that the steam explosion of *S. latissima* for 10 minutes at 130 ^{0}C showed a 20% increase in methane production. Steam explosion for 5 minutes at 200 ^{0}C yielded an 18% higher methane production than untreated *C. linum*. Though the biomethane yield is higher, there are also higher energy costs associated with the process, which need to be considered and compensated for (Maneein et al., 2018). Low-temperature pre-treatment at temperatures between 60 ^{0}C and 90 ^{0}C requires longer contact times. They are most effective when combined with alkaline pre-treatment to increase methane production (Mata-Alvarez et al., 2014).

The high moisture content of seaweed seems suitable for microwave pre-treatment, which facilitates the rapid rise in temperature and pressure of the cell wall, causing it to rupture (Maneein et al., 2018) and break the hydrogen bonds (Thompson et al., 2019). Rapid microwaving heating could also stabilise and minimise the sugar degradation of the seaweed experienced at high temperatures. Romagnoli et al. (2017, cited by Maneein et al., 2018) found that methane production was increased by 92% for *F. vesiculosus* when microwaving was used as a pre-treatment compared to untreated seaweed. Microwave pre-treatment was, however, found to be unsuitable for *Laminaria spp.,* where a 27% reduction in methane production was observed when compared to untreated seaweed.

Other thermal pre-treatments include wet oxidation and plasma-assisted pre-treatment. There is very little information on these methods. Plasma-assisted pre-treatment generates ozone in the reactor, which then reacts and degrades unsaturated organic compounds. Wet oxidation, on the other hand, is a process where water and oxygen are used to fractionate the biomaterial at high temperatures (above 120 ^{0}C) (Maneein et al., 2018).

iv. Biological pre-treatment

In this pre-treatment, microorganisms (fungi or bacteria) or enzymes are applied to the biomass to degrade the lignin and the hemicellulose. Biological pre-treatment can be done in either an aerobic or anaerobic environment. It has been shown to improve the AD process's hydrolysis and biomethane formation (Thompson et al., 2019). There are very few cited cases of biological treatment of macroalgae.

Where biological treatment is completed, white-rot fungi extracted from decaying wood are the most prominently used microbes in biological pre-treatment. The use of this fungi could be attributed to the extensive research completed with other applications, indicating a basic understanding of its mechanism. Treating brown seaweed with white-rot fungi showed a 20% increase in methane formation compared to an untreated sample, as shown in Table 2.5 above.

Table 2.7 gives an overview of combining the pre-treatment process and the effect this had on the methane yield of seaweed. Thermo-chemical pre-treatment processes should have a positive effect on the methane yield. However, a combination of mechanical and biological pre-treatments showed the highest increase in methane yield. This was, however, done at an elevated temperature of 50 ^{0}C to be classified as a combination of thermal, mechanical, and biological pre-treatment. Although the combined processes improve the methane yield overall, the economics must be clearly investigated as it could become very costly. The pre-treatment by-products would also need careful consideration and disposal options.

Table 2.7: Overview of combined pre-treatment processes

Seaweed	Pre-treatment duration	Type of pre-treatment	Effect on methane yield	Reference
F. vesiculosus	90 minutes	Thermo-chemical (0.2M HCl; 80 ^{0}C)	+ 39%	Barbot, Thompson et al., 2015
F. vesiculosus	90 minutes	Thermo-chemical (flue gas condensate; 80 ^{0}C)	+ 24%	Barbot, Thompson, et al., 2015
F. vesiculosus	2 hours	Thermo-chemical (0.2M HCl; 80 ^{0}C)	+ 34%	Barbot, Thompson et al., 2015
F. vesiculosus	24 hours	Thermo-chemical (0.2M HCl; 80 ^{0}C)	+ 36%	Barbot, Thompson et al., 2015
F. vesiculosus		Mechano-biological (mechanical treatment at 1000 bar, incubated at 50 ^{0}C , mixed with four enzymes)	+ 96%	Li, Kjerstadius et al., 2013
G. manilaensis	1 hour	Thermo-chemical (5M HCl; 100 ^{0}C)	+47%	Hessami et al., 2019
G. persica	1 hour	Thermo-chemical (5M HCl; 100 ^{0}C)	+ 77%	Hessami et al., 2019

2.5.2.2 Co-digestion to increase the efficiency of the AD process

The most popular AD substrates include animal manures, organic municipal waste, crops, agro-residues, and slaughterhouse waste. Anaerobic digestion of single substrates has the following disadvantages, as described by Mata-Alvarez et al. (2014):

- Animal manures have a low organic load combined with high nitrogen levels, which could inhibit the methanogenesis process.
- Organic municipal solid wastes could have high levels of heavy metals.
- Crops and agro-industrial wastes are seasonal and might have seasonal compositions.
- Slaughterhouse waste has long fatty acid chains and high nitrogen concentrations, which could inhibit the methanogenesis process.

To improve the efficiency of the methane yield in the AD process, it was proposed to mix the feedstock of garden peas with a co-substrate of brown seaweed to try and overcome some of the problems encountered in optimising the process. Since the availability of a suitable source for the feedstock organic matter is critical to the AD process, co-digestion could be used to supplement the feedstock reserves, thereby alleviating adverse economic impacts due to the seasonal availability of the feedstock (Akunna & Hierholtzer, 2016). It was found that the seaweed initially acted as an inhibitor as upon addition to the AD process, it led to an immediate drop in pH, with a build-up of volatile fatty acids. The methane production was stable, with a seaweed load between 2% and 5% of the total organic load.

Wilkins et al. (2015) used a mixture of digester sludge and organic waste (food waste, xylose, and xylan) to improve the methane yield in the AD process. His combination of organic waste and inoculum showed that all combinations yielded methane, with the food waste and sludge combination performing best per mass. Sarker et al. (2012) investigated the performance of *Ulva Lactuca* (green algae) and *Laminaria digitata* (brown algae) as co-digester with animal (cow) manure. They found that the AD of *Ulva Lactuca* in the thermophilic range (50 ± 2 ^{0}C) produced a methane yield between 157.6 m^3/tonne VS at an 11% Ulva feed rate of seaweed and 70.8 m^3/tonne VS at a 37% seaweed feed rate. The methane drop was attributed to the increase in total VFA, causing instability in the methanogenesis due to the overloading of VFA.

In contrast, the co-digestion of *Laminaria* with animal manure produced methane in the mesophilic range (35 ± 2 ^{0}C) at a relatively stable rate of 138 m^3/tonne VS over 15% to 55% feeding. *Laminaria* in the thermophilic range produced methane yield, ranging from 119 m^3/tonne VS at 15% seaweed biomass feeding to 185.7 m^3/tonne VS at a 55% seaweed feeding rate (Sarker et al., 2012). Seaweed biomass is used not only as a substrate with the primary feedstock in the co-digestion process but also as the main feedstock, co-digested with other waste materials or biomaterials.

Nkemka et al. (2014) investigated a two-stage process for the co-digestion of brown seaweed with solid cow manure using anaerobic digestion. The loading rate ratio was 1:1 based on the volatile solids content of the seaweed and manure. The methane production using seaweed alone is reported at 160 m^3/tonne VS compared to 110 m^3/tonne VS when co-digested with cow manure over 36 days with a hydraulic retention time of nine days. Although the co-digestion methane production was less than that of seaweed alone, the cow manure co-digestion system produced a more stable system at a neutral pH, with low NH_3 concentrations in the digestion system liquids. The resultant biogas production was accompanied by a digestate suitable for farmland fertiliser.

Mata-Alvarez et al. (2014) found that there was very little research done on the modelling of the co-digestion process even though it is crucial to:

- Predict and quantify how two or more substrates interact in the AD process.
- Reduce the time and money associated with laboratory experiments.
- Improve co-substrate selection and dosage rates.

Kinetic modelling, especially, would make predicting the AD process easier. Very little research has been done on this aspect and will be undertaken during this research.

2.5.3 Limitations of the AD process

Limitations of the anaerobic digestion process include the following:

a. Long start-up times: This is mainly due to the hydrolysis of the biomaterial. The start-up time can be reduced by maintaining a higher biomass load during the start-up period.
b. Prolonged recovery time: Should the AD system encounter a problem, the system can take very long to respond and reset itself to optimal conditions.
c. The process is susceptible to environmental conditions: Methanogens are very susceptible to environmental factors, including temperature, pH, and redox potential.
d. The presence of sulphate in the process reduces the methane yield: Methanogens are inhibited by the presence of sulphide, produced by sulphate reducers. The presence of H_2S reduces the quality of the biogas produced and is highly corrosive and malodorous (Khanal, 2008).

2.6 Kinetics of anaerobic digestion

Studies have been performed using cast brown seaweed, assuming hydrolysis was the AD process's rate-limiting step. Further assuming first-order kinetics, the following equation was used by Lymperatou et al. (2022) to describe the kinetics of the hydrolysis process.

$$CMY = BMP(1 - \exp(-kt))$$

Where:

CMY is the cumulative methane formed (ml/g VS)

BMP is the biomethane potential calculated using the Buswell equation (ml/g VS)

k is the hydrolysis rate constant (day^{-1})

t is time (days)

A plot of predicted methane yield using this equation and actual methane yield based on the experimental results would indicate the fitness for the equation’s purpose.

Sigmoidal models are used to evaluate the kinetics of the AD process. These models include the Gompertz, Richards, Stannard and Logistic models (Ware & Power, 2017). These models were limited as they used mathematical parameters rather than parameters specific to biological processes, making it difficult to relate them to biological performance. Zweitering et al. (1990) modified these models to include microbiologically relevant information, as shown in Table 2.8.

Table 2.8: Kinetic models for biological systems

Model name	Model equation	Modified model equation
Logistic	$y = \frac{a}{1 + exp(b - cx)}$	$CMY = \frac{P_{max}}{\left\{1 + exp\left[4R_{max}\frac{\lambdabar - t}{P_{max}} + 2\right]\right\}}$
Gompertz	$y = a * exp[-exp(b - cx)]$	$CMY = P_{max} * \ exp\left\{-exp\left[\frac{R_{max} * e}{P_{max}}\ (\lambdabar - t) + 1\right]\right\}$
Richards	$y = a\{1 + v * exp[k(\tau - x)]\}^{-1/v}$	$CMY = P_{max}\left\{1 + v * \exp(1 + v) * exp\left[R_{\max}\ \ (1 + v)\left(1 + \frac{1}{v}\right) * \lambdabar - t\right]\right\}^{-1/v}$
Stannard	$y = a\left\{1 + exp\left[-\frac{1 + kx}{p}\right]\right\}^{-p}$	$CMY = P_{max}\left\{1 + v * \exp(1 + v) * exp\left[R_{\max}\ \ (1 + v)\left(1 + \frac{1}{v}\right) * \lambdabar - t\right]\right\}^{-1/v}$

Where:

CMY = cumulative methane yield (ml/g VS)

t = anaerobic cultivation period (days)

P_{max} = methane production potential (ml/g VS)

R_{max} = maximum methane production potential (ml/g VS)

e = Eulers constant

$\lambdabar$ = lag phase time (days)

k = first-order reaction rate constant (d^{-1})

The modified Gompertz model is widely used for the kinetic modelling of AD processes, including swine manure, food waste, and wine residues (Pecar & Gorsek, 2020; Romagnoli et al., 2017). This is a first-order kinetic model used to evaluate the performance of a batch AD process and modified to account for changes in growth rate over time by including a parameter for the rate of change (Rangseesuriyachai et al., 2023). The modified Gompertz model assumes that the cumulative biogas production in batch systems results from the methanogenic microorganism growth rate (Pecar & Gorsek, 2020).

Researchers have argued that instead of utilising the BMP of the seaweed in the first-order kinetic equation, it would be best to utilise the P_{max} value from the model that best fits the experimental data (Li et al., 2015; Zhong et al., 2021; Pecar & Gorsek, 2020). The BMP is a theoretical determination based on biomass composition and assuming complete conversion of all sugars. In contrast, the P_{max} value is determined based on actual experimental results obtained for the system.

Sigmoidal models are utilised to predict the performance of the AD process. Based on available experimental data, they can provide a reasonable estimate of the expected methane yield without having to spend days to complete the experiment. In this way, it allows for optimising the process and determining process variables such as feeding rates.

2.7 Carbon intensity and greenhouse gases

Concentrations of greenhouse gases (GHG) in the atmosphere are rising rapidly. The major contributor to this rise is fossil fuel-derived carbon dioxide emissions. Energy derived from biomass can drastically reduce GHG emissions compared to fossil fuels as it utilises locally available resources (Weiland, 2010). The impact of biofuels on GHG emissions depends on the type of technology used, the feedstock, the energy used, and the mode of transportation used (Sudhakar et al., 2018).

To complete a GHG balance on a system, the following is required (Florentinus et al., 2014):

- Analysis of the production chain for energy use
- Whether or not fertilisers were used (Nitrogen)
- Recycling of materials and methods used
- Can the by-products be utilised?
- The materials of construction used in the production chain and the GHG of the production of these materials

Direct GHG emissions are considered to include only the emissions during the application of fertilisation using Nitrogen, where there are direct N_2O emissions (estimated at 33g N_2O produced for every 1 kg Nitrogen fertiliser used). There will be little or no direct GHG emissions for aquatic systems. Indirect GHG emissions are based on the consumption of electricity, methane, and materials used (Florentinus et al., 2014).

A method to reduce carbon dioxide emissions could be to direct the carbon dioxide released from a carbon fuel-burning plant into an open or closed algae system, accelerating the algae growth. Seaweed is an excellent alternative to remove carbon dioxide from the atmosphere due to its higher photosynthetic efficiency and fast growth rate (Sudhakar et al., 2018). It is estimated that 1 ton of seaweed can absorb approximately 960 kg of carbon dioxide during its cultivation. It has been suggested that biogas from seaweed could reduce GHC emissions by 42–82% compared to natural gas (Florentinus et al., 2014). The digestive waste from the AD process contains nitrogen and phosphorus-containing compounds that can be used as fertiliser or biological feedstock, producing additional income (Roesijadi et al., 2010). Biogas from seaweed, when compared to natural gas, can reduce GHG emissions by 42–82% (Maneein et al., 2018).

2.8 Economic considerations

The cost of production of biogas from seaweed is high. Estimates suggest it could be 7–15 times more expensive than natural gas (United Kingdom. The Parliamentary Office of Science and Technology, 2011). For anaerobic digestion to be cost-effective in a commercial application, the cost of the raw material must be reduced by at least 75% over current levels in 2009 (Bruton et al., 2009). For a comprehensive techno-economic assessment, the anaerobic digestion process needs to be evaluated in terms of the material and energy balance, the carbon balance, the costs associated with harvesting the seaweed, and the cost of producing biogas from seaweed (Sudhakar et al., 2018).

Generally, the biomass is converted to a gas with an energy content of 20% to 40% of the lower heating value of the feedstock (McKendry, 2002). To evaluate the performance of the bio-energy system, the following needs to be taken into consideration (McKendry, 2002):

- The entire production chain, from the biomass production to the end-user needs of the energy produced.
- The net energy yield per hectare is the gross energy produced by the biomass, less the energy provided by the fossil fuel used in the production and processing of the biomass.

A detailed analysis of the economic considerations is covered in Chapter Five of the thesis.

2.9 Summary

Seaweed is a promising feedstock for bio-energy production. To utilise seaweed sustainably and efficiently, the following needs to be investigated:

- An increased understanding of the seaweed's morphology, genetics, and biochemistry is required.
- The low bio-conversion of seaweed biomass to biogas results from the complex polysaccharides, which comprise 40 to 60% of the dry weight of the seaweed biomass. These polysaccharides are not easily fermentable, impeding the formation of biogas.
- Pre-treatment methods to increase the bioavailability of organic matter for hydrolysis could increase biogas production. A balance between the pre-treatment and subsequent waste product disposal cost and the biogas yield needs to be established.
- Development of an integrated biochemical and thermochemical conversion process is needed, preferably operating at 80% water so that wet seaweed can be utilised without the need to dry the seaweed.
- A techno-economic study of the overall conversion process from macroalgae feedstock to biofuel production needs to be completed to determine the feasibility of the process and possibly optimise the process.
- A study that determines the impact of large-scale seaweed cultivation on the aquatic systems needs to be performed.
- Dandikas et al. (2015, 2018) suggests an approach to yield high biofuels with the least inhibitory by-products. He recommends performing a primary component analysis of all seaweeds within a sector at various times of the year and determining the correlation of different components to biofuel yields. This can also be done across species. In this way, the characteristics of a suitable seaweed can be identified, which can then be mass-cultivated.

2.10 Significance of research/novelty

In this study, *Ecklonia Maxima* is utilised, along with a synthetic inoculum, to produce biomethane using an anaerobic digestion process. The research intends to identify a possible alternate energy solution for small enterprises in the South African market using an available resource currently under-utilised. It will not affect food security in the country. Pre-treatment processes will be investigated to optimise the AD process utilised for biogas production. The research will determine the cost of producing biogas per mass unit of *Ecklonia Maxima*, which is lacking in current research. Kinetic curve fitting will be used to model the system and predict the biogas production performance. All of the above will contribute to the knowledge base for *Ecklonia Maxima* and the production of biogas via AD.

CHAPTER THREE: METHODOLOGY

3.1 Materials and methods

3.1.1 Materials

Ecklonia Maxima was bought in dry ground form from a company on the West Coast of South Africa in the Western Cape Province. The seaweed was supplied either as a chunky, dried, woody-looking material (Figure 3.1a) or as a ground material (Figure 3.1b) with a smaller particle size, as indicated below.

(a) Raw seaweed received from supplier **(b) 2 mm milled seaweed**

Figure 3.1: Dried *Ecklonia Maxima* (Source: Researcher)

Initially, six potential inocula were identified. This included four synthetic inocula and two complex organic inocula. The inocula were selected based on their availability and price point. The aim was to keep the costs as low as possible, as synthetic inocula cited in literature were usually very expensive and not readily available in South Africa.

The function of the inocula is to assist in degrading the seaweed biomaterial so that hydrolysis can occur.

The synthetic inocula identified included:

- Atlantic BioGanic
    - This is an all-purpose, blended organic fertiliser containing minerals.
    - It is primarily used to assist in the growth of plants and has a high nitrogen content.
- Atlantic Bio Ocean
    - This soil and plant conditioner consists of composting mixtures of kelp, fishmeal, humic acid, and poultry manure.
    - It is bought in pellet form, with a neutral pH and is pathogen-free.

- Wonder Organic Vitaboost
    - This is also a pellet fertiliser, which is primarily used to activate microbes in the soil.
- Makhro Compost activator
    - This is a liquid which is used to biodegrade organic waste.

The complex organic inocula included:

- Municipal sewage starter AD fluid
    - The fluid was collected at the Kraaifontein Municipal Sewage water treatment plant. The RAS composition varies as the operation of the plant varies.
- Cow manure
    - Manure was collected at a dairy farm near Stellenbosch in the Western Cape of South Africa. The cows are grass-fed only.

Glass reactors for the anaerobic digestion process were manufactured by a company in Cape Town (Glasschem). The reactors were manufactured to be airtight with specialised stainless-steel clamps, providing the anaerobic environment needed. Reactors included an inlet feed port and an outlet port for the gases produced, which could be connected to flexible tubing. Clamps were utilised when an inlet or outlet was not needed. A port was also provided for measuring the pH using a pH probe and a port to remove the liquid substrate (Figure 3.2). The gas outlet was connected to a 1 L Supel inert foil SCV gas bag for biogas collection.

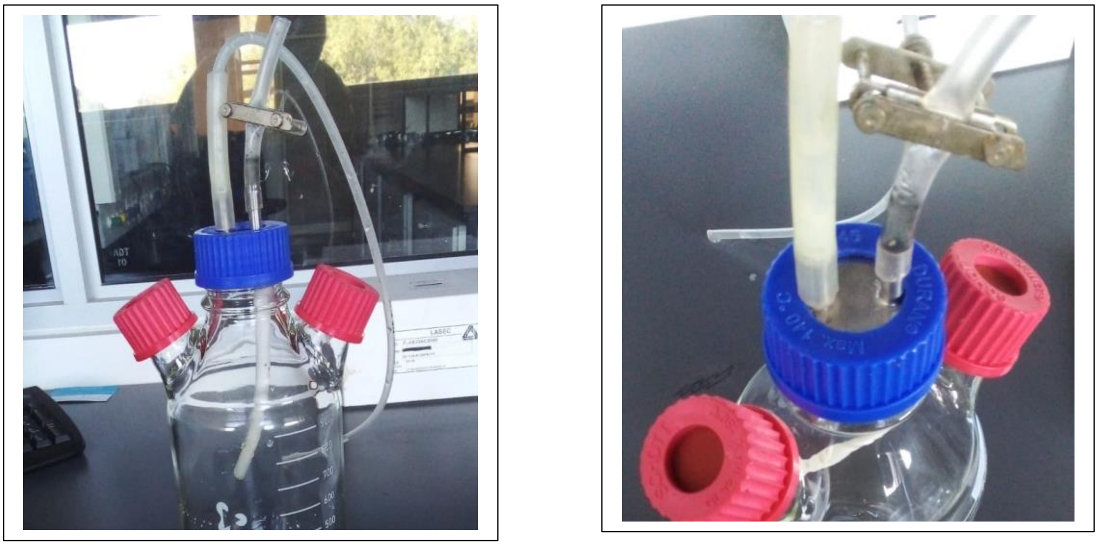

Figure 3.2: AD reactor vessels (Source: Researcher)

pH control was done through the addition of either 2M HCl or 2M NaOH. Distilled water was utilised to dilute and soak the seaweed and prepare the inoculum slurries. pH was monitored using a Hanna Edge pH meter (model HI2002) with an HI11311 electrode. Dissolved oxygen was measured using a Hanna Edge DO meter (model HI2004) with an HI764080 electrode. The temperature of the reactors was controlled by submerging the reactors in a temperature-controlled water bath. Gases produced were collected in 1 L Supel inert foil SCV bags. Biogas formed was analysed using a Biogas 5000 Portable Gas Analyser.

3.1.2 Preliminary tests on seaweed

3.1.2.1 Chemical and organic analysis

The *Ecklonia Maxima* biomass is received dry from the supplier. It is essential to know the chemical composition of the seaweed as it could vary due to seasonal influences and the time of harvesting. The supplier provided a complete organic analysis with the purchase. These included carbohydrates, fats, proteins, and fibre. The chemical analysis of the seaweed was determined using EDS at a UCT laboratory. The EDS is able to read all components on the surface of the seaweed, with the exception of hydrogen.

To determine the basic elemental composition most commonly used in BMP calculations, analysis for CNHS was requested from a Stellenbosch University laboratory. The EDS and CHNS were able to provide all the elements required for BMP analysis based on the chemical analysis of biomass.

3.1.2.2 Lignin tests

Lignin is a complex polymer built up of aromatic alcohol subunits, providing rigidity and support to the fibres of the seaweed. The type of bonds between lignin subunits affects the lignin structure and, therefore, the reactivity of the biomass during lignin content tests. The lignin content was determined using the standard Klason method (Kirk & Obst, 1988), also known as the 71% sulphuric acid method. The major disadvantage is that other components may condense and hydrolyse during the process and can be analysed as Klason lignin, and some of the lignin can also partially solubilise. However, The Klason method gives a good indication of the lignin percentage within a substance. The test was performed three times, and the average value was used as the lignin percentage.

The Klason method: The seaweed was ground and sieved to fit through a 20-mesh screen. Debris and foreign materials were removed. 200mg of seaweed was weighed to 0.1 mg and placed into a beaker. Two (2) ml of 72% sulphuric acid was added to the mixture and placed in a water bath at 30 ^{o}C, with frequent stirring for 1 hour. After 1 hour, the sulphuric acid was diluted to a 3% sulphuric acid solution by adding 56 ml of distilled water (28 ml for every ml of sulphuric acid) to the mixture. The mixture was placed into an autoclave at 120 ^{o}C for an hour to ensure secondary hydrolysis. The solution was filtered and washed with hot water to remove residual acid. The seaweed was transferred to a crucible and dried until it reached a constant weight. The residue was weighed to the closest 1 mg. The weighed sample was combusted at 550 ^{0}C to account for any acid-insoluble inorganics in the seaweed. The residual ash was weighed.

The entire process was repeated twice, and the average of the totals was used to determine the lignin using equation 3.1 below.

Lignin was determined as a percentage according to the following equation:

$$\%Lignin = \frac{W_L - W_A}{W_T} \times 100 \tag{3.1}$$

Where W_L = weight of the lignin in mg = the mass of seaweed after the second hydrolysis and drying to constant weight.

W_A = weight of the ash in mg = the mass of ash collected after incineration.
W_T = weight of the test specimen = mass of the initial seaweed sample.

3.1.2.3 Total volatile solids

The total volatile solids were determined at CPUT via standard experimental procedures.

The TVS was determined as follows:

A known mass (10g) of 2-3 mm ground *Ecklonia Maxima* was weighed and placed in a pre-weighed crucible. The seaweed was heated to 550 ^{0}C and incinerated for an hour. The ash was collected and weighed in a pre-weighed crucible.

The TVS was calculated as follows:

$$TVS = mass\ of\ seaweed - mass\ of\ Ash \tag{3.2}$$

It was assumed that the incinerated portion was the volatile solid component.

3.1.2.4 Bio-methane potential

Determining the biogas production potential could help determine the system’s economics. Bio-methane potential (BMP) is a simple and reliable method that can be used to assess the expected biogas yield of an organic substrate based on either the chemical composition or the organic composition of the substrate (Li, Zhang et al., 2013).

The Buswell formula (Buswell & Mueller, 1952) is used to determine the BMP based on the chemical composition of the substrate:

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right) CO_2 + cNH_3 \qquad (3.3)$$

The equation for BMP is:

$$BMP \left(\frac{ml\,CH_4}{g\,VS}\right) = \frac{22.4x1000x\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{3c}{8}\right)}{12n+a+16b+14c} \qquad (3.4)$$

The values for n, a, b, and c are determined based on the chemical composition of the seaweed.

This version of the Buswell equation does not consider sulphur. A modified version of the Buswell equation, as given by Boyle (Achinas & Euverink, 2016), taking into account sulphur formation, is as follows:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S \qquad (3.5)$$

The theoretical methane production, including sulphur, can be calculated from:

$$TBMP = \frac{22.4x\left(\frac{a}{2}+\frac{b}{8}-\frac{c}{4}-\frac{3d}{8}-\frac{e}{4}\right)}{12.017a+1.0079b+15.999c+14.0067d+32.065e} \qquad (3.6)$$

Kaparaju et al. (2009, cited by Li, Zhang et al., 2013), estimated the BMP based on the organic composition of a substrate as follows:

$$BMP \left(\frac{mL\,CH_4}{g\,VS}\right) = (373 * VFA + 496 * Protein + 1014 * Lipids + 415 * Carbohydrates + 727 * Lignin) \qquad (3.7)$$

Where the components were estimated as follows:

$VFA = C_2H_4O_2$ $\quad$ $Lipids = C_{57}H_{104}O_6$ $\quad$ $Protein = C_5H_7NO_2$
$Carbohydrates = C_6H_{10}O_5$ $\quad$ $Lignin = C_{10}H_{13}O_3$

The equation for BMP, therefore, reduces to:

$$BMP = 373C_2H_4O_2 + 496C_5H_7NO_2 + 1014C_{57}H_{104}O_6 + 415C_6H_{10}O_5 + 717C_{10}H_{13}O_3 \qquad (3.8)$$

The cumulative biomethane yield at a specified time (M) can be determined by (Tabassum et al., 2018):

$$M = P * exp\{-\exp\left(\frac{R_{max} \cdot e}{P}[\Delta - t] + 1\right) \tag{3.9}$$

Where:

P = maximum biomethane potential of the substrate (L CH_4/kg VS)
R_{max} = maximum biomethane production rate (L CH_4/kg VS)
Δ = lag phase (How many days before biomethane production starts)
t = time in days

3.1.3 Inoculum preparation

The total volatile solids of each inoculum were determined using the same method as the seaweed. Each inoculum was mixed with sufficient distilled water to make a 20% (m/m) slurry solution and stored in an airtight container until required. No adjustments to pH were made at this time. Inocula in pellet form were first ground to between 1 and 2 mm in size before mixing with the distilled water.

3.1.4 Methodology: Objective 1 – Identify a suitable inoculum for the anaerobic digestion process

3.1.4.1 Experimental setup

The inocula identified were combined with the seaweed under mesophilic (37 ^{0}C) or thermophilic (52 ^{0}C) conditions and allowed to react under adiabatic conditions for a minimum period of 21 days and a maximum of 42 days.

A semi-batch system was set up using a procedure adapted from the methodology of Montingelli et al. (2017) for anaerobic digestion and refined from Sutherland and Varela (2014) for the inoculum testing. All experiments were duplicated at 37 ^{0}C ± 0.5 ^{0}C or 52 ^{0}C ± 0.5 ^{0}C, depending on which system was running. The loading rate of inoculum to seaweed was 1:1 based on the volatile solids content. The working volume of each reactor was 800 ml, consisting of 400 ml of a 20% (m/m) seaweed slurry and 400 ml of the required inoculum slurry.

Important parameters were identified by performing initial experiments to determine the optimum pH and dissolved oxygen content of the anaerobic digestion process at mesophilic and thermophilic conditions. These were identified as an operating pH of approximately 7 and a DO value of less than 1 ppm.

The initial pH for the combined seaweed and inoculum solution was adjusted to 7 ± 0.2. A similar adjustment was made at each feeding of the reactor. The dissolved oxygen content was measured at the start of the process and at each feeding. The system was purged with nitrogen at this stage if required, until the DO reading was below 1 to ensure an anaerobic system.

3.1.4.2 Experimental procedure

Glass reactors, substrate solutions and inocula were homogenised separately at the correct operating temperature by submerging them in a water bath at the same temperature. Once homogenised, the rectors were set up inside a different water bath. Inlet and outlet ports were connected to feed the reactor and remove samples, and a gas outlet tube was connected to the reactor. The entire system was then purged with nitrogen to remove any oxygen. The correct amount of seaweed slurry and inoculum mix was added to the reactor, and the pH was adjusted. The reactor was purged with nitrogen, and a DO reading was taken to confirm an oxygen content reading of less than 1. A gas bag was connected to the gas outlet, and the system was allowed to react for seven days before the first feeding took place.

Feeding occurred on days 8, 12, 15, 19, 22, 26, 29, 33, 36, and 40, depending on how long each reactor ran. With each feeding, 80 ml of the substrate solution was removed from the system, and 2.7 g of seaweed was added, making up to 20% (m/m) solution, which amounted to 80 ml. The pH was adjusted, and the system purged if needed to obtain a DO reading of less than 1. The gas bag was emptied, and the ml of gas collected was noted.

3.1.4.3 Analysis

The volatile solids content was determined for each substrate sample removed at each feeding so that an accurate accounting could be done for the removal of volatile solids from the system. The gas collected at each feeding was analysed for methane, carbon dioxide, hydrogen sulphide and oxygen. The amount of other gases present was noted. Since each reactor was duplicated, the total methane formation was averaged for each inoculum and seaweed combination at the given temperature. The analysed gas percentages and the volatile solids removed and added to the system with each feeding were utilised to determine the methane formation at standard conditions at each feeding. The cumulative methane collected after the required reactor time, measured as ml/g VS, was used to determine the overall methane formation at standard volume, temperature, and pressure conditions.

3.1.5 Methodology: Objective 2 – Optimisation of biogas production

3.1.5.1 Operating temperature

Mesophilic and Thermophilic temperature experiments were conducted for all combinations of inoculum and seaweed, as described above. Once the experimentation had been completed in duplicate, a decision could be made on whether mesophilic or thermophilic conditions yielded more methane gas. These experiments were all performed at the optimal pH of 7 to 7.2.

3.1.5.2 Pre-treatment processes

Pre-treatment of the seaweed biomass was performed to increase the methane yield. The pre-treatment processes included mechanical, chemical, and thermal processes. All experiments performed were duplicated as before, with a contact time of 28 days. The same processes were followed as described in section 3.4.2.2 above, except for the pre-treatment steps described below, which were performed before the inoculum and seaweed slurries were homogenised in the reactors. All experiments were completed at both mesophilic and thermophilic temperatures.

i. Mechanical pre-treatment

The seaweed and sieve were ground to the required size distribution. Initial experimentation had been completed using a seaweed size of 2 mm. Additional size distributions of 2 to 3 mm for experiment one and less than 2 mm for experiment two were prepared. The seaweed was prepared as a 20% (m/m) solution and homogenised at the required temperature before mixing with the inoculum solution.

ii. Chemical pre-treatment

The seaweed was prewashed with acid for one set of experiments and a base for another set of experiments.

For acid pre-treatment, two concentrations (0.15M HCl and 0.30M HCl) were used, and experiments were performed at mesophilic and thermophilic temperatures for each. The ground seaweed (2 mm) was mixed with the required acid concentration and boiled for 1 hour (Maneein et al., 2018). The solution was allowed to cool. The seaweed was drained, and the biomass dried until a constant weight had been reached. The pre-treated seaweed was stored in an airtight container until required.

For basic pre-treatment, two concentrations (0.15M NaOH and 0.3M NaOH) were used, and experiments were performed at both mesophilic and thermophilic temperatures for each. The ground seaweed was mixed with the required base concentration and soaked until saturated (Maneein et al., 2018). The excess solution was drained off, and the seaweed was dried until a constant weight had been reached. The pre-treated seaweed was stored in an airtight container.

iii. Microwave pre-treatment

The required amount of dry seaweed was soaked in distilled water overnight (or until saturated). The excess water was drained off. Three separate samples were prepared. The first sample was microwaved at 100% power (750W) for 30 seconds. The second sample was 60 seconds, and the third was 90 seconds. Only the samples to be used on that day were prepared, with a 20% (m/m) slurry used in the same way as before and homogenised with the inoculum at the operating temperature before the start of each experiment.

3.1.5.3 Analysis

Analysis was done as before for volatile solids and methane production at standard conditions.

3.1.6 Methodology Objective 3 – Kinetics of the AD process

Data collected from both the initial and pre-treatment experiments were utilised to determine if the data are described as zeroth-order, first-order, or second-order.

For zeroth order kinetics, a plot of cumulative methane vs. time.
For first-order kinetics, a plot of ln (cumulative methane) vs. time.
For second-order kinetics, a plot of the inverse (cumulative methane) vs. time.

A trend line was drawn through the data points. A linear trend line with a high R-squared value would indicate the order of kinetics.

Once the kinetics order was determined, sigmoidal equations corresponding to the kinetics order would be utilised to fit the experimental data to predict the data's maximum methane production, lag time, and methane potential.

3.1.7 Statistical analysis

The data collected were compared for differences in mean values by means of a one-way analysis of variance (ANOVA) using the SPSS v28 software of 2022-2023. The ANOVA describes the statistical variance of the effect of the added inoculum on biomethane yield. The significance level was set at 0.05 (95% confidence interval).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Objective 1: Identifying a suitable inoculum for the anaerobic digestion process

4.1.1 Introduction

Four South African Inocula were identified for use in the anaerobic digestion of *Ecklonia Maxima* to produce biogas. These included two synthetic (Vitaboost and BioGanic fertilisers) and two biological (Cow manure and AD starter fluid) inocula. The first objective was to select a single inoculum for the anaerobic process of biogas formation. The selection was based on the total biogas formation (bio-methane) per gram of volatile solids of the *Ecklonia Maxima* biomass. The performance of each combination of seaweed and inoculum was compared to the calculated bio-methane potential of the seaweed to determine if the inoculum had any effect on the overall bio-methane yield.

4.1.2 Composition of *Ecklonia Maxima*

A laboratory along the West Coast of South Africa completed the organic analysis. The analysis was provided with the purchase of the dried *Ecklonia Maxima,* as indicated in Table 4.1. The carbohydrate content is high at 50% on a dry weight basis, making it ideal for biogas production. According to Sudhakar et al. (2018), the average carbohydrate content of brown seaweed is between 30% and 50%. *Ecklonia Maxima* is at the upper limit of this range. It is expected that biogas production could be easily achieved. The analysis does not indicate the ease of carbohydrate conversion. The protein content is in the middle of the range expected at 9% on a dry weight basis. There is a significant amount of ash at 28% (Table 4.1).

Table 4.1: Comparison of *Ecklonia Maxima* organic analysis

	Organic analysis (g/1000g seaweed)			
Component	**Mass (g)**	**%**	**% dry basis**	**Wang et al. (2020) analysis (% dry basis)**
Protein	78	7.8	9.08	12.01
Fat	4.5	0.45	0.52	
Carbohydrates	431.5	43.15	50.2	51.83
Fibre	102	10.2	11.9	
Moisture	141	14.1	-	
Ash	243	24.3	28.3	25.52
Total	**1000**	**100**	**100**	

Ideally, the seaweed would need low ash content for optimum biogas production (Sudhakar et al., 2018).

Brown macroalgae are reported to have ash contents ranging between 3.5% and 46%. However, the anaerobic digestion process is tolerant to the higher ash content of *Ecklonia Maxima* (Ghadiryanfar et al., 2016). The protein present in the *Ecklonia Maxima* in Table 4.1 is significant at 7.8%. During the AD process, protein is hydrolysed to amino acids, then acidified to volatile fatty acids, which are converted into methane (Wang et al., 2020). Since carbohydrates hydrolyse in less time than lipids and proteins (Li et al., 2020), the protein present could slow down the overall hydrolysis of the AD process and impact on methane production.

The analysis received compares well to the proximate composition of *Ecklonia Maxima* as stipulated by Wang et al. (2020), who experimentally determined the protein content to be 12.01% ± 0.18, the ash content to be 25.52% ± 0.40, and the carbohydrate content to be 51.83% ± 0.48 (Table 4.1). The slight differences could be attributed to the harvesting time and the different environmental conditions.

The analysis of the ash showed trace amounts of the following components, which accounted for just over 1% of the total ash content:

Table 4.2: Analysis of the ash in *Ecklonia Maxima*

Component in Ash	Amount (mg)
Barium	47
Boron	6
Calcium	27.1
Cobalt	8
Copper	16
Fluorine	10
Iodine	700
Iron	1.1
Magnesium	7.4
Manganese	210
Molybdenum	9
Nickel	8
Phosphorous	3
Potassium	130
Selenium	0.4
Sodium	40
Strontium	10
Sulphur	12
Zinc	60

The organic analysis did not provide information on the chemical analysis for a bio-methane potential calculation to be completed, as described in equation 3.4. The *Ecklonia Maxima* was sent for Energy Dispersive Spectroscopy (EDS) to determine the elemental analysis of the surface of the macroalgae, as shown in Figure 4.1.

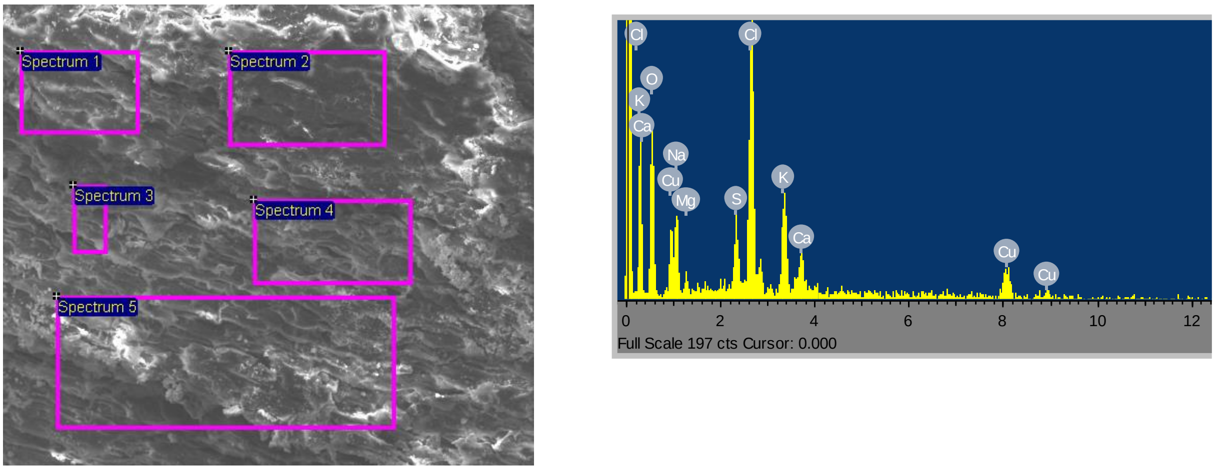

Figure 4.1: EDS surface zones and analysis of *Ecklonia Maxima*

The EDS analysis (Table 4.3) showed very high carbon and oxygen content. While the high carbon content was expected, the high oxygen content was unexpected.

Table 4.3: EDS elemental analysis of *Ecklonia Maxima*

Spectrum	C	O	Na	Mg	S	Cl	K	Ca	Cu
					Weight %				
1	48.82	32.6	2.69	0.59	1.01	7.15	3.85	1.88	1.4
2	50.76	30.54	4.67	0.58	0.75	7.98	3.31	1.41	0
3	43.02	28.38	2.85	0.47	1.22	12.24	8.47	3.36	0
4	46.43	29.11	2.74	0	1.28	9.48	5.31	1.79	3.85
5	46.77	29.59	3.3	0.45	1.64	7.9	3.55	1.42	5.39
Mean	**47.16**	**30.04**	**3.25**	**0.42**	**1.18**	**8.95**	**4.9**	**1.97**	**2.13**
Standard Deviation	2.89	1.63	0.83	0.24	0.33	2.03	2.14	0.8	2.41
Maximum	50.76	32.6	4.67	0.59	1.64	12.24	8.47	3.36	5.39
Minimum	43.02	28.38	2.69	0	0.75	7.15	3.31	1.41	0

However, The EDS is not a true reflection of the oxygen content as elements like hydrogen and nitrogen were not accounted for in the analysis. The higher-than-expected oxygen content could influence the anaerobic environment needed for biogas production.

The *Ecklonia Maxima* was further sent for C-H-N-S analysis at the ICP unit at Stellenbosch University, South Africa, as shown in Table 4.4 below. Even though the oxygen content could not be determined, the total weight percentage of the C-H-N-S is only 87.371%.

Table 4.4: C-H-N-S analysis of *Ecklonia Maxima*

	Name	Weight mg	N (%)	C (%)	H (%)	S (%)
QC value	**Cert Ref Std sulfamethazine**		20.67	52.84	4.91	11.52
	QC Analysed	5.03	19.99	51.15	4.72	11.51
	LOD		*0.03*	*0.45*	*0.22*	*0.15*
	% Recovery calculated		***96.7***	***96.8***	***96.1***	***99.9***

Assuming all the other elements besides carbon in Table 4.3 account for the other 12.629%, it can be assumed that the oxygen content is approximately 30% of this value. The overall analysis for *Ecklonia Maxima* to determine the theoretical bio-methane potential is estimated at 51.15% C, 19.99% N, 3.789% O, 4.719% H, and 11.512% S on a mass basis. The other 8.84% is a combination of trace metals and other components. A comparison of *Ecklonia Maxima* chemical analysis is given in Table 4.6. Cited analyses and the one completed in this study show marked differences in the carbon, nitrogen, and oxygen content. Darko et al. (2022) collected the *Ecklonia Maxima* from the West Coast of SA, which was the same collection area for this study. Darko et al. (2022) used residual *Ecklonia Maxima* after it had been subjected to polysaccharide extraction. Polysaccharide (or carbohydrates) removal would negatively affect the biogas production and reduce the carbon content of the substrate. In this case, the chemical analysis for Darko et al. (2022) shows a 50% decrease in carbon content compared to the *Ecklonia Maxima* (Table 4.5) before polysaccharide extraction. Seasonal variation in the carbon content of *Ecklonia Maxima* collected along the West Coast of SA differed from month to month, as well as from the southwest and west side of the coast. The composition of the seaweed varies depending on the location it is collected as well as the season when it is collected.

Table 4.5: Comparison of *Ecklonia Maxima* elemental analysis

	Chemical composition of *Ecklonia Maxima* (weight %)				
	C	N	O	H	S
This study	51.15	19.99	3.789	4.719	11.512
Darko et al., 2022	24.990	1.770	58.548	4.709	BDL
Smith, 2007	33.82	1.770			

4.1.3 Bio-methane potential

The bio-methane potential is calculated using equation 3.4:

$$BMP\left(\frac{ml\ CH_4}{g\ VS}\right) = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c}$$

It is based on equation 3.3, which is:

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3$$

This relation does not include the sulphur content of the *Ecklonia Maxima*.

A modified version of the Buswell and Mueller equation by Boyle includes sulphur, which allows for the fraction of ammonia and hydrogen sulphide to be estimated in the product stream. This calculation method is preferred since the estimated sulphur percentage is significant at 11.5%.

The Theoretical Biochemical Methane Potential is calculated using equation 3.6:

$$TBMP = \frac{22.4 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12.017a + 1.0079b + 15.999c + 14.0067d + 32.065e}$$

It is based on equation 3.5:

$$\begin{aligned} &C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \\ &\qquad \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \end{aligned}$$

Using the estimated values for C-H-N-O-S as stipulated above (section 4.1.2) and converting the mass fractions to mole fractions, the chemical formula for *Ecklonia Maxima* is $C_{0.0426}H_{0.0472}N_{0.0143}O_{0.0024}S_{0.0036}$.

The stoichiometric equation based on the Buswell equation, utilising the calculated values of the hydrogen, oxygen, nitrogen, and sulphur, is:

$$C_{0.0426}H_{0.0472}O_{0.0024}N_{0.0143} + 0.0404H_2O \rightarrow 0.0213CH_4 + 0.0214CO_2 + 0.0143NH_3 \qquad (4.1)$$

The calculated value for BMP is 598.04 L/kg VS. The expected carbon conversion is based on the coefficients for both CO_2 and CH_4. It is estimated as follows:

$$Carbon\ conversion\ to\ methane = \frac{fraction\ of\ methane\ formed}{methane\ fraction + carbon\ dioxide\ fraction} \qquad (4.2)$$

It is estimated that 49.89% of the carbon is converted to methane gas.

The stoichiometric equation based on Boyles modified Buswell equation is:

$$C_{0.0426}H_{0.0472}O_{0.0024}N_{0.0143}S_{0.0036} + 0.0422H_2O \rightarrow 0.0204CH_4 + 0.0223CO_2 + 0.0143NH_3 + 0.0036H_2S \qquad (4.3)$$

The calculated value for BMP is 499.64 L/kg VS. In the same way, it is estimated that 47.78% of the carbon is converted to methane gas using the Boyle-modified Buswell equation. The calculated amount does not consider the hydrolysis stage and is therefore estimated to be higher than the actual yield. The difference between the two calculations is attributed to the inclusion of sulphur in the calculations for the modified Buswell equation. Both the Buswell and the modified Buswell equations assume that the composition of the feedstock only consists of the identified elements in the estimated chemical formula. This is not the case, as the components only account for 91% of the feedstock, including the sulphur. Therefore, this lower percentage is expected to influence the actual bio-methane formed. According to Milledge et al. (2019), the actual methane yield in the anaerobic digestion process could vary between 19–81% of this theoretical amount determined.

The calculated amounts are compared to the actual bio-methane produced in section 4.1.6. *Ecklonia Maxima*, using a modified version of the Buswell equation utilising only C-N-H-S data, estimated the stoichiometric methane potential to be 990.85 L/kg VS (Darko et al., 2022). The difference could be because the seaweeds were harvested in different areas and under different conditions, and different plant parts have been used to determine the methane potential. Tabassum et al. (2018) reported a BMP for the brown seaweeds *A. Nodosum* at 95 L CH_4/kg VS and *S. latissima* at 342 L CH_4/kg VS. The large discrepancy is mainly attributed to the differences in composition across the plant (frond, stripe, and holdfast) and the composition differences for each seasonal harvest.

4.1.4 Lignin analysis

Lignin content was determined using the Klason method (Kirk & Obst, 1988). The lignin analysis of the seaweed was determined to be 29%. This value was averaged over a total of three tests performed. Macroalgae are reported to have low levels of lignin (Dave et al., 2013; Obata et al., 2020; Ra et al., 2016; Pfromm et al., 2011; Yanagisawa et al., 2013; Ghadiryanfar et al., 2016). Rabemanolontsoa and Saka (2013) reported the lignin content of Sargassaceae, which is a brown seaweed, determined using a modified version of the Klason method, as between 6% and 12.9% of the dry weight of the seaweed. This is significantly less than the amount of lignin determined experimentally for the *Ecklonia Maxima*. The difference can be attributed to different process conditions, as the same process was not used in both cases. Red and Green seaweeds had a lignin content ranging between 1.1% and 3% of the dry weight of the seaweed.

Comparisons can be difficult among macroalgae for lignin content as well as chemical composition due to the varying composition of the seaweed due to different environmental conditions, which would affect the growth of the seaweed. However, the higher-than-expected lignin content of the *Ecklonia Maxima* suggests that a pre-treatment step is necessary to optimise biogas production, as it might be more challenging for the biomass to release fermentable sugars.

4.1.5 Statistical analysis of data

A normally distributed population was assumed for each experimental run. This was tested by drawing a histogram for each experimental run and looking for a normally distributed curve. A paired sample t-test analysis was performed using Microsoft Excel to test for statistical variance between the mesophilic and thermophilic bio-methane yields.

- The null hypothesis: The digestion temperature does not affect the biogas yield.
- Alternate analysis: The digestion temperature affects the biogas yield.

Table 4.6: Comparison of the F-Test Two-Sample for Variances

F-Test Two-Sample for Variances						
	Cow Manure		**Vitaboost**		**BioGanic**	
	Meso	**Thermo**	**Meso**	**Thermo**	**Meso**	**Thermo**
Mean	5.77	12.36	4.29	35.82	26.31	171.83
Variance	25.90	234.03	46.03	1405.20	244.62	9709.09
Observations	10	10	10	10	10	10
Df	9	9	9	9	9	9
F	0.111		0.033		0.025	
P(F<=f) one-tail	0.0015		0.00001		0.000004	
F Critical one-tail	0.3146		0.31457		0.315	

A 95% confidence interval was used. The null hypothesis is rejected if the calculated P-value is less than 0.05 and the alternate analysis is valid. Should the P-value be more than 0.05, the null hypothesis is valid. The P-value for cow manure, Vitaboost and BioGanic is less than 0.05 (Table 4.6). The null hypothesis is therefore rejected, and the alternate hypothesis is accepted. Temperature affects the bio-methane yield for all three inoculum/seaweed combinations.

4.1.6 Comparison of the performance of inocula in anaerobic digestion of *Ecklonia Maxima*

Initially, five inocula were identified to introduce the biological environment for the AD process. These included two biological inocula and three synthetic inocula. The biological inocula included cow manure from grass-fed cows and starter fluid from the municipal sewer. It was challenging to collect additional starter fluid required to duplicate the experiments as the municipal sewer had fluctuating biological compositions and performance issues of the RAS. After the first three experimental runs, the starter fluid was rejected as a possibility due to these reasons. The synthetic inocula used were as described in Chapter Three. The Bio Ocean inoculum was rejected as it contains Kelp, which is a brown seaweed. Adding another seaweed could influence the results and give an inaccurate representation of the performance of adding inoculum to the *Ecklonia Maxima* during a comparative analysis with other inocula. The performance of the Bio Ocean inoculum is available as a stand-alone article, which has been published in a peer-reviewed journal. All calculations for the biogas produced were standardised at STP (0 ^{o}C; 101.3 kPa). The calculations considered the volatile solids due to the seaweed present during sampling. Experimentation was performed at mesophilic and thermophilic conditions to determine if temperature affected the biogas yield.

4.1.6.1 Mesophilic temperature biogas production

Figure 4.2 provides an overview of the cumulative methane formation in ml per gram of volatile solids at mesophilic temperatures. All inoculum/seaweed slurries showed a lag phase of at least 8 to 12 days (Figure 4.2) before any biogas was produced, which could result from the first feeding of the anaerobic digestion system only taking place on day 8. The methane production was initially slow, which was expected as the AD system was still breaking down and hydrolysing the organic matter.

The accumulative methane production for the cow manure seemed very low compared to the other inocula. It was also low compared to what is cited in the literature. Vergara-Fernandez et al. (2008) stipulate a biogas formation of 179 ml/g VS over 31 days using the brown seaweed *Durvillea antarctica*. In the exact text, *Macrocystis pyrifera* yielded 181 ml/g VS of biogas. This aligns with the biogas produced using *Ulva lactuca*, which was 196 ml/g VS, but over 82 days (Costa et al., 2012). It is not easy to compare yields based on literature as the yields were determined using varied methodologies.

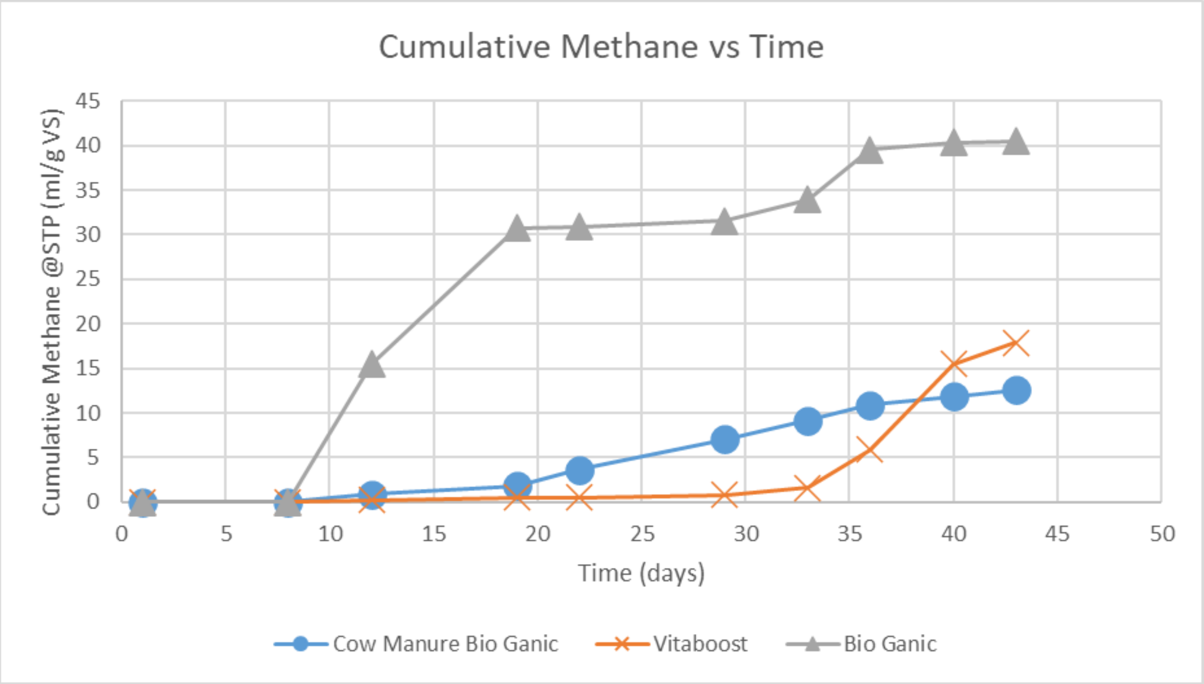

Figure 4.2: Cumulative methane production vs. time under mesophilic conditions

The biogas production using *Ecklonia Maxima* increased steadily throughout the contact time. A longer contact time could see a continued upward trend with a higher biogas yield. The slow biogas formation could be due to the manure's relatively low organic load with a high nitrogen concentration, which could result in the initial inhibiting of the formation of biogas. The methane production could significantly increase with continued contact and extended process time. As the period was not increased, this is purely an observation based on the methane yield trend seen. The cumulative methane yield over the 42 days for cow manure was low at 12.5 ml/g VS. The VS content of the cow manure was 0.6 g/g solids. BioGanic performed well when compared to the two other inocula. The biogas production increased until day 35, when it started stabilising. Continuing with experimentation would be unnecessary once the biogas yield started levelling off, as microbial growth has moved from the exponential phase to the stationary phase.

This was noted on day 36 for the BioGanic inoculum. Methane production averaged 37% of the entire biogas production, with the balance being carbon dioxide, hydrogen sulphide and other gases. This is below the expected calculated value of 49.89%, which assumed that only methane and carbon dioxide would be produced, which is not the case. The discrepancy could be due to these other gases forming during the AD process. There are no documented cases of this synthetic inoculum being utilised in producing methane using an AD system, so comparisons with literature and performance expectations cannot be discussed in detail.

The lag phase for the Vitaboost was more prolonged (19 days) than the other two inocula, indicating a more extended period of acclimatisation. Therefore, the upward trend remained prevalent after 42 days, indicating an exponential phase. As indicated in Table 4.7, Vitaboost shows a relatively low VS content at 0.287 g/g solid compared to BioGanic at 0.72 g/g solid. The Vitaboost may need more VS in the system to produce significant amounts of methane as the organic load is too low. This could account for the relatively low methane yield of 17.9 ml/g VS compared to the 40.5 ml/g VS methane yield for the BioGanic (Table 4.7).

Table 4.7: Cumulative methane production over 42 days for mesophilic conditions

Cumulative Biogas Production (ml/g VS)

Day	Vitaboost	Cow Manure	BioGanic
1	0.000	0.000	0.000
8	0.000	0.000	0.000
12	0.232	0.852	15.508
19	0.485	1.788	30.748
22	0.527	3.631	30.894
29	0.796	7.062	31.537
33	1.603	7.062	33.968
36	5.888	9.120	39.566
40	15.462	10.889	40.358
43	17.911	11.844	40.478

Vitaboost showed a 3% yield of the calculated methane potential. The cow manure showed a 2.4% yield, and the BioGanic showed an 8.1% yield.

As previously discussed, Vitaboost showed a more prolonged lag phase, only producing significant amounts of biogas after 30 days. This could be because the inoculum was palletised instead of granulated, which increased the degradation time required, thereby increasing the hydrolysis time required. A longer contact time could lead to increased biogas production. Mechanical pre-treatment of the inoculum and increasing the surface area could also increase biogas production. Once started, the Vitaboost performed better than the manure but not as well as the BioGanic. There was, however, a very sharp upward trend in biogas production after day 35 for the Vitaboost, which indicates improved biogas production after the long acclimatisation period.

Overall, the daily methane production for the BioGanic was higher than those of the manure and Vitaboost. Maximum daily methane production for the BioGanic was on days 12 through 19. Afterwards, there was a steady decline in methane production, even with periodic feeding of the system with seaweed, as seen in Figure 4.3 below, after which the system started to show an upward trend again. This is likely due to the introduction of organic matter and volatile solids from the seaweed biomass. Cow manure was consistently low, whereas the Vitaboost peaked at day 36.

Figure 4.3 shows a normal distribution of the yield data, which means the data could be further analysed using an ANOVA for variance. This is in line with Khanal (2008), who stipulated that when both acidogens and methanogens exist in the same culture, the optimum pH for anaerobic digestion is between 6.8 and 7.4. Even though all inoculum/seaweed combinations operated within the expected pH range, the BioGanic displayed a narrower pH range of 7.0 and 7.5.

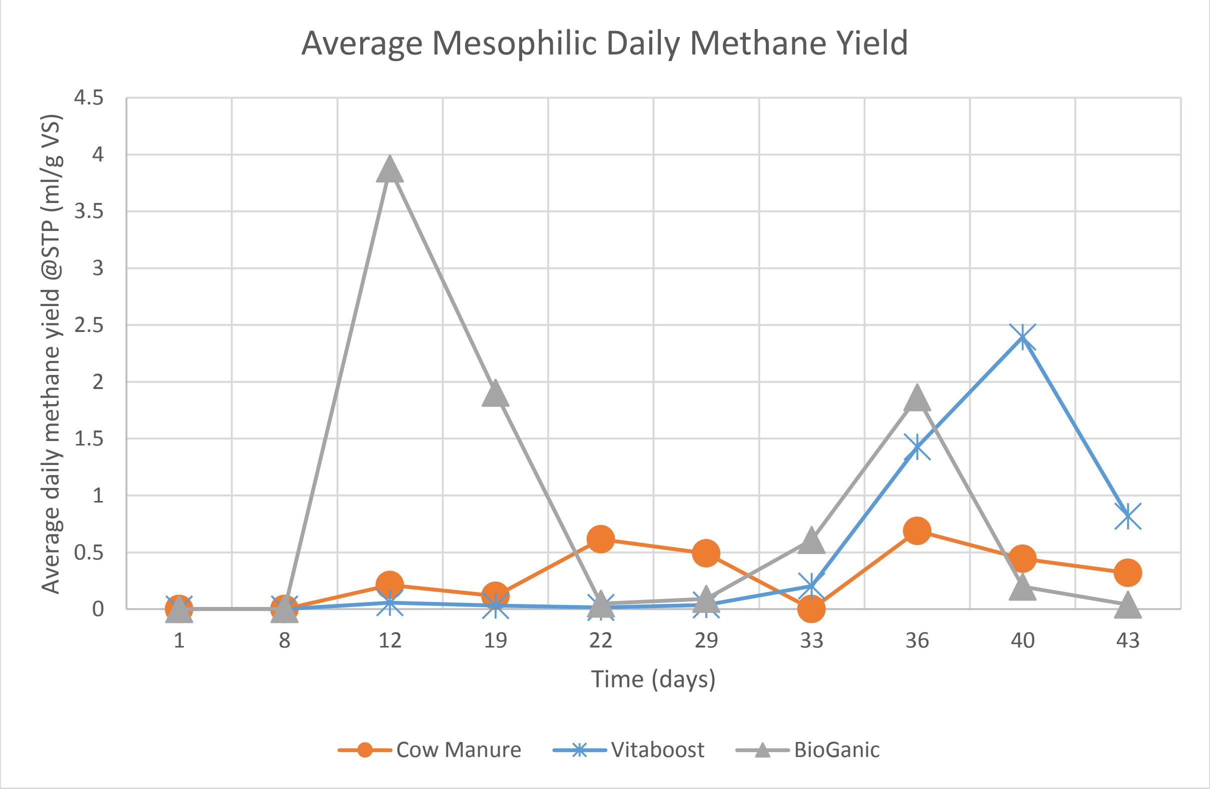

Figure 4.3: Average daily methane yield under mesophilic conditions

Both figures 4.4 and 4.5 indicate an optimum pH range of 6.9 and 7.6 for biogas production using the AD process.

There was a pronounced dip in the pH within the first eight days. This is because the system had not been fed and was slightly acidic due to the possible onset of acidogenesis. As the biomaterial degraded over the first eight days, the amount of volatile fatty acids increased, which could also contribute to a lowered pH value. An alkaline pH yielded a higher biogas amount when compared to a neutral and acidic pH using cow manure (Bahira et al., 2018). A pH value of 8.52 saw biogas production start within 24 hours, while biogas production was delayed by up to eight days for an acidic pH of 4.52.

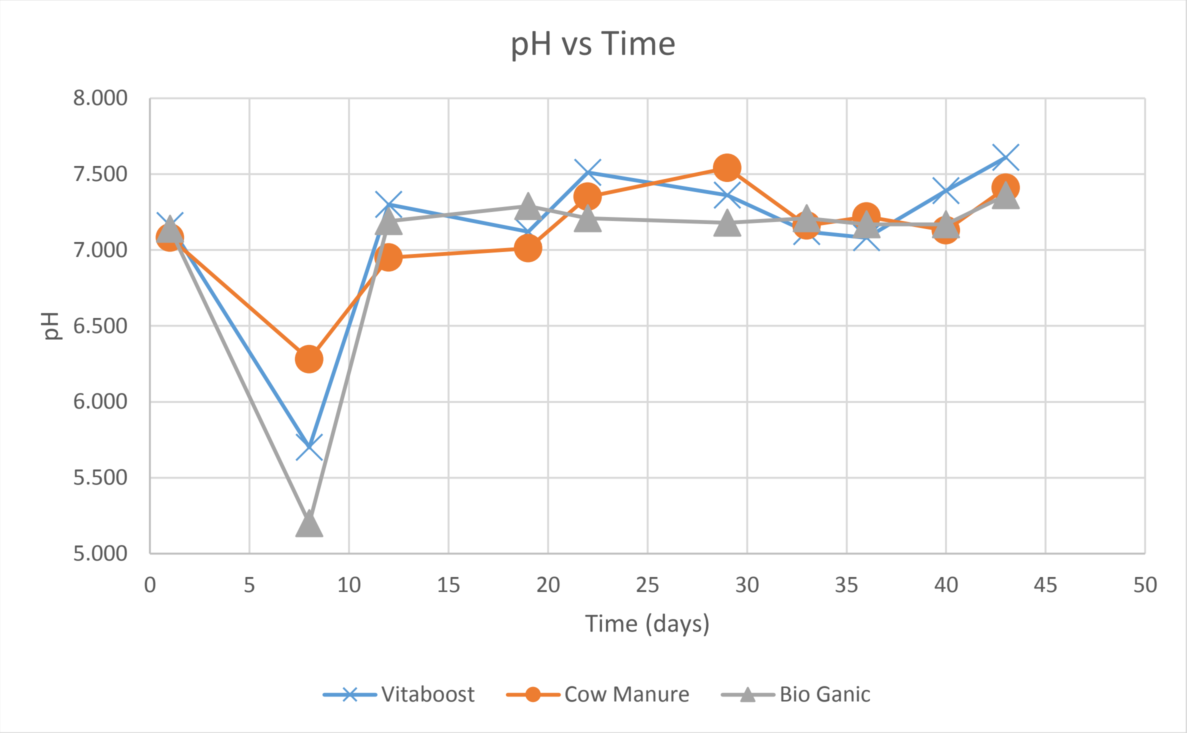

Figure 4.4: pH vs. time for mesophilic conditions

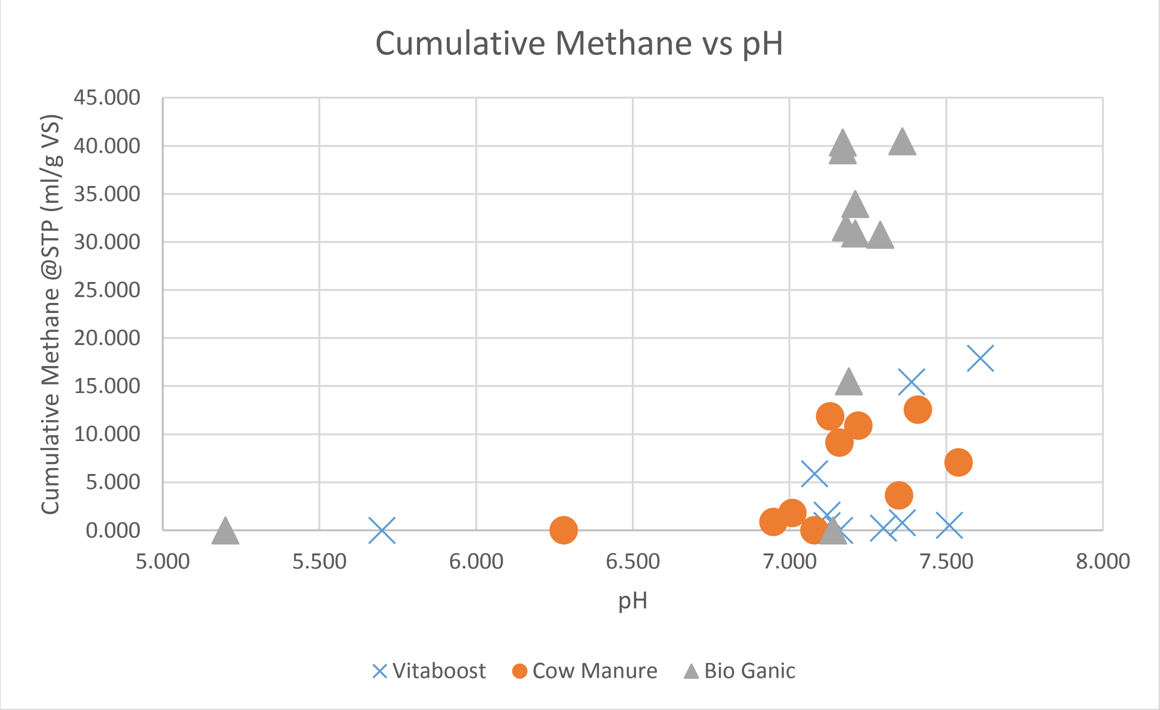

Figure 4.5: pH vs. cumulative methane production under mesophilic conditions

The lower yield at acidic conditions is corroborated in literature by Budiyano et al. (2014) and Augenstein et al. (1976), who suggested that during anaerobic fermentation, micro-organisms require a natural or mildly alkaline environment for efficient gas production.

Within the timeframe parameters of 42 days, the BioGanic out-performed the other inocula for the mesophilic temperature. This is based solely on the bio-methane yield as described above and is not conclusive evidence that it is the best-performing inoculum to improve the biogas yield of *Ecklonia Maxima* using the AD process.

4.1.6.2 Thermophilic temperature biogas production

A similar lag phase as noted in the mesophilic temperature range was evident using a higher temperature for the AD process. The biogas yield using a cow manure/seaweed slurry was low compared to the other two inocula. The lag phase was long at 15 days (Figure 4.6). At higher temperatures, researchers have observed an increase in the concentration of free ammonia, which acts as an inhibitor to the AD process (Babaei & Shayegan, 2019). At the higher temperature of 52 ^{o}C, a yield of 37.3 ml/g VS was observed using the cow manure inoculum. This is three times the yield for the mesophilic temperature. Even though the lag phase was longer than that observed at the lower temperature, the higher biogas yield was observed over a shorter period. The increased yield is due to the higher temperature, which improves the biomaterials' degradation rate, leading to faster hydrolysis and an increased biogas yield (Mao et al., 2015). The extended lag phase could be attributed to acclimatisation, as the inocula are typically reactive at the lower mesophilic temperatures. Based on the results presented in Table 4.8, the Vitaboost inoculum yielded 18% of the calculated methane potential.

Table 4.8: Cumulative methane production over 42 days for thermophilic conditions

Day	Vitaboost	Cow Manure	BioGanic
1	0.000	0.000	0.000
8	0.000	0.000	10.670
12	6.619	0.000	95.902
15	7.741	0.387	147.635
19	8.572	1.269	202.123
22	8.963	1.502	210.978
26	10.610	2.032	216.376
29	25.321	4.746	222.696
33	53.466	16.696	233.836
36	79.053	29.091	239.115
40	86.164	32.991	248.283
43	90.032	37.354	254.680

Cow manure yielded 7.5% of the calculated methane potential, and BioGanic yielded the highest conversion at 51% of the calculated methane potential.

In the same way as seen in the mesophilic temperature range, biogas formation goes through an exponential phase, followed by a stationary phase. Once this stationary phase is reached, continuing with the AD process would only be recommended if additional feedstock (seaweed) and inoculum are introduced into the system to jumpstart the microbial growth.

The lag phase for the BioGanic inoculum was less than in the mesophilic temperature range (8 days instead of 12), and biogas formation started immediately after feeding. For the higher temperature, there are no inhibitory conditions to the AD process, with the breakdown of the biological materials not being affected. Rapid biogas yield occured between days 10 and 19, indicating an exponential phase of the inoculum growth, followed by slower biogas yield rates for the BioGanic inoculum, indicating a stationary phase of inoculum growth (Table 4.8).

In a study by Tabassum et al. (2018), BioGanic yields compared well with the brown seaweed BMP. The bio-methane yield was 74% of the BMP for *S. latissima* and much higher at nearly three times the BMP amount for *A. nodosum*. The yield was only 26% of the BMP for the *Ecklonia Maxima*, as cited by Darko et al. (2022). Vitaboost showed rapid biogas yields between days 26 and 36. It can be assumed that at this stage, degradation of the biological material was complete (hydrolysis), which means that biogas formation could commence.

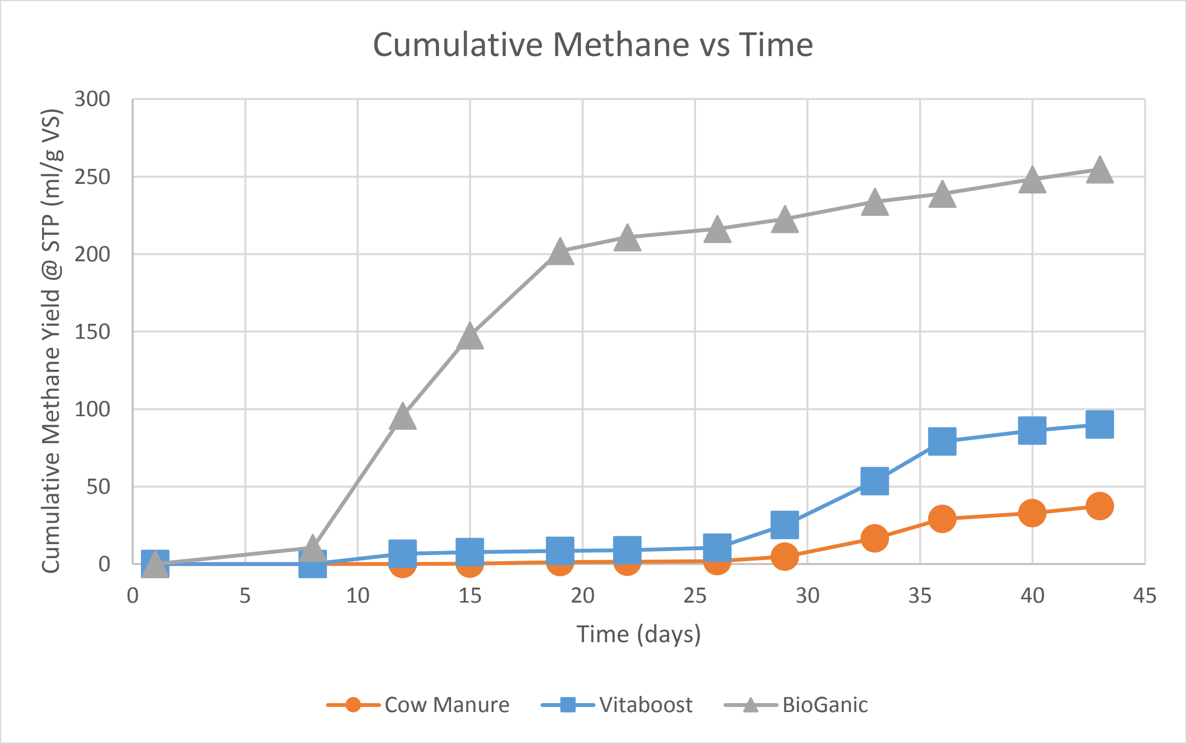

Figure 4.6: Cumulative methane production vs. time for thermophilic conditions

The lag phase for the BioGanic inoculum was less than in the mesophilic temperature range (8 days instead of 12), and biogas formation started immediately after feeding. For the higher

temperature, there are no inhibitory conditions to the AD process, with the breakdown of the biological materials not being affected. Rapid biogas yield occured between days 10 and 19, indicating an exponential phase of the inoculum growth, followed by slower biogas yield rates for the BioGanic inoculum, indicating a stationary phase of inoculum growth (Table 4.8).

BioGanic yields compared well with the brown seaweed BMP that Tabassum et al. (2018) stipulated. The bio-methane yield is 74% of the BMP for *S. latissima* and much higher at nearly three times the BMP amount for *A. nodosum*. The yield is only 26% of the BMP for the *Ecklonia Maxima*, as cited by Darko et al. (2022).

Vitaboost shows rapid biogas yields between days 26 and 36. It can be assumed that at this stage, degradation of the biological material is complete (hydrolysis), which means that biogas formation can commence.

Similar trends in higher yields described for the cow manure are noted for the other two inocula. Bio-methane yield using Vitaboost was 90 ml/g VS (Table 4.9), five times the mesophilic yield observed. The BioGanic yield was 254.7 ml/g VS, 6.3 times the mesophilic yield observed. This indicates that the increased temperature positively affects the biogas yield for all inocula. BioGanic shows the highest bio-methane yield over the timeframe investigated.

Table 4.9: Comparison of bio-methane yield for mesophilic and thermophilic conditions

Inoculum	**Cow Manure**		**Vitaboost**		**BioGanic**	
Temperature	37 ºC	52 ºC	37 ºC	52 ºC	37 ºC	52 ºC
Bio-methane Yield (ml/g VS @ STP)	11.844	37.354	17.911	90.032	40.478	254.580
Ratio (52 ºC/37 ºC)	3.15		5.03		6.29	

For the BioGanic inoculum, biogas production peaked at 12 days and then decreased slowly until day 19. After that, there was quite a sharp decline in bio-methane production despite the introduction of additional VS with the seaweed feeding, indicating a death phase in the inoculum growth. No new inoculum was introduced into the system, which could be required to assist in the conversion of seaweed to biogas at the higher operating temperature. The inoculum introduces the initial microbial environment to assist in the degradation of the seaweed. With a higher temperature, the degradation rate is increased, but microbes are sensitive to temperature. The synergistic effect of the microbes can change with an increase in temperature, leading to variations in methane production and process stability (Li et al., 2020). The variations in bio-methane production are evident for all three inocula (Figure 4.7).

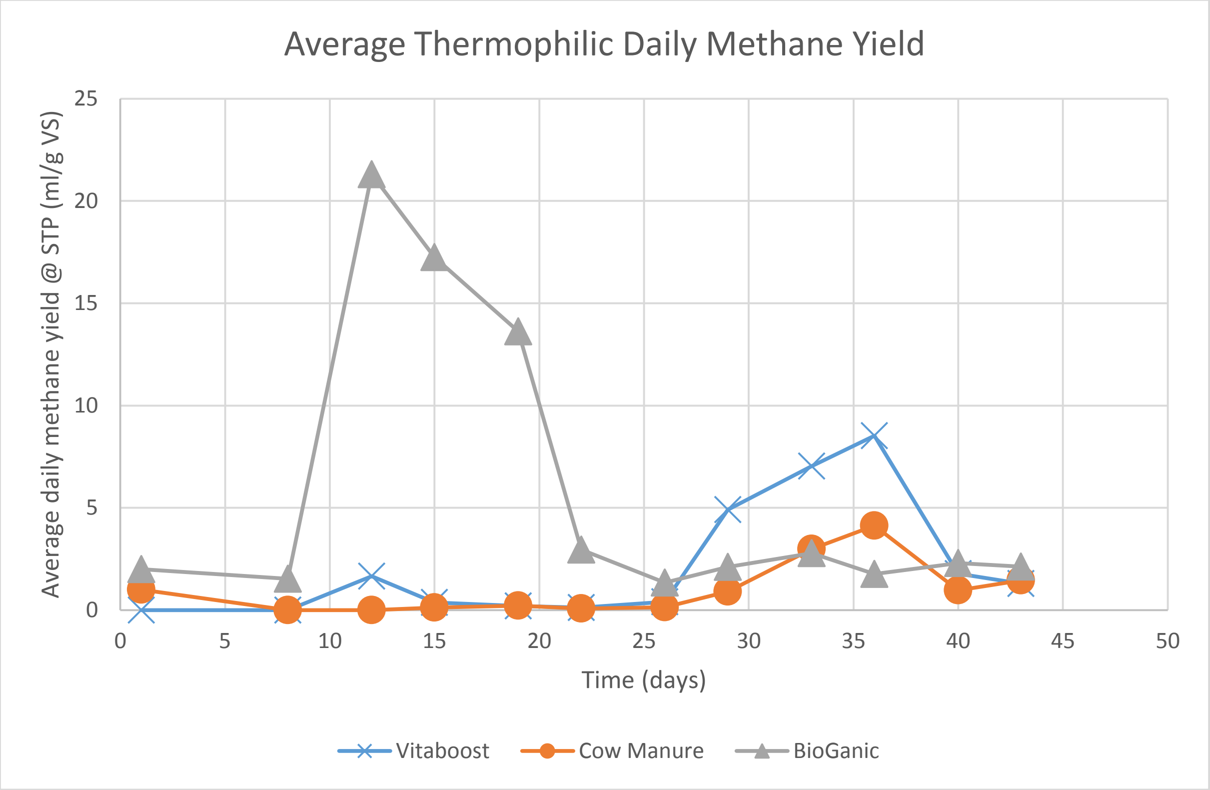

Figure 4.7: Average daily methane yield for thermophilic conditions

Biogas production occurred between the pH range of 6 and 7.7 (Figures 4.8 and 4.9). The upper limit is similar to the mesophilic pH range. However, the lower limit is lower than the 6.9 observed for the mesophilic range. Initially, at a lower pH value, biogas yields are low. Generally, when the pH increases to between 7 and 7.7, the biogas yield increases at an elevated temperature. Biogas production is still within the optimum range for AD, according to Khanal (2008).

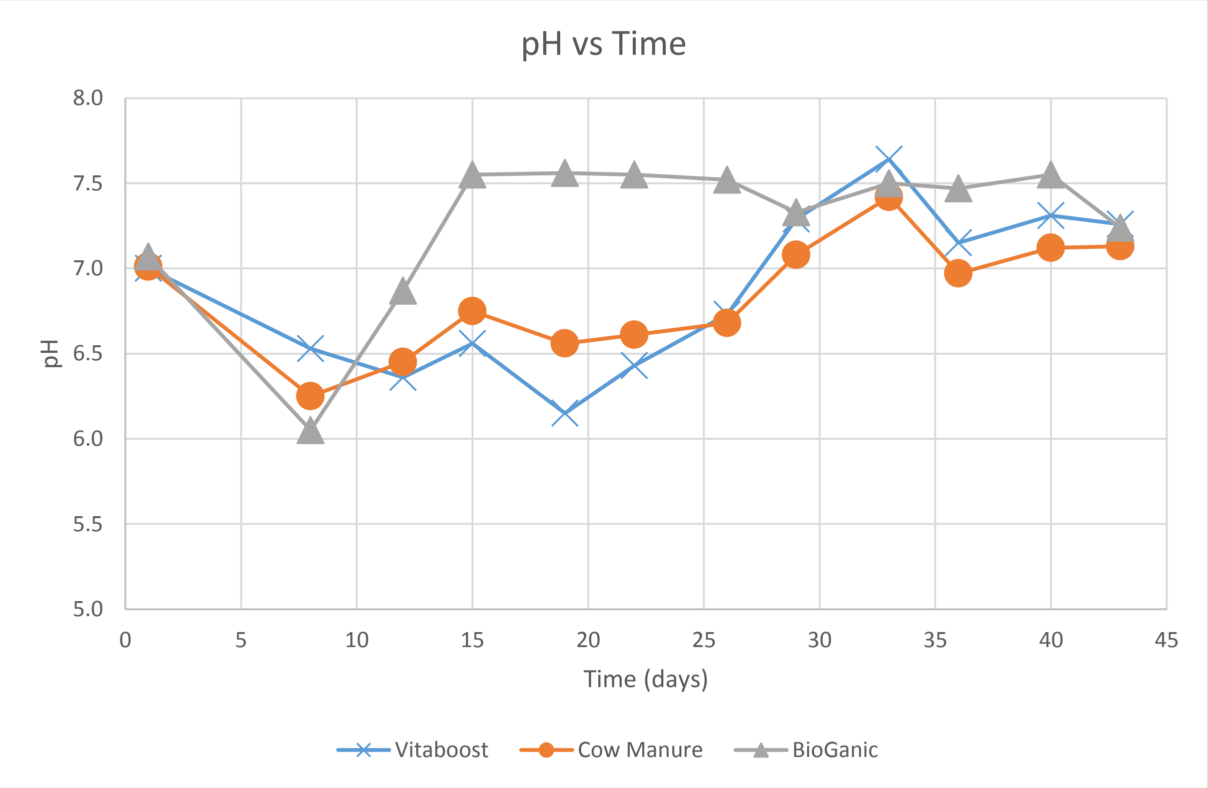

Figure 4.8: pH vs. time for thermophilic conditions

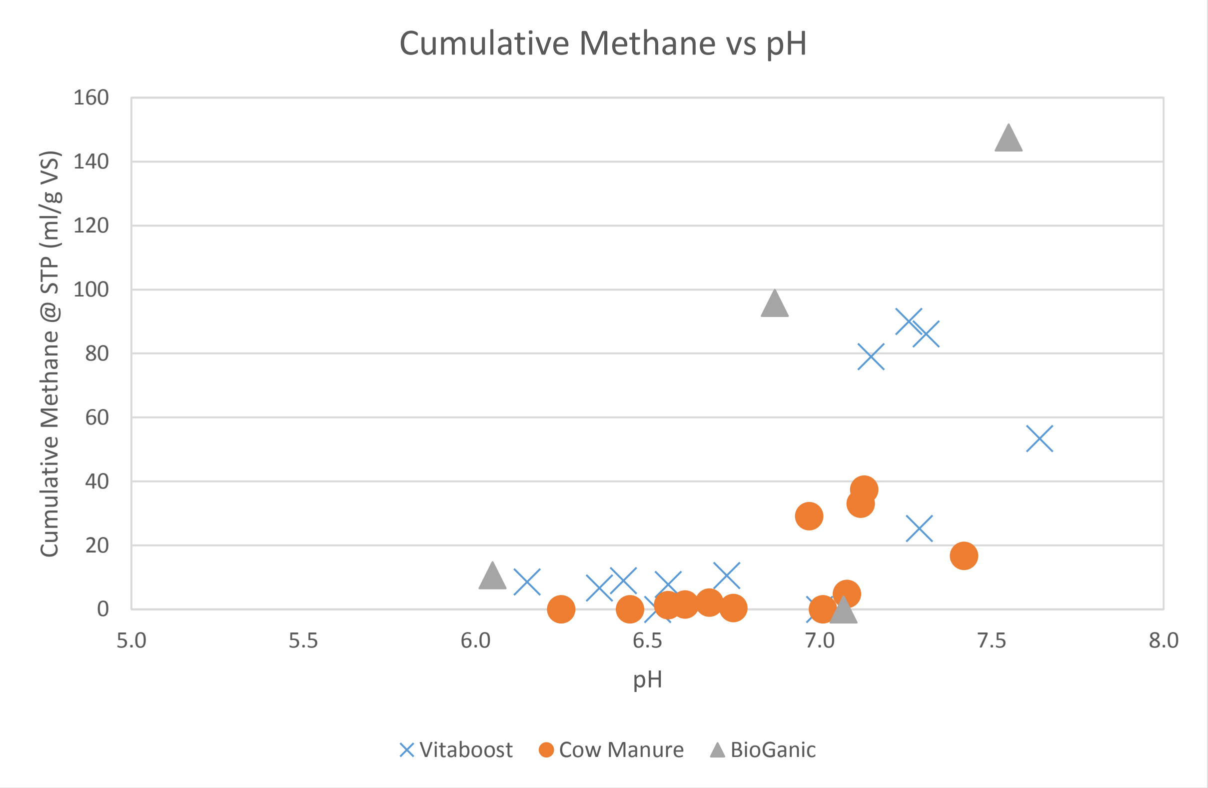

Figure 4.9: pH vs. cumulative methane production for thermophilic conditions

4.1.7 Conclusion

Based solely on the temperature data collected, the BioGanic inoculum/seaweed slurry yielded the highest bio-methane yield at both the mesophilic (40.478 ml/g VS) and thermophilic (254.580 ml/g VS) temperature ranges. The bio-methane yield was highest for all three inocula in the thermophilic range. The statistical analysis performed corroborates the fact that temperature affects the bio-methane yield. The increased operating temperature could have significant operating challenges and associated increased operating costs. This could be mitigated by increasing the biogas yield for the mesophilic temperature range, which could be accomplished by pre-treating the seaweed biomaterial to try and increase the rate of the hydrolysis stage, which is the rate-limiting step of the AD process.

4.2 Objective 2: Investigating pre-treatment processes to optimise the production of biogas from seaweed

4.2.1 Introduction

The BioGanic inoculum yielded the highest amount of biogas, based on section 4.1 above. The BioGanic/seaweed combination was selected to investigate pre-treatment processes to improve the algal biomass's methane yield. The thermophilic temperature range showed the highest yield based on initial experimentation, but it was decided to apply the pre-treatment processes for both temperature ranges, as only selecting the higher temperature range could have additional cost and operational implications. Should the pre-treatments applied bring the biogas yield for the mesophilic range to within the yield range for the thermophilic temperature range, it might be feasible to utilise the lower temperature and the additional pre-treatment step. This can only be established once a full suite of pre-treatment results are obtained.

Three different pre-treatment methods were utilised: mechanical, chemical, and thermal. Statistical analysis was performed where a simple F-Test, Two-Sample for Variances, was performed at a 95% confidence interval for each pre-treatment process.

The null hypothesis: Pre-treatment of the biomass does not influence the biogas yield.
The alternate hypothesis: Pre-treatment of the biomass does influence the biogas yield.

Should the p-value be less than 0.05, the null hypothesis is rejected, and the alternate hypothesis is valid.

4.2.2 Mechanical pre-treatment

Grinding was selected as the mechanical pre-treatment. The aim was to break down the cell walls to increase biodegradability during anaerobic digestion (Tedesco et al., 2014). Seaweed biomaterial was ground and sieved to 1.7–3 mm and less than 1.7 mm. These two size distributions were combined with the BioGanic inoculum and anaerobically digested at both mesophilic and thermophilic temperatures for 28 days. The results are illustrated in Figure 4.10 below.

The <1.7 mm size distribution range performed best in the mesophilic temperature range based solely on cumulative methane yield. This was followed closely by the thermophilic 1.7–3 mm size distribution range. The smaller particle size was expected to show improved hydrolysis and, therefore, a higher yield, as the particles are characterised by larger surface area and degrade quicker. This would increase the hydrolysis rate and increase the biogas yield. However, it was surprising that the increased temperature and the small-size distribution's surface area did not show the highest biogas yield. At higher temperatures (thermophilic), the VFA production increased when compared to the mesophilic temperatures (Cho et al., 2013). Combining the higher temperature with the increased hydrolysis and acidogenesis as a result of the reduced particle size lead to a double effect on the increase of VFA, which could ultimately hamper methanogenesis, decreasing the overall biogas formation (Montingelli et al., 2016). Careful consideration must be given to the particle size distribution and other factors such as pH, inoculum selection and reactor type, which could all affect the AD process (Maneein et al., 2018).

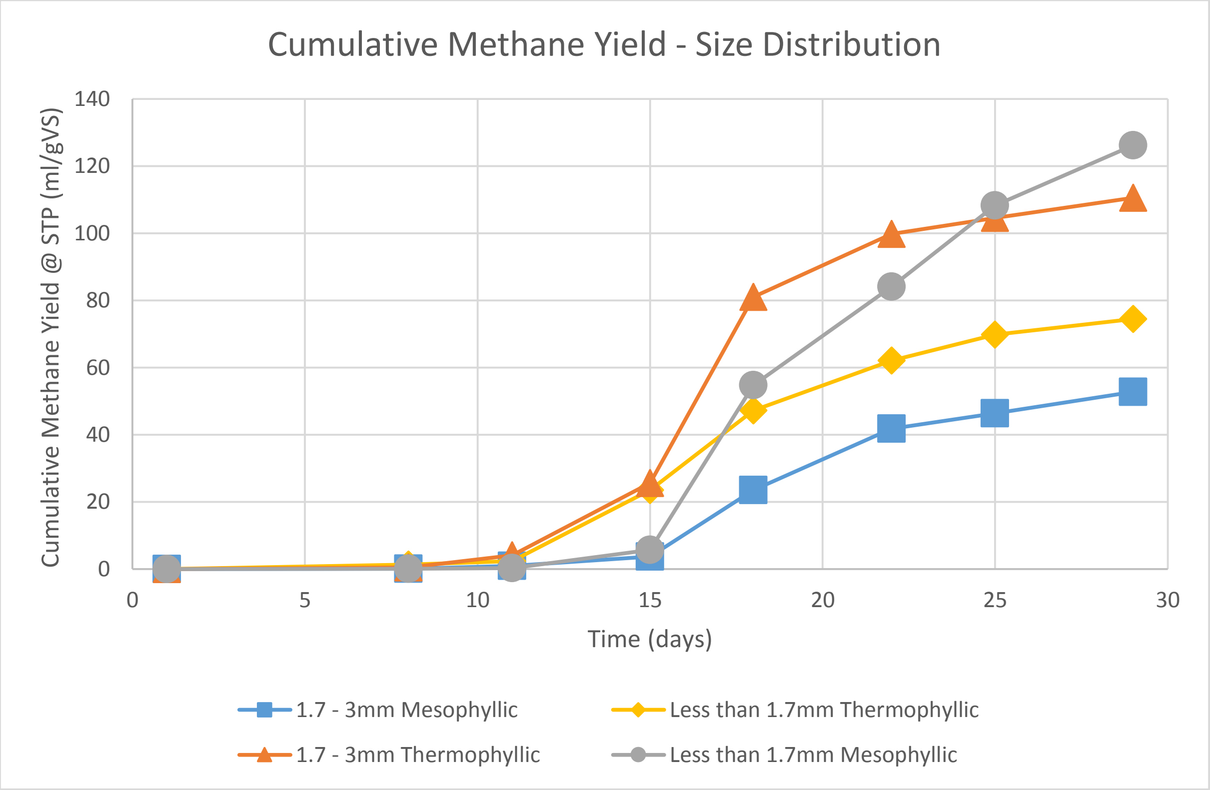

Figure 4.10: Cumulative methane yield at various particle size distributions

Table 4.10 and Figure 4.11 compare the methane yield of the two size distributions. Table 4.10 also compares the methane yield to a raw seaweed size of approximately 2 mm. In the mesophilic temperature range, decreasing the size distribution to an almost fine powder significantly increases the methane yield compared to results obtained for the raw seaweed size distribution. An increase is also noted for the mixed ratio containing smaller and larger particles. In the thermophilic temperature range, only 33% to 50% of the raw seaweed yield is noted for the two size distribution profiles.

Table 4.10: Comparison of methane yield at various size distribution

	Size Distribution					
	< 1.7 mm		**1.7–3 mm**		**Raw Seaweed ±2 mm**	
	Meso	**Thermo**	**Meso**	**Thermo**	**Meso**	**Thermo**
Methane Yield (ml/g VS)	126.157	74.48	52.740	110.59	31.537	222.696
% of Raw Seaweed Yield	400	33	167	50		

A significantly reduced size and a combination of very fine and bigger particles negatively impact the methane yield at higher temperatures, which corroborates the statements made by Montingelli et al. (2016) and Maneein et al. *(*2018) above. The finer particles clog up the system, making it difficult for the gases to be released.

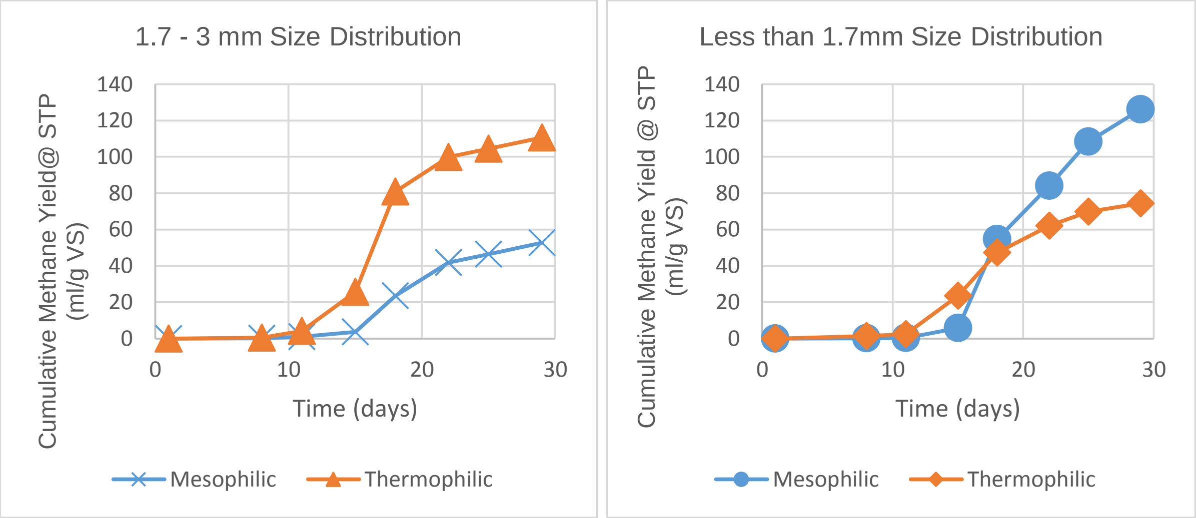

Figure 4.11: Comparison of methane yield vs. time for mesophilic and thermophilic temperature ranges for varying size distributions

There are no documented studies where *Ecklonia Maxima* was mechanically (grinding) pre-treated and then utilised in the AD process to yield biogas, making it difficult to compare the results obtained with those in the literature directly. There are limited studies on brown macroalgae that can be utilised. Montingelli et al. (2016) found a 27% reduction in bio-methane yield using ball-milled 1 mm *Laminaria spp.* compared to untreated seaweed at mesophilic conditions. There was also a 21% reduction in bio-methane yield when a 2 mm size distribution was utilised. Initially, there was an increase in biogas yield (after three days), which was attributed to a faster hydrolysis stage due to the smaller size distribution. However, ultimately, the pre-treatment did not increase the overall biogas yield.

On the other hand, ball milling showed a slight increase when compared to untreated seaweed, but this increase was found not to be statistically significant. This is opposite to what was found in this study for mesophilic conditions, where an increase in biogas yield was observed, with a decrease in yield for the thermophilic temperature range. The differences can be attributed to the fact that three types of seaweed were used to make up the *Laminaria spp.* mixture, and the inoculum used was sewage sludge. This shows that the type of seaweed and the composition at harvesting, combined with the inoculum selection, dramatically affect the AD process. The inoculum in this study also had a standard reproducible composition, whereas the starter fluid’s composition could vary with time, depending on the performance of that live biological system.

In this study, the pH distribution for the mesophilic and thermophilic temperature ranges was between 7 and 7.5, as shown in Figure 4.12 below. The pH range was expected to be in the same range as the initial experiments, as no changes to the chemical structure of the seaweed were made. The two size distributions in Figure 4.12 do not differ in the optimal pH operating range.

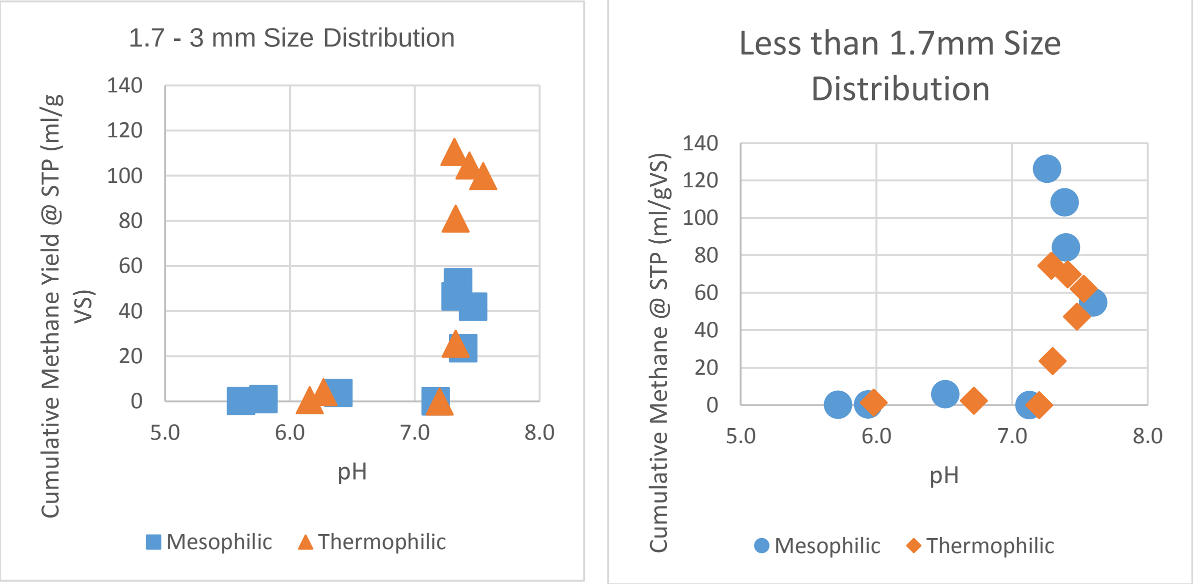

Figure 4.12: Comparison of cumulative methane vs. pH for mesophilic and thermophilic temperature ranges for varying size distributions

Statistical analysis was performed as described previously, and the results are shown in Table 4.11. For mesophilic temperatures, the P-value is less than 0.05, at only 0.02. The null hypothesis is therefore rejected, and the alternate hypothesis is accepted. The size of the biomass does influence the biogas yield.

For thermophilic temperatures, the P-value is more than 0.05 at 0.13. The system has variances, which means the null hypothesis is valid. The size distribution of the biomass does not influence the biogas yield. The difference in statistical analysis implies that the higher temperatures affect the methane yield more than the size distribution. At higher temperatures, the hydrolysis phase is more rapid; degradation of the seaweed takes place quicker, and the carbohydrates required for methane production are released and converted faster.

Table 4.11: Comparison of F-Test Two-Sample for Variances using various size distributions

F-Test Two-Sample for Variances				
	Mesophilic		**Thermophilic**	
	<1.7 mm	**1.7–3 mm**	**<1.7 mm**	**1.7–3 mm**
Mean	47.428	21.161	35.106	53.279
Variance	2822.953	524.555	1029.779	2522.263
Observations	8	8	8	8
Df	7	7	7	7
F	5.382		0.408	
P(F<=f) one-tail	0.02054		0.13008	
F Critical one-tail	3.787		0.264	

4.2.3 Chemical pre-treatment

Polysaccharides such as alginate and laminarin must be broken down during hydrolysis before methane production. Hydrolysis remains the rate-limiting step of the AD process; therefore, any process enhancing this step would be beneficial (Obata et al., 2015).

4.2.3.1 Acid pre-treatment

Pre-treating seaweed with acid prior to the hydrolysis stage has been shown to increase the methane yield of seaweeds such as *Laminaria digitata* and *A. nodosum* by enhancing the hydrolysis stage of the AD process (Obata et al., 2015).

Pre-treating *Ecklonia Maxima* with dilute acid at mesophilic temperatures saw an increase in methane yield from 31.5 ml/g VS to 60.3 ml/g VS using 0.15M HCl and an increase to 41.3 ml/g VS methane using 0.3M HCl. Conversely, the thermophilic temperature range methane yield dropped from 222.7 ml/g VS to 58 ml/g VS using 0.15M HCl and 70.3 ml/g VS using 0.3M HCl. The mesophilic acid pre-treatment showed a 131% to 191% increase in methane yield compared to the initial experiments performed with no pre-treatment. The methane yield was only 26% to 32% of the methane yield obtained with no pre-treatment. The decrease in methane yield compared to the raw seaweed during the elevated temperature could be explained by considering the solution chemistry of the system. An increase in temperature generally causes a shift in the system's equilibrium, leading to the formation of H^+ ions decreasing the solution pH. It was shown in section 4.1 that the optimum pH for the AD process is between 7 and 7.6. An operating pH below this value will lead to a decrease in methane yield over time. For the thermophilic range, the pH remained consistently below 7.4 until day 18 of the experiments, with adjustments being made, indicating that the system was forming H^+ ions, which inhibited the hydrolysis stage (Figure 4.13).

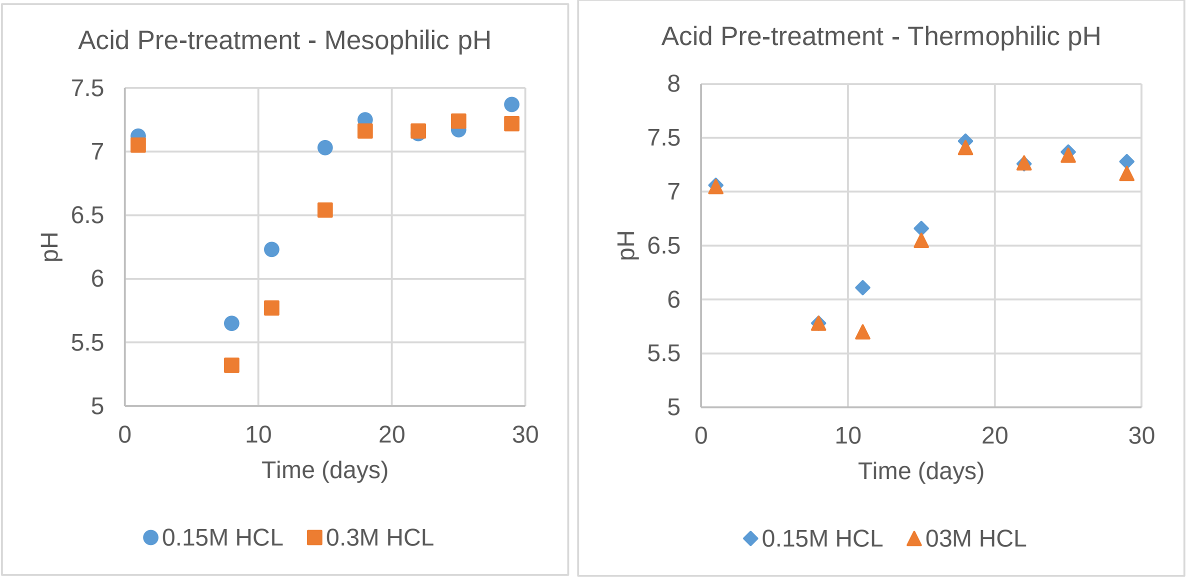

Figure 4.13: pH vs. time for acid pre-treated *Ecklonia Maxima*

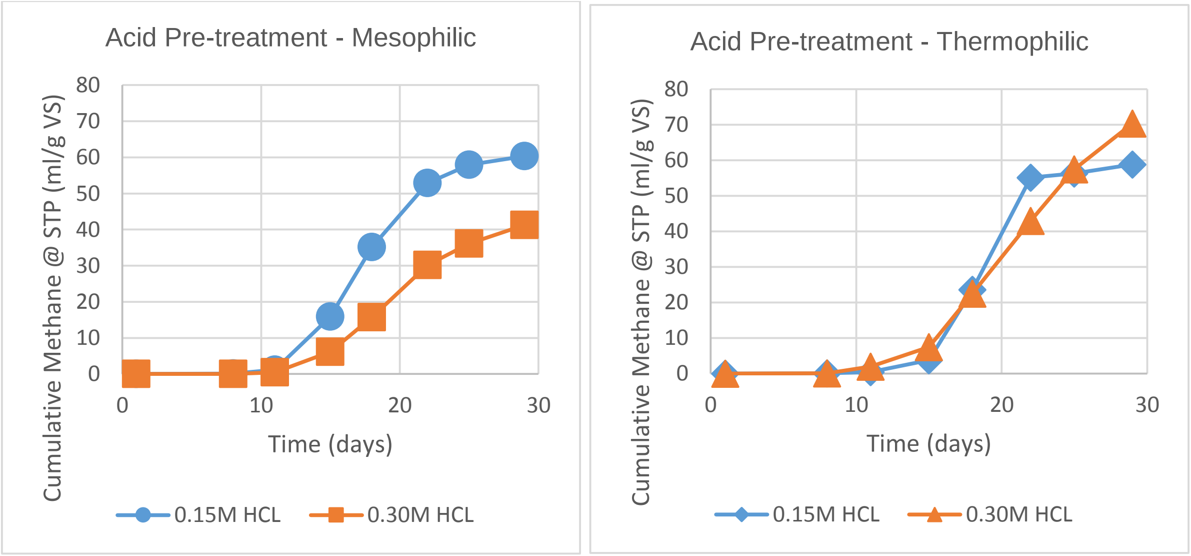

Figure 4.14: Cumulative methane yield for acid-pre-treated *Ecklonia Maxima* for mesophilic and thermophilic temperatures

When comparing the mesophilic acid pre-treatment results, the 0.15M HCl performed better than the 0.3M HCl. Comparing the thermophilic acid pre-treatment results, the 0.3M HCl performed slightly better than the 0.15M HCl (Figure 4.14).

The overall results for the 0.15M HCl pre-treatment show that both the mesophilic and thermophilic temperature ranges yielded similar results for methane yield. The results for the 0.3M HCl pre-treatment showed the thermophilic temperature methane yield to be slightly higher than the mesophilic temperature yield (Figure 4.15). There is an inverse relationship between pH and temperature. An increase in temperature causes a decrease in pH. An increase in the acid concentration also causes a decrease in pH as the number of protons increases. This double effect causes the acidogenic effect to prolong, therefore delaying the methanogenesis stage of AD using the higher concentration and higher temperature. It is not easy to compare methane yields with those recorded in the literature, as similar process conditions have not been replicated. Obata et al. (2015) recorded increased methane yield using H_2SO_4 pre-treatment at an elevated initial pre-treatment temperature of 120 °C for one hour. Once pre-treatment was complete, the AD process was run at mesophilic temperatures to determine methane yield.

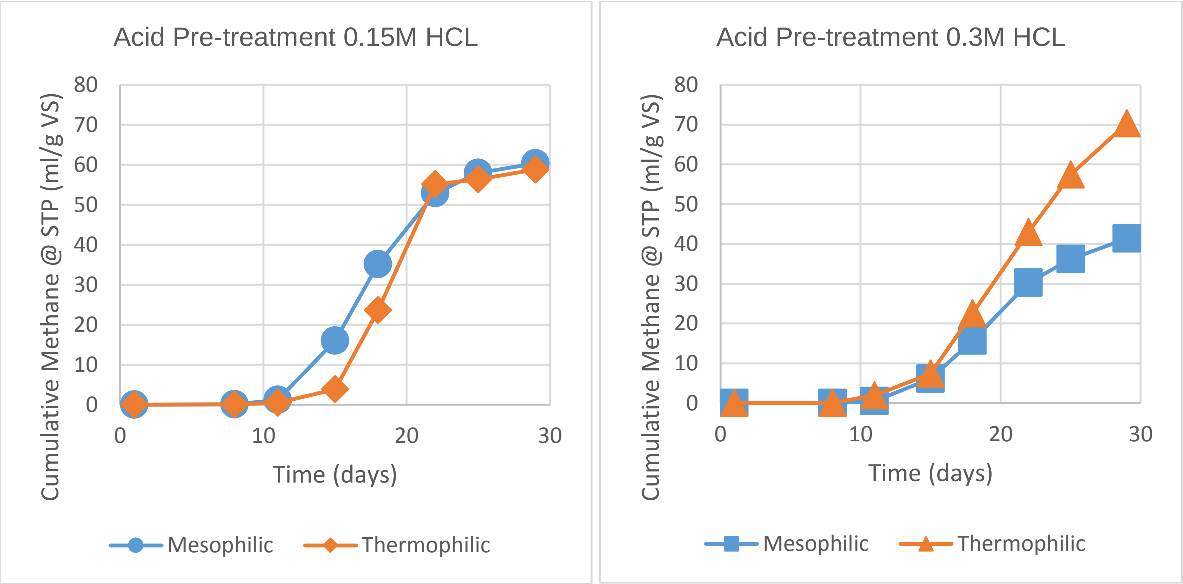

Figure 4.15: Cumulative methane yield for 0.15M HCl and 0.3M HCl for mesophilic and thermophilic temperature profiles

Barbot, Falk et al. (2015) recorded an increase in overall methane yield using 0.2M HCl pre-treatment at 80 °C of between 115 and 147% compared to an untreated *Fucus vesiculosus* (brown seaweed) sample. The AD process occurred at mesophilic temperatures, with a contact period between 2 and 12 hours. Lymperatou et al. (2022) found that when treating a brown cast seaweed mix of the genre *Ectocarpales*, with 0.15M HCl for 24 hours at 80°C, an increase of 78% in methane yield over the raw seaweed yield was seen, with AD taking place at 37 ^{0}C. From the results obtained and cases cited in the literature, acid pre-treatment (at elevated temperatures) and allowing the AD process to take place at 37 ^{o}C leads to an increase in methane yield. There are no documented studies where acid pre-treatment and the AD process at thermophilic conditions have been attempted for seaweed, except for review papers on past work.

The mesophilic results obtained at 0.15M HCl and the thermophilic results obtained at 0.3M HCl are comparable in cumulative methane yield (Figure 4.16). Maintaining the lower operating temperature at a lower acid pre-treatment concentration would be a more manageable system combination, as the construction materials for the system would not have to withstand the elevated temperatures or higher acid concentration for the pre-treatment process. This is a consideration which should be made when the up-scaling and cost of the process are being considered.

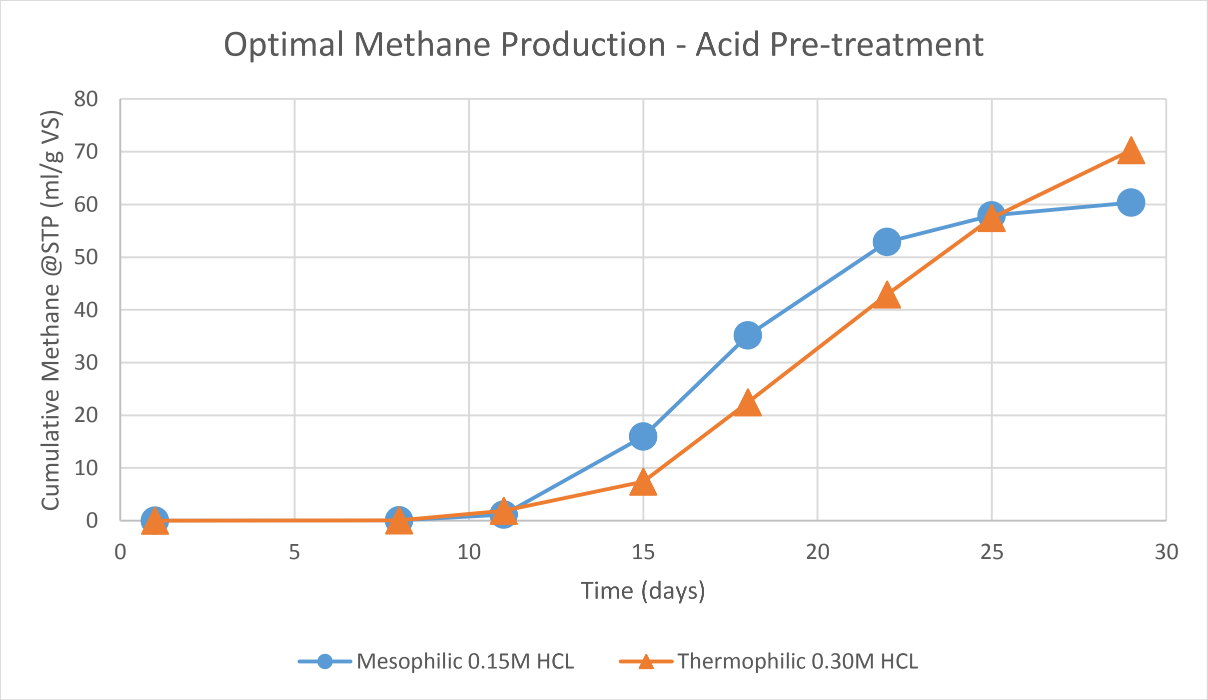

Figure 4.16: Optimum cumulative methane yield for acid pre-treatment at mesophilic and thermophilic temperature ranges

Statistical analysis (Table 4.12) shows that for both mesophilic and thermophilic temperatures, the P-value is more than 0.05. This indicates that the system has variances, which means the null hypothesis is valid in both cases. Acid pre-treatment of the biomass does not influence the biogas yield in this instance.

Table 4.12: Comparison of F-Test Two-Sample for Variances using acid pre-treatment

F-Test Two-Sample for Variances				
	Mesophilic		**Thermophilic**	
	0.15M HCl	***0.3M HCl***	***0.15M HCl***	***0.3M HCl***
Mean	27.928	16.258	24.774	25.326
Variance	719.846	299.642	762.115	788.739
Observations	8	8	8	8
Df	7	7	7	7
F	2.402		0.966	
P(F<=f) one-tail	0.135		0.482	
F Critical one-tail	3.787		0.264	

The pH is an essential consideration in the AD process. The methodology included pH adjustment at regular intervals. Therefore, the pH was constantly reset to within the optimum pH range. This could be the reason that the acid pre-treatment did not prove to be statistically significant.

Thus far, particle size distribution has the most significant effect on methane yield. Even though there is an increase in methane yield at mesophilic temperatures with acid pre-treatment compared to the initial experiments where no pre-treatment took place, this increase is not statistically significant according to the data analysis.

4.2.3.2 Alkaline pre-treatment

Alkaline pre-treatment disintegrates biomass over a fixed digestion time to release AD products (Li et al*.,* 2012). Low-dose alkaline pre-treatment has been utilised since 1997, mainly using NaOH for sludge treatment.

Figure 4.17 shows the comparison of base pre-treatment at mesophilic and thermophilic temperatures. Both show an upward trend after an initial lag phase. As expected, the lag phase for the higher temperature is less than that of the lower temperatures due to the increase in kinetic energy leading to an increase in the hydrolysis stage. The higher temperature also shows a higher overall methane yield than the lower temperatures. The yield observed from the 0.15M pre-treated seaweed stabilised after 20 days at a higher temperature.

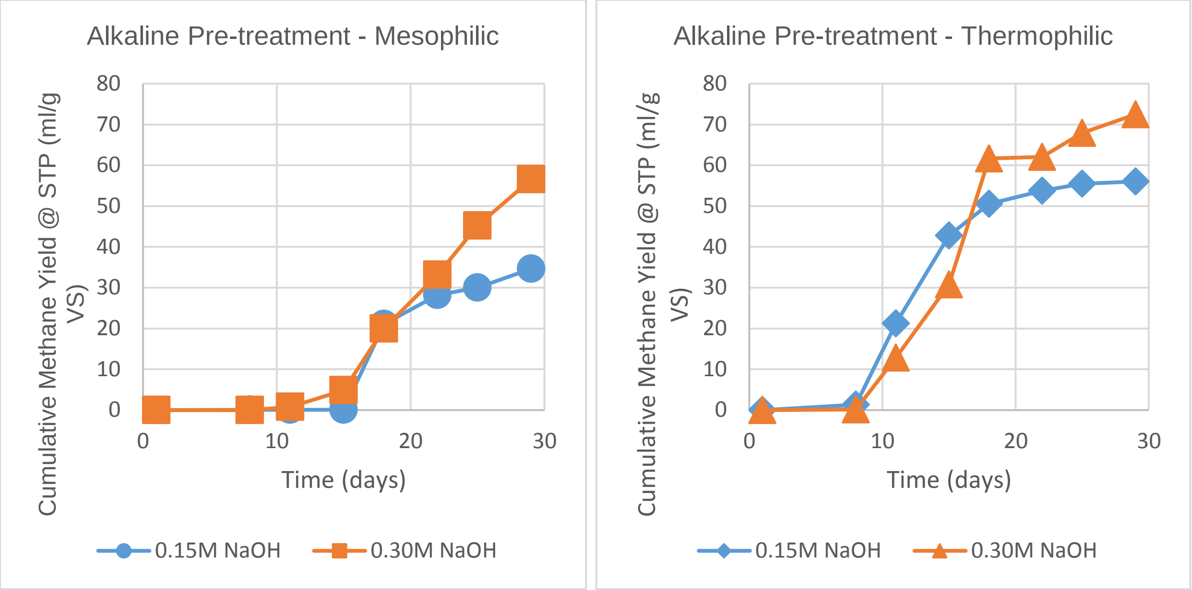

Figure 4.17: Cumulative methane yield for alkaline pre-treated *Ecklonia Maxima* for mesophilic and thermophilic temperatures

The thermophilic methane yield for the 0.15M NaOH and the 0.3M NaOH pre-treatment experiments is similar to those obtained for the thermophilic acid pre-treatment at just over 70ml/g VS (Figure 4.18). This indicates that for chemical pre-treatment at elevated temperatures, the temperature and not the chemical pre-treatment is of more significance.

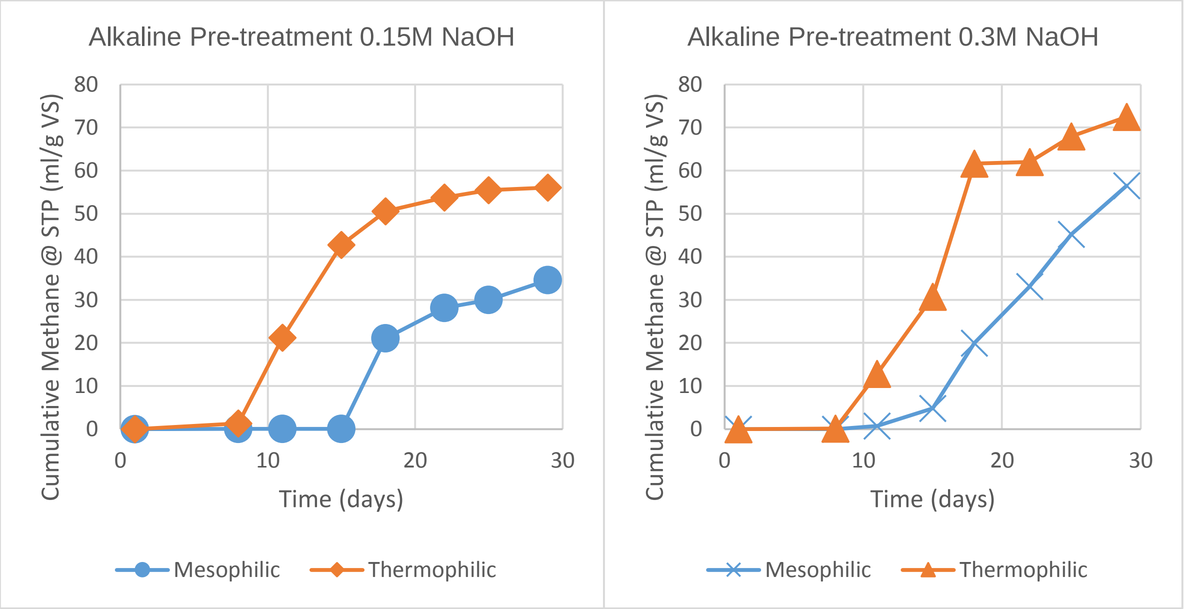

Figure 4.18: Cumulative methane yield for 0.15M NaOH and 0.3M NaOH for mesophilic and thermophilic temperature profiles

When comparing the mesophilic results with the raw seaweed methane yield, we see that at 0.15M NaOH, the methane yield is similar to the base results obtained for the raw seaweed. For the acid, methane yield is almost twice that of the raw seaweed using 0.15M HCl. These observations were also noted by Li et al. (2012) when using chemical pre-treatment concentrations of between 0.05 and 0.5M NaOH and 0.3M HCl. The 0.3M NaOH methane yield was higher at 181% of the raw seaweed yield than the 131% recorded for the 0.3M HCl.

Documented yields using pre-treated brown seaweed have been hard to find over the last five years. Cho et al. (2013) found that as the pH increased from 9 to 13, the methane yield decreased from 363 to 213 ml/g VS using a pre-treated NaOH seaweed blend. At a pH of 9, the yield was already less than the raw seaweed results, emphasising that the methane yield is negatively affected once the AD process operates outside of its optimum pH range. This was further corroborated by Li et al. (2012), who found that at low doses of NaOH pre-treatment, there was insufficient breakdown of biomass to affect hydrolysis. At higher doses of NaOH, the AD process was inhibited. Methane yield decreased due to an increase in pH. The control of the alkaline pre-treatment process is complex as residual NaOH in the system after degradation can destroy the bicarbonate buffer of the system, leading to a higher pH, which can inhibit microorganisms and negatively affect the AD process (Li et al*.,* 2012). Maneein et al. (2018) reported decreases in methane yield between 21% and 27% for NaOH pre-treated red seaweed. There was no mention of pH control in all cases above, meaning the system had to regulate its pH, which is the main reason for the difference between reported cases and the research presented. In the research presented, the pH was adjusted to the optimum pH every time a sample was taken.

Figure 4.19 shows the pH profile for the alkaline pre-treatment. There was a pronounced dip over the first eight days when the system was allowed to run without interference. Once the pH adjustments were made, starting at day 8, the system slowly increased over ten days of adjustments in pH until the optimum operating pH was reached. At this stage, the biomethane yield also increased. When a different alkaline treatment was used with ammonia, there was a 36% increase over raw cast seaweed from the Baltic Sea. The *Ectocarpales* species was treated with a 15% ammonia solution for four days and then anaerobically digested to form methane at 37 ^{o}C (Lymperatou et al*.,* 2022). This increase did not warrant the additional costs of chemicals needed for the pre-treatment process.

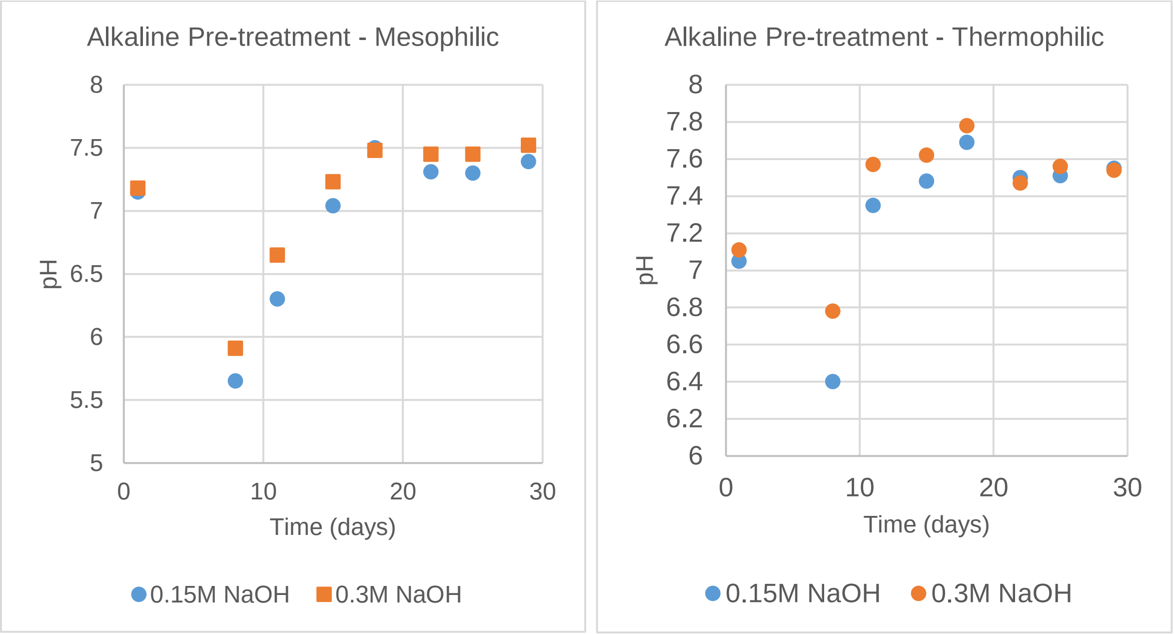

Figure 4.19: pH vs Time for alkaline pre-treated *Ecklonia Maxima*

This study’s best-performing pre-treatment process regarding methane yield was 0.3M NaOH at an elevated temperature, followed by 0.15M NaOH at thermophilic temperatures (Figure 4.20).

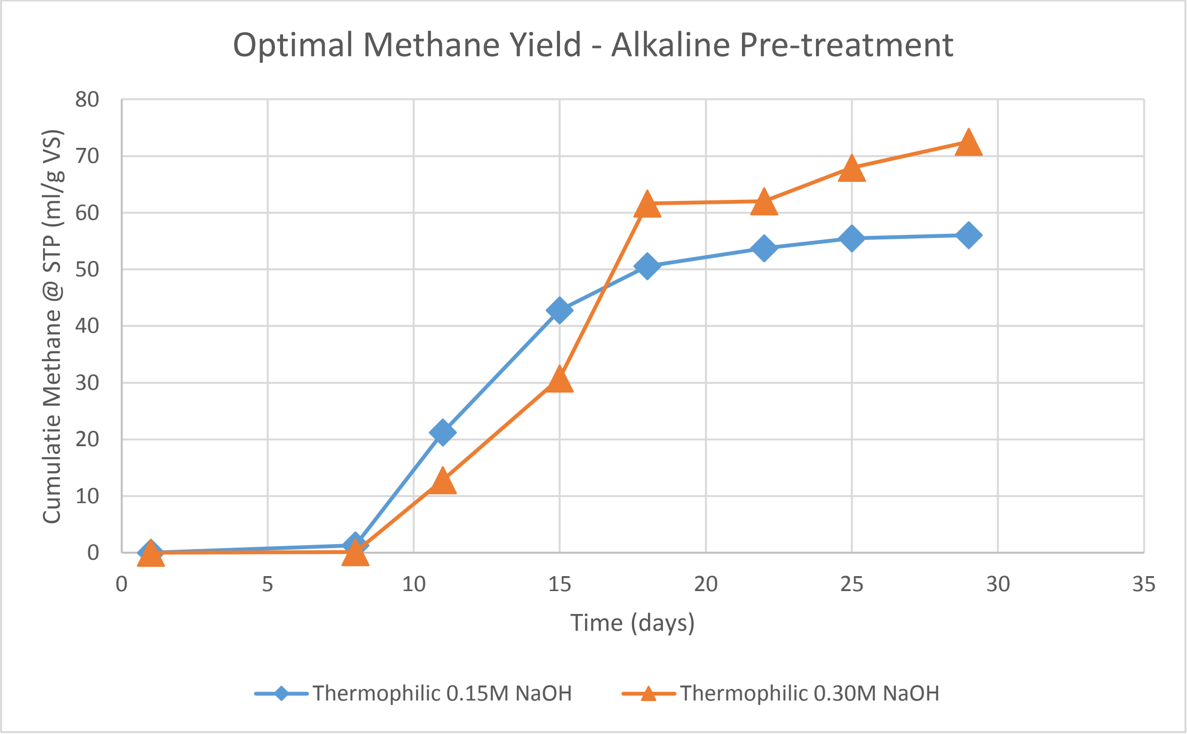

Figure 4.20: Optimum cumulative methane yield for alkaline pre-treatment at mesophilic and thermophilic temperature ranges

Since it was suggested above that at elevated temperatures, the chemical pre-treatment is less of a consideration than the temperature, the statistical analysis would be an essential factor to consider.

Statistical analysis (Table 4.13) shows that for both mesophilic and thermophilic temperatures, the P-value is more than 0.05. This indicates that the system has variances, which means the null hypothesis is valid in both cases. Alkaline pre-treatment of the biomass does not influence the biogas yield.

Table 4.13: Comparison of T-Test two Sample for Variances using alkaline pre-treatment

F-Test Two-Sample for Variances				
	Mesophilic		**Thermophilic**	
	0.15M NaOH	***0.3M NaOH***	***0.15M NaOH***	***0.3M NaOH***
Mean	14.235	20.041	35.148	38.477
Variance	244.434	506.069	580.871	969.630
Observations	8	8	8	8
Df	7	7	7	7
F	0.483		0.599	
P(F<=f) one-tail	0.179		0.258	
F Critical one-tail	0.264		0.264	

4.2.3.3 Comparison of acid and alkaline pre-treatments

Table 4.14 compares the performance of the optimal mesophilic and thermophilic chemical pre-treatment results. The highest thermophilic individual yield amounts at the higher chemical pre-treatment concentrations. However, it is only a fraction of the yield obtained when compared to the performance of the raw seaweed for both the acid and the alkaline pre-treatments. When considering only the acid pre-treatment, the mesophilic range yield is almost double that of the raw seaweed.

Table 4.14: Comparison of acid and alkaline optimal yields

Comparison of Acid and Alkaline pre-treatments						
	Optimal Acid		**Optimal Alkaline**		**Raw Seaweed ±2 mm**	
	Meso 0.15M HCL	**Thermo 0.3M HCL**	**Thermo 0.15M NaOH**	**Thermo 0.3M NaOH**	**Meso**	**Thermo**
Methane Yield (ml/g VS)	60.325	70.3	56.05	72.5	31.537	222.696
% of Raw Seaweed Yield	191%	31.6%	25%	33%		

Pre-treating the *Ecklonia Maxima* with 0.15M HCl shows the highest biomethane yield compared to the raw seaweed. It is therefore the recommended chemical pre-treatment process when considering acid and alkaline pre-treatment processes.

4.2.4 Microwave pre-treatment

Microwaving increases the kinetic energy of the water within the biomass cells, leading to a rapid increase of heat and pressure in the cell, forcing compounds out of the biological matrix of the material. This is called cell hydrolysis (Romagnoli et al., 2017). Figure 4.21 shows the methane yield for microwave pre-treated seaweed at 30 seconds, 60 seconds, and 12 seconds. There is a reasonably long lag time for all three timeframes, with measurable methane yields recorded at 15 days. The 60-second pre-treated seaweed performs well, reaching approximately 40 ml/g VS after 22 days and stabilising. The 120-second pre-treated seaweed still shows an upward trend after 28 days, with the methane yield almost linear over time. There are still sugars available for hydrolysis even after 28 days. The 30-second pre-treated seaweed performs poorly, producing less than 20 ml/g VS methane in 28 days. This could be attributed to the fact that the cell wall was not wholly ruptured, as described above.

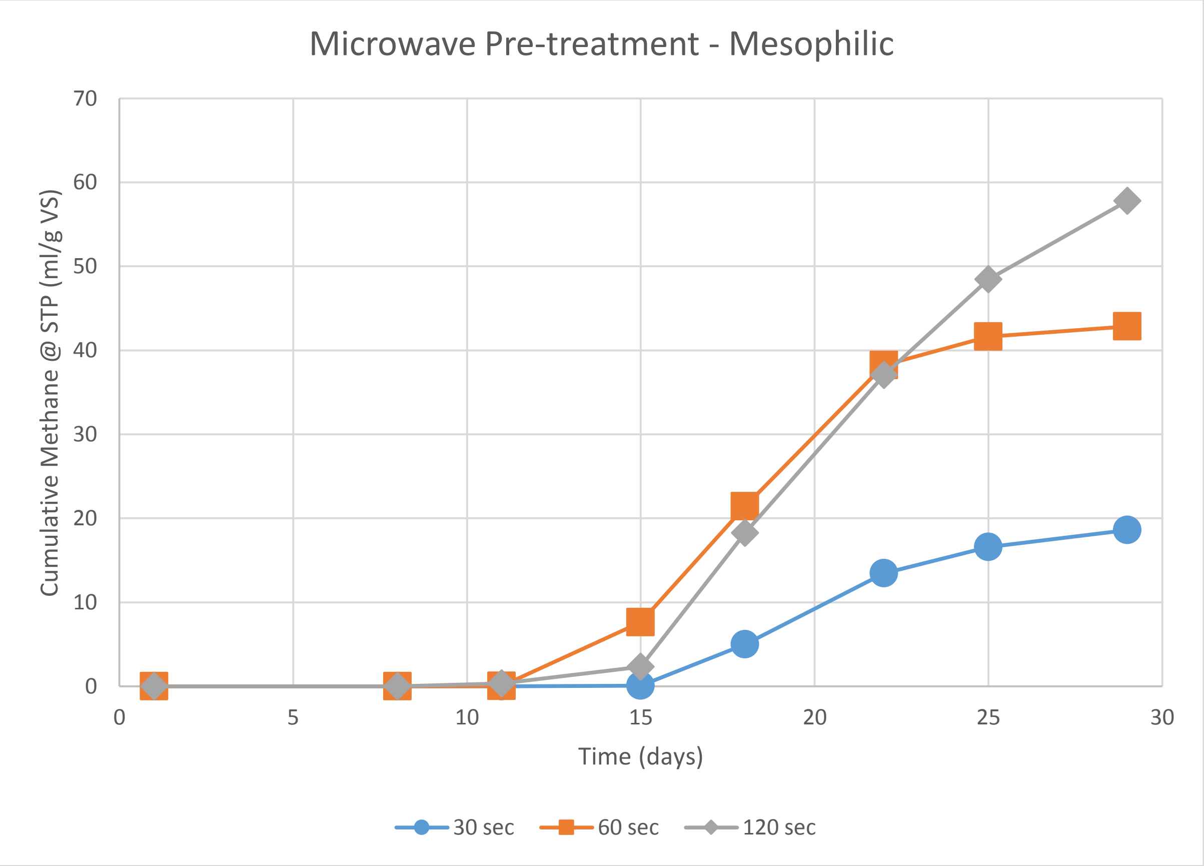

Figure 4.21: Cumulative methane yield for microwave pre-treated *Ecklonia Maxima* for mesophilic conditions

When comparing the methane yield of the microwave pre-treated seaweed to the raw seaweed (Table 4.15), it is seen that there is a decrease in methane yield for the 30-second pre-treated seaweed and increases for both the 60-second and 120-second pre-treated seaweed yields. The decrease could be due to the partial rupture of the cell wall at the shorter period, not fully releasing the sugars for hydrolysis. The extended periods could indicate a higher release of available sugars from the biomass wall, increasing the biogas yield.

Table 4.15: Comparison of methane yields at various microwave pre-treatment times for mesophilic temperatures

	Microwave pre-treatment with Mesophilic AD			
	30 seconds	**60 seconds**	**120 seconds**	**Raw Seaweed**
Methane Yield (ml/g VS@ STP)	18.595	42.862	57.795	31.537
% of Raw Seaweed Yield	58.96	135.91	183.25	

This corresponds to research which shows increased accumulated yields of biomethane using a combination of microwaving pre-treatment between 90 and 180 seconds. Romagnoli et al. (2017) reported a 7.8 to 43.7% increase in methane after microwave pre-treatment for 90 seconds and a 37.2–45.2% increase in methane yield after microwave pre-treatment for 180 seconds using *Fucus vesiculosus*. Montingelli et al. (2017), on the other hand, reported a decrease of 25.6% in methane yield when *Laminaria spp.* was pre-treated for 30 seconds. This corroborates the findings of this research, which showed a similar decrease when a 30-second microwave pre-treatment was applied.

The microwave-pre-treated seaweed at thermophilic temperatures all show the same curve of lag phase, followed by an exponential phase and, finally, a stationary phase (Figure 4.22). The methane yields are all within 5 ml/g VS of each other. When compared to the raw seaweed, all three yields are less than the yield obtained for the raw seaweed at thermophilic temperatures (Table 4.16), with the maximum yield only attaining 25% of the value of the raw seaweed. Yields recorded at 60 and 120 seconds are comparable, indicating that microwaving is not necessary past 60 seconds for elevated AD temperatures. Since the yield is so low for all three-time intervals, microwave pre-treatment is insignificant to the biogas yield for thermophilic conditions.

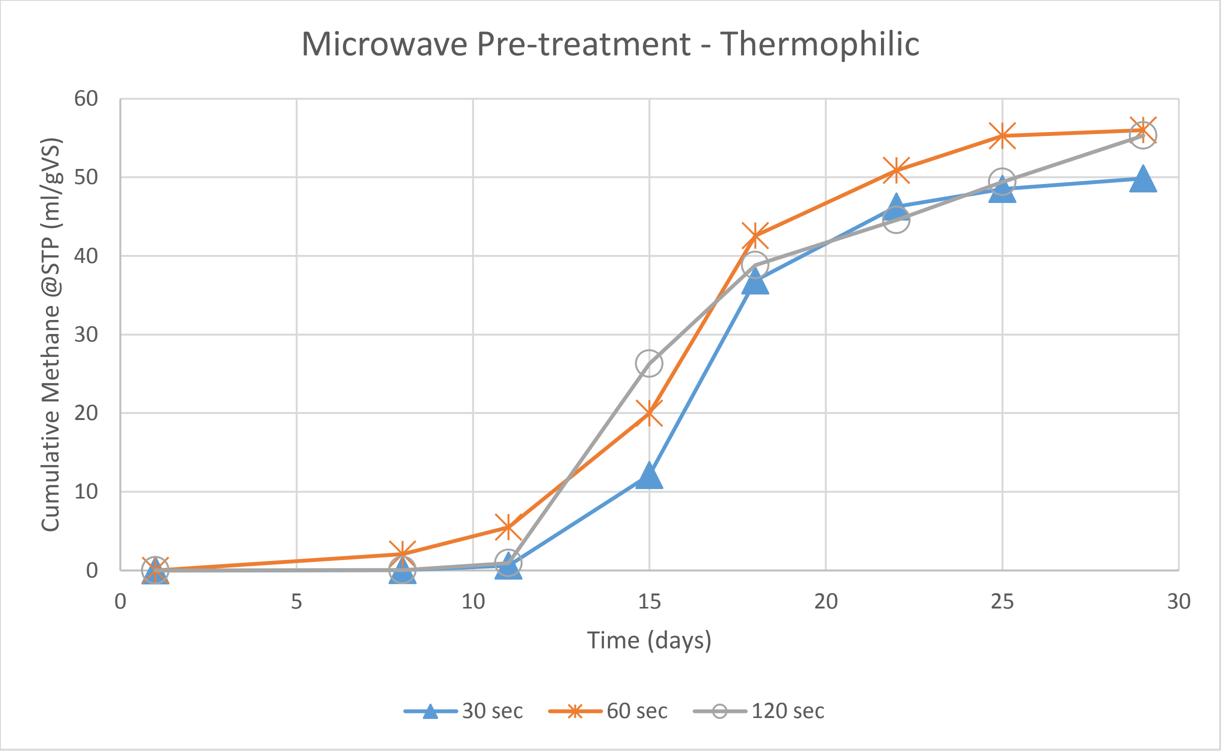

Figure 4.22: Cumulative methane yield for microwave pre-treated *Ecklonia Maxima* for thermophilic conditions

Table 4.16: Comparison of methane yields at various microwave pre-treatment times for thermophilic temperatures

	Microwave pre-treatment with Thermophilic AD			
	30 seconds	**60 seconds**	**120 seconds**	**Raw Seaweed**
Methane Yield (ml/g VS@ STP)	49.843	55.995	55.323	222.696
% of Raw Seaweed Yield	22.38	25.14	24.84	

Increasing the microwave pre-treatment time for the thermophilic region has a marginal effect on the yield obtained. In contrast, the yield increases with increased pre-treatment time in the mesophilic region (Figure 4.23). The combination of microwave energy to rupture the biomass wall and the high operating process conditions became too destructive for the biomass material and the sugars required for hydrolysis, inhibiting biogas formation.

At lower temperatures, it is advantageous to have the cell walls ruptured to allow for the release of the sugars required for hydrolysis. At higher operating temperatures, the kinetic energy within the structure of the seaweed will allow for the release of the sugars required for hydrolysis, and additional energy is not required from microwaving.

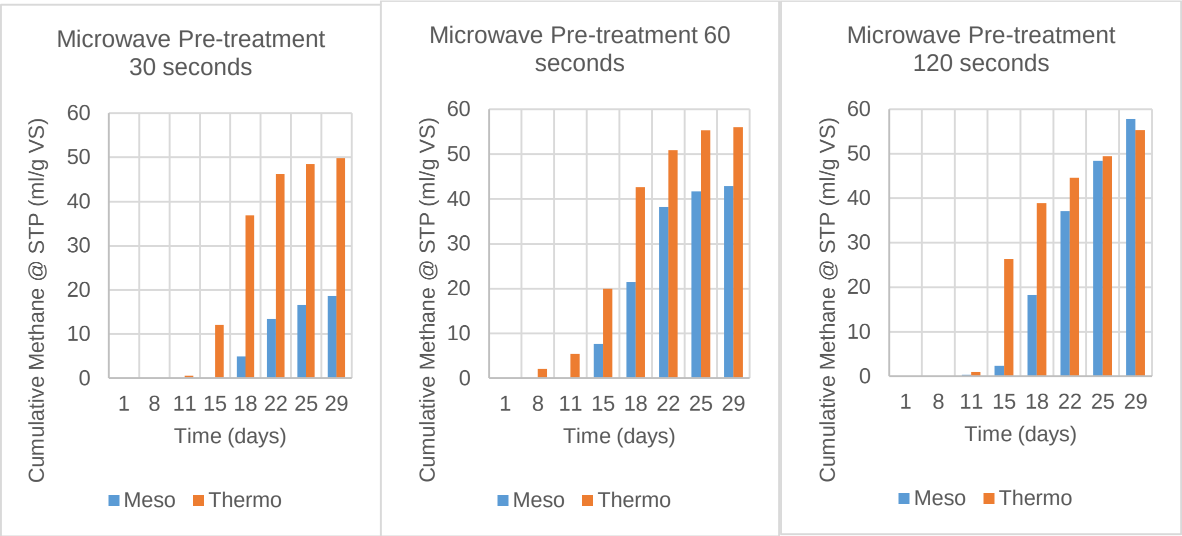

Figure 4.23: Comparison of microwave pre-treated methane yields for mesophilic and thermophilic temperatures

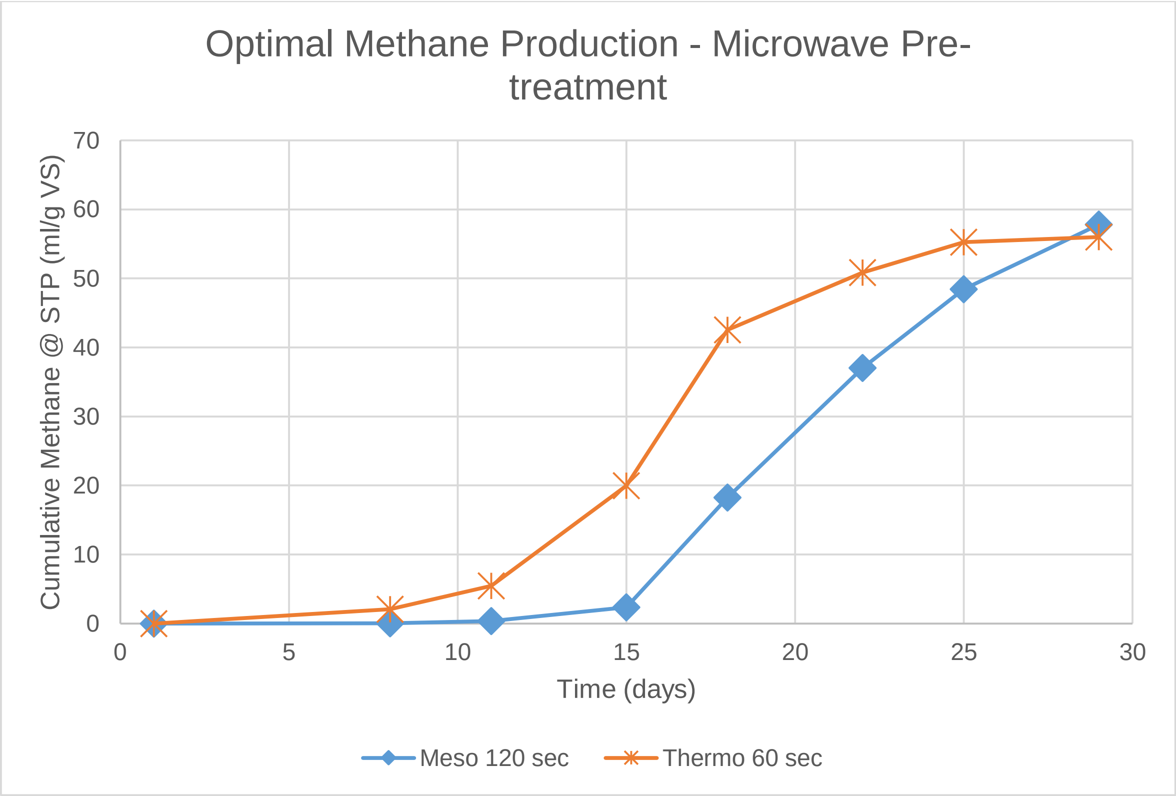

Figure 4.24: Comparison of optimal methane yield for microwave-pre-treated *Ecklonia Maxima*

Comparing only the methane yields over 28 days, it is observed that the optimal yields for the mesophilic and thermophilic ranges are similar (Figure 4.24). If the operating conditions are considered, operating the AD system at a lower temperature would be more straightforward and cost-effective. The growth part of the mesophilic yield curve is almost linear after the initial lag phase, indicating a clear relationship between time (independent variable) and cumulative methane (dependent variable).

Statistical analysis (Table 4.17) shows that for the mesophilic temperatures, the system has variances when comparing the 30-second with the 60-second and the 30-second with the 90-second pre-treatment. That microwave pre-treatment does affect methane yield. This is proven in the yield obtained, where the methane yield was almost half compared to raw seaweed at 30 seconds but nearly a third more than the yield obtained for the raw seaweed at 60 seconds. The yield was 80% more than the raw seaweed at 120 seconds.

Table 4.17: Comparison of F-Test Two Sample Variance for microwave pre-treated *Ecklonia Maxima* at mesophilic temperatures

F-Test Two-Sample for Variances						
	Comparison of		***Comparison of***		***Comparison of***	
	30 sec	***60 sec***	***30 sec***	***120 sec***	***60 sec***	***120 sec***
Mean	6.712	18.984	6.712	20.534	18.983	20.534
Variance	66.583	381.171	66.583	575.100	381.171	575.100
Observations	8	8	8	8	8	8
Df	7	7	7	7	7	7
F	0.175		0.1158		0.663	
P(F<=f) one-tail	0.0174		0.00543		0.300	
F Critical one-tail	0.264		0.264		0.264	

However, when comparing the 60-second pre-treatment to the 120-second pre-treatment, the null hypothesis is valid, which means that microwaving between those times has no effect on methane yield.

When analysing the thermophilic results (Table 4.18), it is seen that the p-value is more than 0.05 in all cases. The null hypothesis is valid, and microwave pre-treatment does not influence methane yield at thermophilic AD operating temperatures.

Table 4.18: Comparison of F-Test Two-Sample Variance for microwave pre-treated *Ecklonia Maxima* at thermophilic temperatures

F-Test Two-Sample for Variances						
	Comparison of		***Comparison of***		***Comparison of***	
	30 sec	***60 sec***	***30 sec***	***120 sec***	***60 sec***	***120 sec***
Mean	24.275	29.024	24.275	26.923	29.024	26.923
Variance	538.236	611.614	538.236	555.836	611.614	555.836
Observations	8	8	8	8	8	8
Df	7	7	7	7	7	7
F	0.880		0.968		1.100	
P(F<=f) one-tail	0.435		0.484		0.451	
F Critical one-tail	0.264		0.264		3.787	

4.2.5 Conclusion

The overall results based solely on the methane yield are listed below for all the pre-treatment processes. Comparing the results to the raw seaweed shows that all attempts at pre-treating the seaweed for thermophilic conditions saw a decline in methane yield.

Table 4.19: Overall pre-treatment results for *Ecklonia Maxima*

	Cumulative Methane @ STP (ml/g VS)	
	Mesophilic	**Thermophilic**
Raw Seaweed	31.537	222.696
Mechanical Pre-treatment	126.157 @ <1.7 mm	110.59 @ 1.7–3 mm
Acid Pre-treatment	60.325 @ 0.15M HCl	70.3 @ 0.3M HCl
Alkaline Pre-treatment	59.8 @ 0.3M NaOH	72.5 @ 0.3M NaOH
Microwave Pre-treatment	57.79 @ 120 seconds	55.99 @ 60 seconds

All pre-treatment processes applied to the seaweed in the mesophilic temperature range show an increase in methane yield, with the highest increase corresponding to the size reduction of the seaweed to below 17 mm.

Microwave pre-treatment yielded similar results at both thermophilic and mesophilic temperatures, meaning that the system could be operated at a lower temperature while yielding the same results, which would be beneficial not only in terms of the cost of the system but also in terms of the ease of operation of the AD system.

Although mechanical pre-treatment at mesophilic conditions shows the highest methane yield, this value is still less than that of raw seaweed at thermophilic operating conditions. Other factors such as cost, operating procedures, energy consumption, environmental impact and process up-scaling will have to be considered before a final decision can be taken on the best system to implement for the highest possible yield obtainable.

4.3 Objective 3: Kinetics of the anaerobic digestion process

4.3.1 Introduction

Determining the kinetic parameters is essential for analysing the process and optimising the process for scale-up purposes (Pecar & Gorsek, 2020). An analysis of kinetics shows the rate of biodegradability of the AD process and can indicate the mechanism of the AD process (Rangseesuriyachai et al., 2023). The discussion is restricted to identifying the order of kinetics and then fitting the data to a sigmoidal curve to predict specific AD process parameters.

4.3.2 Kinetic models and curve fitting

First-order kinetic equations are generally used to describe the kinetics of the AD process (Pecar & Gorsek, 2020; Zhong et al., 2021; Lymperatou et al., 2022). To verify if the first-order kinetics relationships are suitable to describe the kinetics of the AD process, primary first and second-order curves were plotted for the cumulative methane vs. time, and linear trend lines fitted to the curves. The R-squared value indicated the fit of the data. A plot of the cumulative methane yield vs. time was drawn for first-order kinetics. For second-order kinetics, a plot of (Cumulative methane yield)$^{-1}$ vs. time was drawn. As shown in Figure 4.25 below, the results indicate that the first-order kinetics fit better than the second-order kinetics for both mesophilic and thermophilic temperature ranges. While both the plots have a low R^2 value, the first-order plots are a slightly better fit with higher R^2 values.

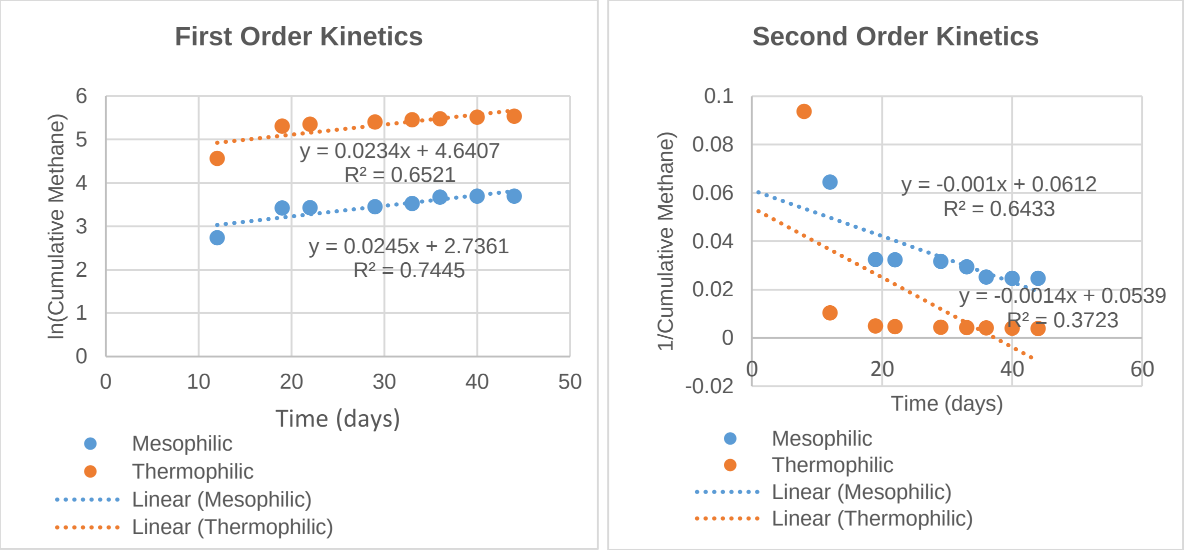

Figure 4.25: First and Second-order kinetics curves for *Ecklonia Maxima* at both mesophilic and thermophilic temperatures

These basic plots in Figure 4.25 do not consider the AD process's biological activity. They are merely a theoretical analysis to select a more appropriate model to describe the system's kinetics. The modified Gompertz model is a first-order kinetics model specifically developed for batch AD processes. This model was tested for optimum fit of the AD process and adapted if needed.

The fitted modified Gompertz curves (Figure 4.26) allow for the determination of the maximum methane production potential, the production rate, as well as the lag phase of the AD system. Solver was utilised to fit the modified Gompertz equation to the experimental data and to determine the variables for P_{max}, R_{max} and ƛ (Tables 4.20 and 4.21). The relative root mean square was determined using (Romagnoli et al., 2017):

$$RMSE = \left[\frac{1}{m}\sum_{j=1}^{m}\left(\frac{d_j}{y_j}\right)^2\right]^{1/2}$$

where d_j is the deviation between the measured and the predicted values for cumulative methane,

m is the number of experimental values, and

Y_j is the measured cumulative methane value.

Table 4.20: Mesophilic modified Gompertz constants

Mesophilic Gompertz constants				
	Pmax ml/g VS	**Rmax ml/g VS**	**ƛ day^{-1}**	**R^2**
Raw seaweed	37.653	13.329	7.360	0.996
Pre-treated mechanical (1.7-3mm size)	52.089	31.074	14.599	0.991
Pre-treated mechanical (<1.7mm size)	131.312	57.977	14.293	0.997
Pre-treated acid (0.15M HCL)	61.917	32.098	12.692	0.992
Pre-treated acid (0.3M HCL)	44.859	17.720	13.716	0.998
Pre-treated base (0.15M NaOH)	31.313	38.814	15.393	0.951
Pre-treated base (0.3M NaOH)	69.743	20.404	14.044	0.996
Pre-treated microwave (30 seconds)	19.053	11.230	15.977	0.995
Pre-treated microwave (60 seconds)	44.255	25.773	13.860	0.993
Pre-treated microwave (90 seconds)	63.244	25.901	14.994	0.998

The methane production potential using the modified Gompertz equation for the mesophilic temperature ranges between 19.053 ml/g VS and 131.312 ml/g VS (Table 4.20). The actual experimental values were between 18.595 ml/g VS and 126.157 ml/g VS. There is a 2.5% to 4% difference between the predicted and actual methane yields. The lag phase is between 7 and 16 days. This is a function of the experimental design, where the first feeding occurred on day 8 of the AD process. Adjusting the system with an earlier feeding could significantly reduce the lag phase.

Table 4.21: Thermophilic modified Gompertz constants

Thermophilic Gompertz constants				
	Pmax ml/g VS	**Rmax ml/g VS**	**ƛ day^{-1}**	**R^2**
Raw Seaweed	248.776	66.159	6.542	0.999
Pre-treated mechanical (1.7-3mm size)	106.899	96.793	13.743	0.971
Pre-treated mechanical (<1.7mm size)	75.459	35.636	11.806	0.994
Pre-treated acid (015M HCL)	58.879	55.746	15.937	0.981
Pre-treated acid (03M HCL)	86.096	26.012	14.108	0.999

Pre-treated base (0.15M NaOH)	55.455	34.102	8.221	0.999
Pre-treated base (0.3M NaOH)	72.388	33.895	9.825	0.996
Pre-treated microwave (30 seconds)	49.040	43.782	13.693	0.972
Pre-treated microwave (60 seconds)	57.300	30.616	11.484	0.991
Pre-treated microwave (90 seconds)	52.198	28.731	11.010	0.991

Similarly, the methane potential for the thermophilic temperature range was between 49.040 and 248.776 ml/g VS (Table 4.21). The actual methane yield was between 49.843 and 222.696 ml/g VS. There is a 1.6% to 10.5% difference between the predicted and actual methane yields. The lag phase is between 6.5 and 16 days and can be shortened again with an earlier feeding, as described before.

The modified Gompertz equation shows less deviation from the actual values for the mesophilic temperature than for the thermophilic temperature. In both instances, the fit is above 90%, which indicates that the modified Gompertz curve can be utilised to predict the performance of the AD process (Figures 4.26 and 4.27). The curves fit well for the raw and pre-treated experimental results, with P_{max} for the raw seaweed at thermophilic temperature showing the highest yield. This is followed by methane at a size distribution less than 1.7 mm for the mesophilic temperature range, with no chemical or thermal pre-treatment.

The mesophilic curves show a mixture of three zones, namely the lag phase, growth phase and stagnant phases. The stagnant phase indicates that the AD process can be stopped, or more inoculum could be fed to the system to jumpstart the biological reactions. For the size plots in Figure 4.26, only the stagnant and growth regions are evident. The same is seen for the acid pre-treated plots. The alkaline pre-treated plots show a stagnant stage for the lower concentration of NaOH but a growth phase for the higher concentration. Microwave pre-treatment is mixed with the 60-second pre-treated plots, showing a stagnant phase after 22 days, but the other two plots indicate a growth phase past day 28.

For the size plots in Figure 4.27, only the stagnant (stationary) and growth (exponential) regions are evident. The acid pre-treated plots indicate that at a higher acid concentration, the growth phase is still evident after 28 days, whereas the stagnant phase is reached after 22 days. The alkaline pre-treated plots show that the stagnant phases are reached at both concentrations after 22 days. Microwave pre-treated plots are mixed, with a stagnant zone evident after 22 days at 30 seconds pre-treatment, but the growth phases are still evident in the other two plots. The Gompertz model could be used to predict the day on which the maximum predicted methane yield would be obtained.

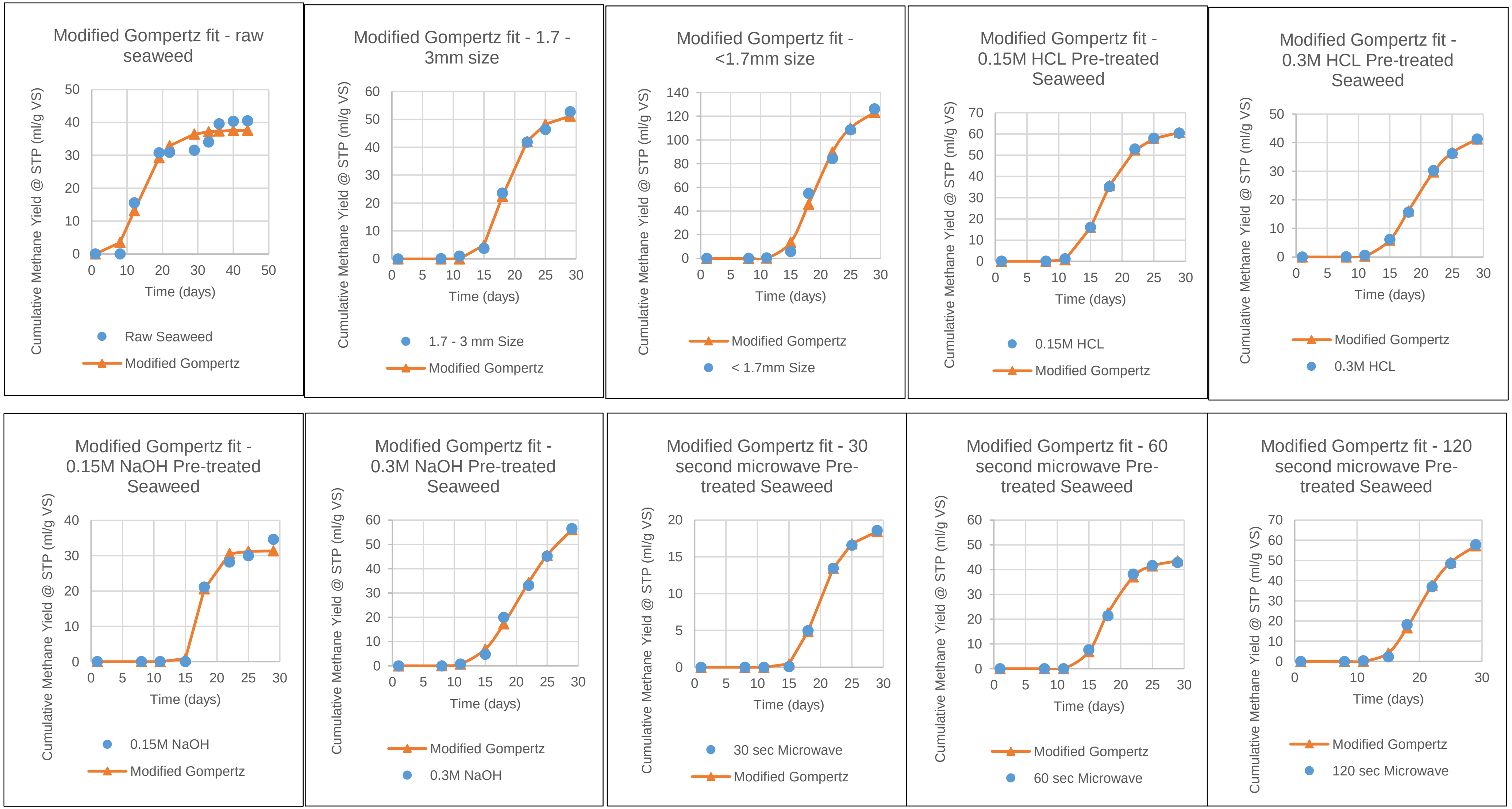

Figure 4.26: Modified Gompertz curves fitted to experimental data of *Ecklonia Maxima* at mesophilic temperature

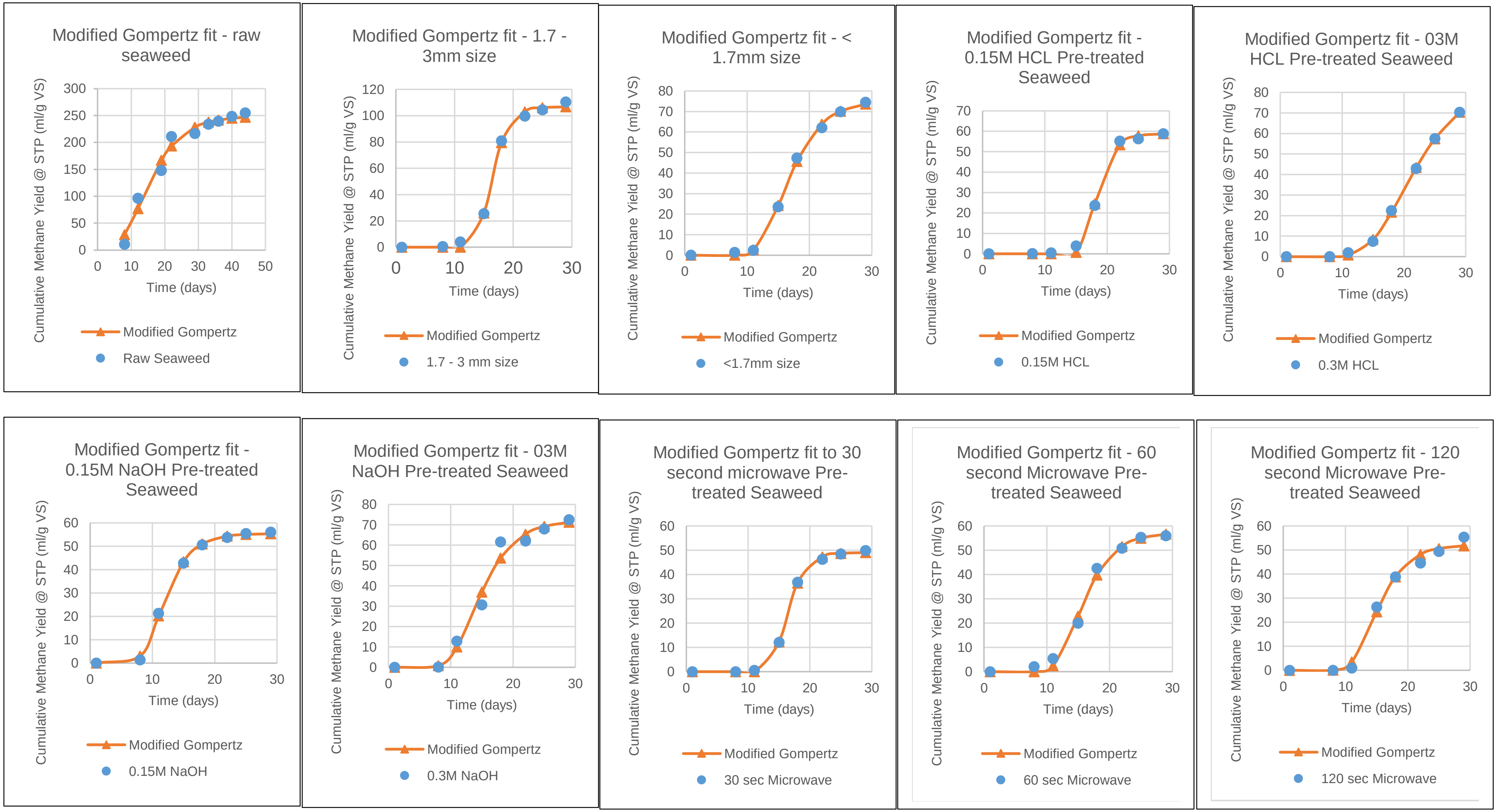

Figure 4.27: Modified Gompertz curves fitted to experimental data of *Ecklonia Maxima* at thermophilic temperature

Table 4.22 shows the predicted time for the system to reach the maximum cumulative methane for the <1.7 mm size distribution. Although the maximum methane yield is only reached after 42 days (P_{max}), very little methane is produced after day 35, indicating that the system has reached the stagnant phase.

Table 4.22: Example of modified Gompertz curve fitting using Solver in Excel

Seaweed condition	Predicted day for maximum methane Yield< 1.7 mm Size	Modified Gompertz	Residual	Residual square
1	0	1.51421E-33	0.000	0.000
8	0.064	0.000	0.064	0.004
11	0.285	0.243	0.041	0.002
15	5.720	13.566	-7.846	61.564
18	54.763	45.639	9.123	83.236
22	84.120	89.681	-5.561	30.920
25	108.317	109.953	-1.636	2.676
29	126.157	123.164	2.993	8.956
30		124.9519593		
35		129.5011839		
37		130.2210792		
39		130.6554297		
42		131.0056787		
Pmax	Rmax	ƛ		SSR
131.312	57.977	14.293		187.358

A distinction must be made between the maximum methane yield and the optimum methane yield before an adjustment is made to the system.

4.3.3 Conclusion

First-order kinetics described the AD process for this system, including the pre-treated experiments run. The modified Gompertz model showed an excellent fit to the experimental data and allowed a calculation of the maximum expected methane yield based on experimental data. This model allowed for the prediction of the AD system, given parameters such as lag time and expected k values. The curve fitting was based on experimental results, including a long lag time, which was partly determined by the experimental methodology. The expected lag time can be reduced to what is stipulated in the literature of between six and ten days (Pecar & Gorsek, 2020) if the system is fed earlier, encouraging hydrolysis to start earlier.

CHAPTER FIVE: TECHNO-ECONOMIC STUDY

5.1 Introduction

This chapter gives an overview of the techno-economic evaluation of the AD process for biomethane production. The emphasis is on comparing the evaluation to two industrial-scale biogas plants in the Western Cape, South Africa.

The economic feasibility of full-scale biogas production from macroalgae is poorly understood (Dave et al., 2013). Even though full-scale biofuel plants operate in Europe and Asia, the feedstock used is diverse and usually consists of food waste, animal manure, and food energy crops (Sheets & Shah, 2018). The market for biogas produced from solid waste in South Africa is emerging.

The Western Cape of South Africa currently has several small-scale operating AD processes that produce biogas for on-site usage, generating electricity and heat (GreenCape, 2017). Figure 5.1 provides an overview of operating biogas plants in SA. The bulk of the AD plants are currently centred in Gauteng and the Cape Town area. In the Western Cape, feedstocks consist of, but are not limited to manure, slaughter waste, and fruit waste. There are no biogas plants that utilise macroalgae as a feedstock.

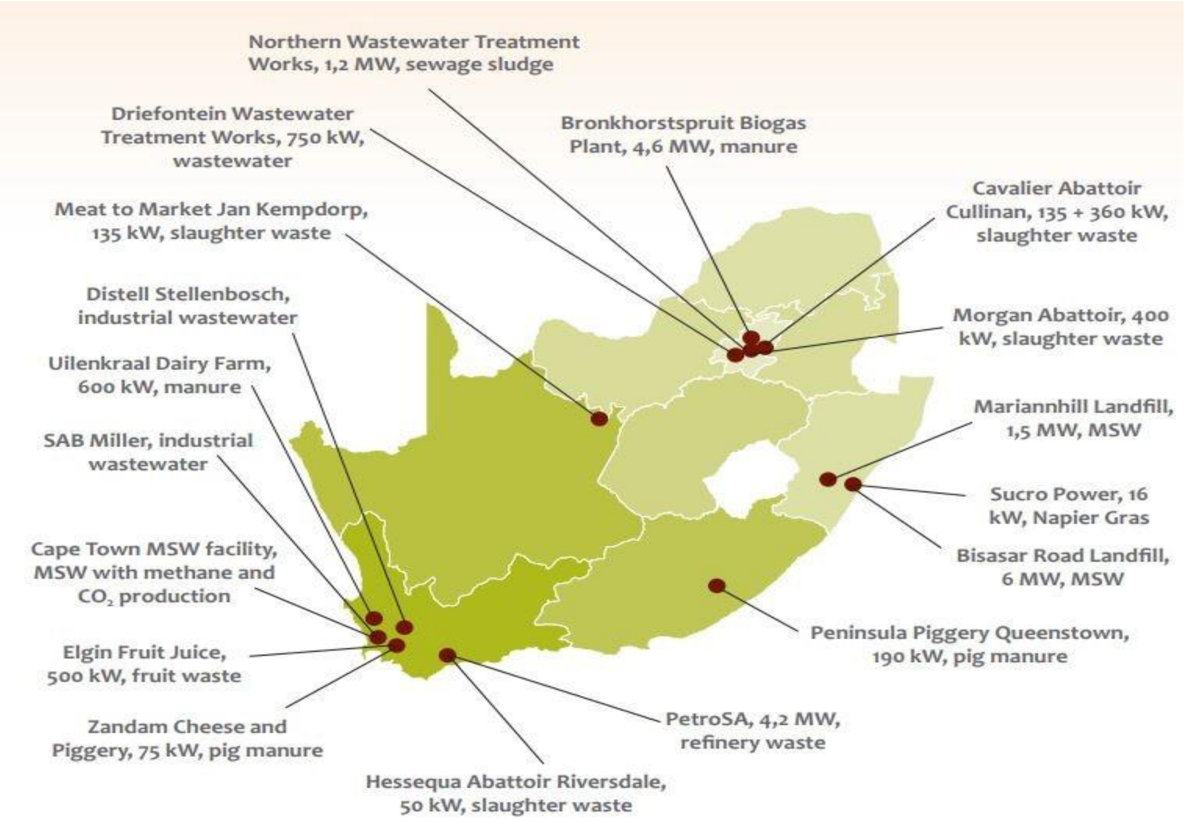

Figure 5.1: Overview of selected biogas plants in South Africa (South Africa. Department of Forestry, Fisheries & the Environment, 2021)

The South African coastline has a cold-water climate, suitable for cultivating brown macroalgae (Dave et al., 2013; Rothman et al., 2017). The big drawback in using macroalgae in commercial or industrial applications is the high moisture content, which impacts on the economic feasibility of any process utilising seaweed as a feedstock for energy production. Having a process capable of using seaweed as a wet feedstock would be advantageous, making the anaerobic digestion process a suitable application.

A Combined Heat and Power (CHP) unit utilising biomethane could be an economically competitive unit for community-based energy applications (Dave et al., 2013), provided the feedstock can be sustainably sourced at a reasonable price.

The current AD and CHP units in the Western Cape for the two processes under review are based on a co-ownership agreement between the manufacturer and owner (GreenCape, 2017). Both case studies used animal manure as the feedstock, sourced on-site or nearby. This removed the need for transport costs of the feedstock. The research compares the feasibility of replacing the feedstock of these two operational units in the Western Cape with a similar unit, using seaweed as a feedstock, while maintaining the original designs. Capital costs (CAPEX) are noted for the case studies, while operating costs (OPEX) are determined for the seaweed system and compared to the existing AD units. The assumption is that the capital costs for identical systems would be approximately equal when the present value of the CAPEX is determined. The operating costs would account for the significant differences. The currency used for the study is the South African Rand (R).

5.2 Economic analysis

Net Present Value (NPV) and Internal Rate of Return (IRR) are important parameters used to determine how much profit can be achieved from an industrial application. These two factors are used to assess the two scenarios using seaweed to replace the feedstock at Uilenkraal Dairy and Zandam Cheese and Piggery farms. Economic parameters are evaluated using a material and energy balance as a basis for the seaweed system, as well as the biomethane potential of the seaweed. Parameters include operating and maintenance costs, costs for raw materials and chemicals, and transport costs of the seaweed. The break-even electricity selling costs (BESC) are determined using the Net Present Value (NPV) method. The primary economic indicator wis the Levelised Cost of Electricity (LCOE).

5.2.1 Net present value (NPV)

The NPV is used to evaluate the feasibility of a project. The equation used is (Tan et al., 2022):

$$NPV = \sum_{t=0}^{N} \frac{CF_n}{(1+k)^n} \tag{5.1}$$

Where: CF_n is the cash flow in the year t (R)

n is the year the cost of revenue occurs (year)

k is the discount rate (%)

The discount rate is the interest rate used to calculate the present value. If the NPV is positive, the project is profitable. If the NPV is negative, the project capital costs cannot be covered by the return on investment (ROI).

5.2.2 Levelised cost of electricity (LCOE)

The LCOE is used to estimate the cost per kWh of electricity generated over the lifetime of the AD process (CSIR Enterprise Creation for Development, 2022). The benefit of using LCOE is the inclusion of CO_2 emissions of the renewable technology. When comparing technologies, it considers the different capital and operating costs, construction times and plant load factors. The equation used is (NIRAS-LTS et al., 2021).

$$LCOE = \frac{\sum_{t=1}^{n} \frac{C_t}{(1+DR)^t}}{\sum_{t=1}^{n} \frac{E_t}{(1+DR)^t (1+IR)^t}} \tag{5.2}$$

Where: C_t = costs incurred in the year

DR = discount rate

E_t = energy consumed in a year

IR = annual inflation rate

5.2.3 Internal rate of return (IRR)

The IRR is the discount rate at which NPV is zero. It is, therefore, the value when the present value of the costs and the present value of the benefits are equal. The investment should be viable if the IRR is more significant than the project's discount rate. The IRR can be calculated from (Garcia, 2014):

$$IRR = NPV = \sum_{t=0}^{N} \frac{CF_n}{(1+k)^n} = 0 \tag{5.3}$$

5.2.4 Payback time

This is the number of years required to recover the investment costs. It can be estimated as the ratio of the total amount invested and the estimated annual cash flow. An alternate way would be to determine the break-even analysis, which can be determined by:

$$Break - even\ analysis = \frac{fixed\ costs}{price\ per\ unit - cost\ to\ produce\ a\ unit} \tag{5.4}$$

5.2.5 CAPEX costs

CAPEX costs are used in each scenario for Uilenkraal Dairy Farm as well as Zandam Cheese and Piggery Farm. The present value of the investment in 2023 is estimated using the average inflation rate of South Africa since commissioning. It is assumed that the same industrial plant layout is utilised, with the same control systems set out in each farm. The only difference is the feed to the plant, as it will has to be done manually in both cases instead of the automated system currently used.

5.2.6 Assumptions for the economic evaluation of using seaweed as a feedstock

The economic evaluation is based on a combination of Farm brief factors and literature assumptions:

i. The AD plant is located within 50 km's of Cape Town, in the Western Cape of South Africa.
ii. CAPEX costs are present values estimated from the initial plant costs listed for Zandam and Uilenkraal farms. No ownership agreement is considered, and the entire CAPEX amount is considered as the total investment.
iii. The average inflation rate from 2016 to 2023 was 4.86% per annum (SA inflation calculator, 2023).
iv. The average inflation rate from 2014 to 2023 was 5.01% per annum (SA inflation calculator, 2023).
v. The predicted electricity produced is based on the BMP of the seaweed. The algal conversion rate to biogas is assumed to be 65% within 15 days (Dave et al., 2013).
vi. Electricity produced is utilised on-site for the AD process and farm operations as determined by Uilenkraal and Zandam farms.
vii. Any surplus electricity is sold back into the electricity grid, as this is now allowed through the City of Cape Town. The buy-back rate will be R1.24 per kWh (2023).
viii. Seaweed is bought in, dry harvested, and must be soaked and mixed with water to constitute a 20% (m/m) slurry.
ix. Inoculum loading is only done on the initial start-up of the AD plant to introduce the biological culture.
x. The payback time for the CAPEX costs will be determined, assuming the seaweed feedstock replaces the manures used in Uilenkraal and Zandam farms.
xi. Plant expected lifetime is 30 years.

5.3 Case Study AD systems in the Western Cape

5.3.1 Zandam Cheese and Piggery

Zandam Farm is located 17 km east of Durbanville, in the Northern Suburbs of Cape Town. It uses pig manure in an operating AD process, where the resulting biogas is used in a CHP machine to produce electricity and heat energy.

The manufacturer of the AD plant is IBERT (Pty) Ltd, which designs and manufactures small-scale biogas plants in South Africa. The biogas plant at Zandam Farm was commissioned in 2016 on a joint-ownership agreement. IBERT paid for the initial movable equipment, including the CHP unit, stirrers, the control system, etc. Zandam Farm paid for the immovable equipment like the reactors (GreenCape, 2017). At the time, the total cost of the equipment and installation was R9,200,000. IBERT paid 68% of this amount, and Zandam Farm paid 32% of the CAPEX amount. The individual investments were R6,256,000 for IBERT, financed through a banking institution with a five-year payback plan, and R2,944,000 for Zandam Farm (SAGEN, 2017).

Table 5.1: Plant operating conditions at Zandam Cheese and Piggery

Project Owner	IbertZandam (Pty) Ltd
Feedstock	Pig Manure (40 tonne/day) 6,650 sows producing approximately 22 tonne of manure (@12% solids)
Technical Specifications	Dual Chamber Anaerobic Digester (Bio-reactor) Capacity of Bio-reactor = 500 m^3 200 m^2 Storage Tank 75 kWe CHP unit Thermo-Gas-Lift technology for heating, mixing and desulphurisation Screw Press to remove liquid in the feed and increase solids concentration Digester operating temperature = 37–38 ^{o}C HRT = 26 days
Energy Output	75 kWe base load electricity 100 kWth average for heating

The digestate from the AD process is separated into solids and liquids. The liquid is transferred to maturation dams and used for pasture irrigation, while the solids (organic fertiliser) are sold to a tomato farm nearby (SAGEN, 2017).

The biogas production is approximately 41 m^3/hr. The CHP unit consumes approximately 36 m^3/hr. The thermal energy generated is used to pre-heat water to approximately 85^{o}C, used in processes including pasteurisation, steam generation and hot water supply for cleaning. The electricity produced is also utilised to replace the diesel, which was previously used to fuel the boiler system (GreenCape, 2017).

It should be noted that at the time (2016), there was no provision to feed electricity back into the power grid. The power utility in South Africa, ESKOM, did not allow for excess power generation to be fed back into the power grid. This meant that the AD process could not produce electricity above that which would be utilised on-site. The system was, therefore, set up so that the maximum electricity production did not exceed 95% of the required electricity for the farm (GreenCape, 2017). The throttling system incurred an additional expense and also meant that the Farm could only partially be self-sustainable in terms of electricity generation capacity.

The throttling of the electricity produced could now be removed as the Western Cape Government has put a system in place to purchase surplus electricity from private producers to help alleviate the load shedding and energy crisis in the country as of 2023. When the system for Zandam Farm was designed, plans were put in place to install an Organic Rankine Cycle (ORC) to utilise the heat produced that the farm cannot currently utilise should the opportunity arise to feed back into the electrical grid (SAGEN, 2017).

The joint ownership agreement between Zandam Farm and IBERT (Pty) Ltd also set out the facility’s operational costs. IBERT did not own the immovable property and would rent the equipment from Zandam Farm for a set monthly fee. Zandam Farm paid IBERT a fixed amount for electricity generation per kWh. The costs of electricity generation would increase annually at the prime interest rate of the country. This was advantageous to Zandam Farm, as the power utility ESKOM has been increasing electricity tariffs far above the prime interest rate of the country since before 2016. Table 5.2 details the prime lending rate as well as the ESKOM tariff hike in electricity since 2016, when the plant at Zandam Farm was commissioned.

The initial cost of electricity to Zandam Farm from IBERT was R1.01 in 2016, while ESKOM charged R1.417 per kWh to commercial entities. The cost of electricity to Zandam Farm was estimated for 2017 to 2020 using the inflation rate per annum in Table 5.2. Figure 5.2 gives an overview of the difference in costs that Zandam Farm pays compared to the ESKOM tariffs charged between 2016 and 2022. There was an initial cost saving to Zandam Farm, which increased annually with the above-inflation increases implemented by ESKOM. Figure 5.3 shows the above-inflation increases implemented by ESKOM between 2016 and 2022. The ESKOM increases in electricity are consistently higher than the annual interest rate, except for the 2017/2018 year.

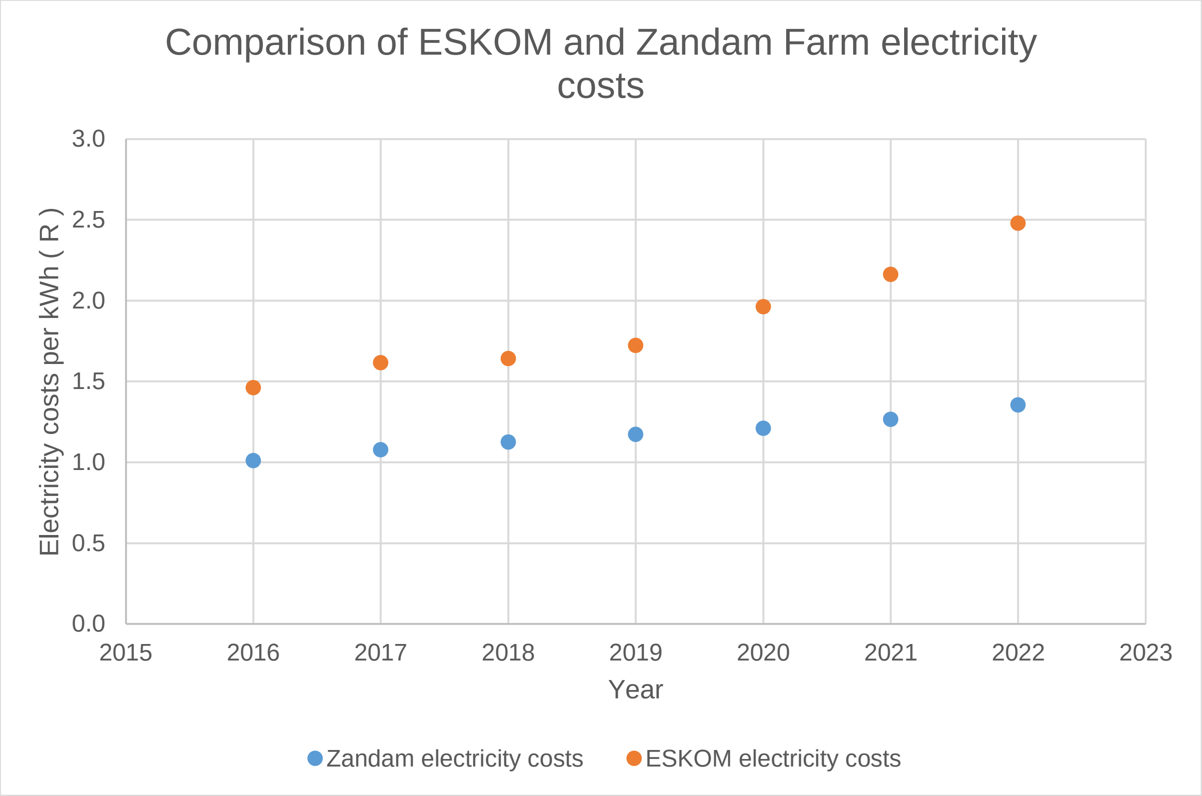

Figure 5.2: Zandam Farm electricity costs compared to ESKOM electricity costs between 2016 and 2022

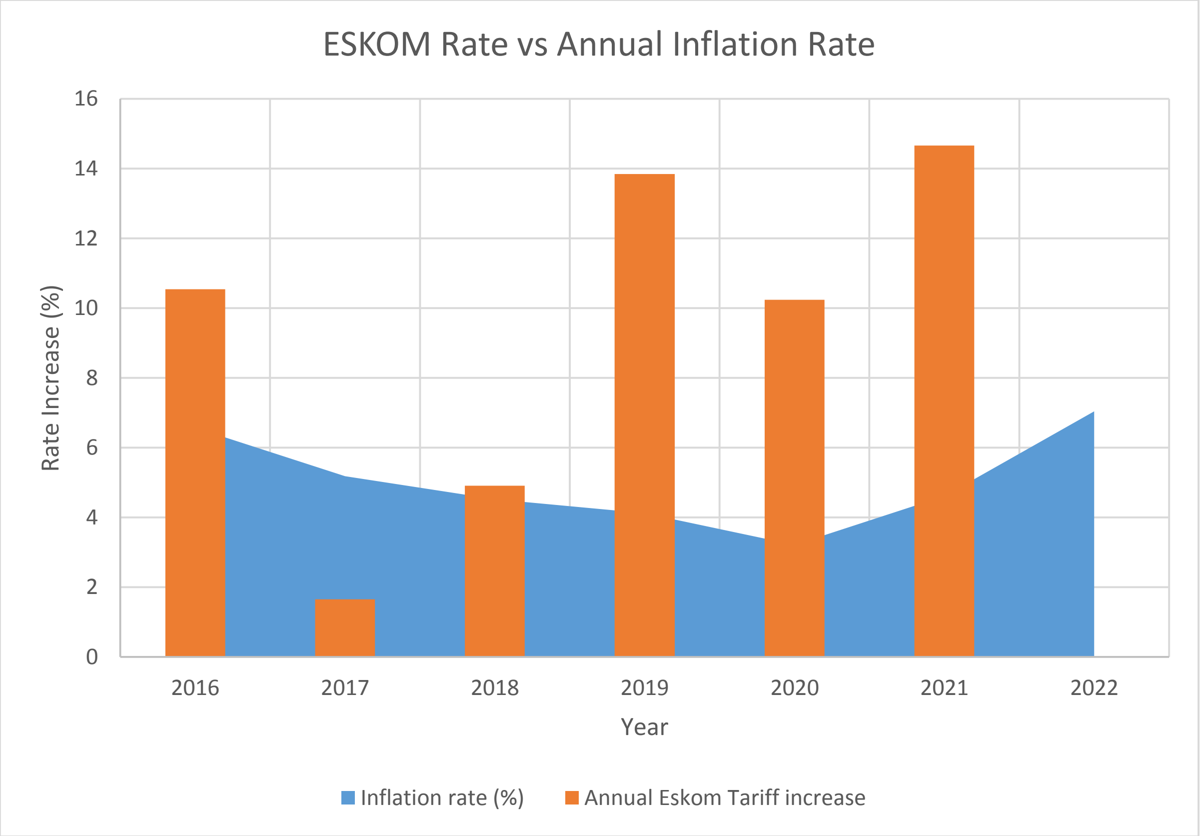

Figure 5.3: ESKOM annual increase vs. the annual inflation rate – Zandam

Table 5.2: Comparison of Zandam Farm electricity tariffs and ESKOM tariffs

Year	Inflation rate (%)	Charge per kWh to Zandam Farm (R)	ESKOM charge per kWh (R)		Annual Eskom tariff increase	kWh cost savings (R)	Possible cost savings per annum (R) x10^3	Actual cost savings per annum (R) x10^3		Cumulative cost savings (R) x10^3		Savings at 55% efficiency (R) x10^3	Cumulative cost savings at 55% efficiency (R) x10^3
		Electricity costs (increase at prime rate)	Excl. VAT	Incl. VAT (14% until 2018; 15% after)	Eskom annual % increase in electricity charge	IBERT charge - ESKOM charge	Based on the total electricity needs of Farm (1430000 kWh)	One CHP unit @75 kWh; 657000 kWh per annum	Max yield of 834435 kWh	One CHP unit @ 75 kWh per day; 657000 kWh per annum	Max yield of 834435 kWh	One CHP unit @ 75 kWh per day; 657000 kWh per annum	One CHP unit @ 75 kWh per day; 657000 kWh per annum
2016	6.57	1.01	1.28	1.46		-0.45	-645	-296	-377	-297	-377	-163	-163
2017	5.18	1.08	1.42	1.62	10.54	-0.54	-770	-354	-450	-651	-826	-195	-358
2018	4.52	1.13	1.43	1.64	1.65	-0.52	-739	-340	-431	-990	-1258	-187	-545
2019	4.12	1.17	1.50	1.72	4.91	-0.55	-788	-362	-460	-1352	-1718	-199	-744
2020	3.21	1.21	1.71	1.96	13.84	-0.75	-1075	-494	-628	-1847	-2345	-272	-1016
2021	4.61	1.26	1.88	2.16	10.23	-0.90	-1282	-589	-748	-2436	-3094	-324	-1340
2022	7.04	1.35	2.16	2.48	14.66	-1.12	-1608	-739	-939	-3175	-4032	-406	-1746
		1.17		1.86		-0.69	-987	-454	-576	-3628	-4608	-249	-1996

(Source: *South African Energy Price Report, 2021)

Zandam Farm utilises approximately 120,000 kWh per month or 1,430 MWh per year. Based on the savings listed per kWh in Table 5.2, the projected savings on only the electricity costs is almost R2,000,000 from commissioning to 2022, assuming a 55% efficiency in the CHP unit. The projected amount does not consider the diesel cost savings or the savings due to the heating costs. It also does not consider the rental amount received from IBERT for the immovable equipment. It was challenging to include the rental information as it was not freely available and formed part of the confidential contracts between Zandam Farm and IBERT.

Two-thirds of the original CAPEX investment of R2,944,000 was recovered by the electricity cost savings alone within six years of commissioning of the AD plant. It was estimated that the CAPEX costs would be recovered within ten years. The plant currently (2023) produces 834,435 kWhe per annum and 1074,570 kWht per annum. This is only 58% of the required electricity per annum. The actual saving for the farm is less than the projected amount as the process was not producing optimally. The throttling originally installed also affected the overall electricity output.

Assuming a basic production using only a single CHP unit, the break-even analysis of Zandam Farm can be determined as follows:

Table 5.3: Break-even analysis of Zandam Farm

Fixed costs	R2,944,000
Price per unit of electricity (ESKOM) Averaged from 2016 to 2023	R1.86
Price per unit of electricity (IBERT) Averaged from 2016 to 2023	R1.17
Break-even calculation	*BEA = 294,4000/(1.86-1.17) = 4266,666 kWh*
Using one CHP producing 75 kWh per day, and 657000 kWh per annum	BEA = 4266,666/(657,000*0.55) **BEA = 11.81 years**

Zandam Farm can recover the initial investment of R2,944,000 based solely on electricity generated by the AD plant within 11.81 years, as shown in Table 5.3 and Figure 5.4.

The LCOE was not considered as the farm purchases its electricity at a fixed rand value based on the co-ownership agreement.

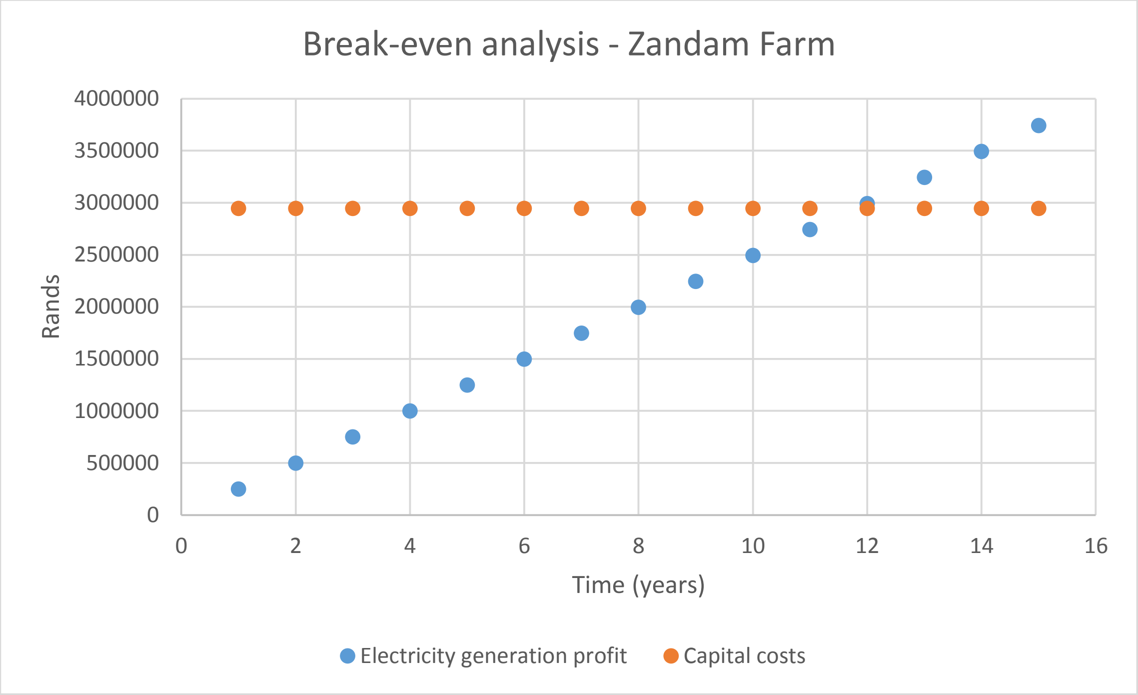

Figure 5.4: Break-even analysis Zandam Farm

5.3.2 Uilenkraal Dairy

Uilenkraal Dairy is located in Darling, in the Western Cape of South Africa. It uses an AD process to produce biogas using a total mixed ration (TMR) dairy cattle feedstock. Uilenkraal is a significant milk producer, supplying up to 30% of one of the major milk distributors' milk supply in the Western Cape.

The 7,000 m^3 lagoon digester was manufactured by Cape Advanced Engineering (CAE). The feedstock is cow manure collected from 1,900 lactating cows housed inside (NIRAS-LTS et al., 2021). CAPEX costs amounted to a cash amount of R11,000,000, provided by Uilenkraal (GreenCape, 2017).

Uilenkraal owns the fixed assets, while CAE provides the technology and technical support and manages the system. This is estimated at 33% of the overall project development costs. CAE is responsible for routine operation and generator maintenance and repair. Uilenkraal Farm is responsible for digester feeding, major equipment replacements and upgrades (NIRAS-LTS et al., 2021).

Generated electricity is used to offset the electricity obtained from the power utility ESKOM. The annual average demand for the farm is approximately 2,593 MWhe (NIRAS-LTS et al., 2021). The operational costs were agreed upon in the ownership agreement. Uilenkraal paid CAE R0.50/kWhe when the plant was commissioned. This amount increased to R0.74 by 2021, which is still below the cost of electricity received from ESKOM. In 2021, the cost per kWh from the national grid was already R1.85, which was more than double the costs Uilenkraal paid to CAE.

Table 5.4: Plant operating conditions at Uilenkraal Dairy

Project Developer	Cape Advanced Engineering
Feedstock	180–320 m^3 of slurry per day from 1,900 contributing cattle
Technical Specifications	*7,000 m^3 lagoon digester
	*Hybrid mixed, heated, plug flow reactor
	# x 250 kWe CHP units
	*500 kWe installed capacity
	*Electrical system designed for up to 1MW (with the addition of CHP units)
	+Digester operating temperature = 37 °C
	*HRT = 20–40 days
Energy Output	º 1,848 MWe/yr
	72% of the annual requirement in 2021

+ Farmers Weekly (2015); * GreenCape (2017); # South African Energy Price Report (2021)

Using the cost of electricity paid between 2014 and 2021, an average percentage increase in electricity was determined to be 6.857% per annum. There was an initial saving to Uilenkraal Dairy compared to ESKOM, which increased significantly with the above inflationary increases implemented by ESKOM in the last few years. An overview of the difference in costs between the Uilenkraal Dairy farm and ESKOM is given in Figure 5.5. Comparing the ESKOM rate of increase in electricity costs and the annual inflation rates in Figure 5.6, we see that the ESKOM rate of increase is far above the inflation rate.

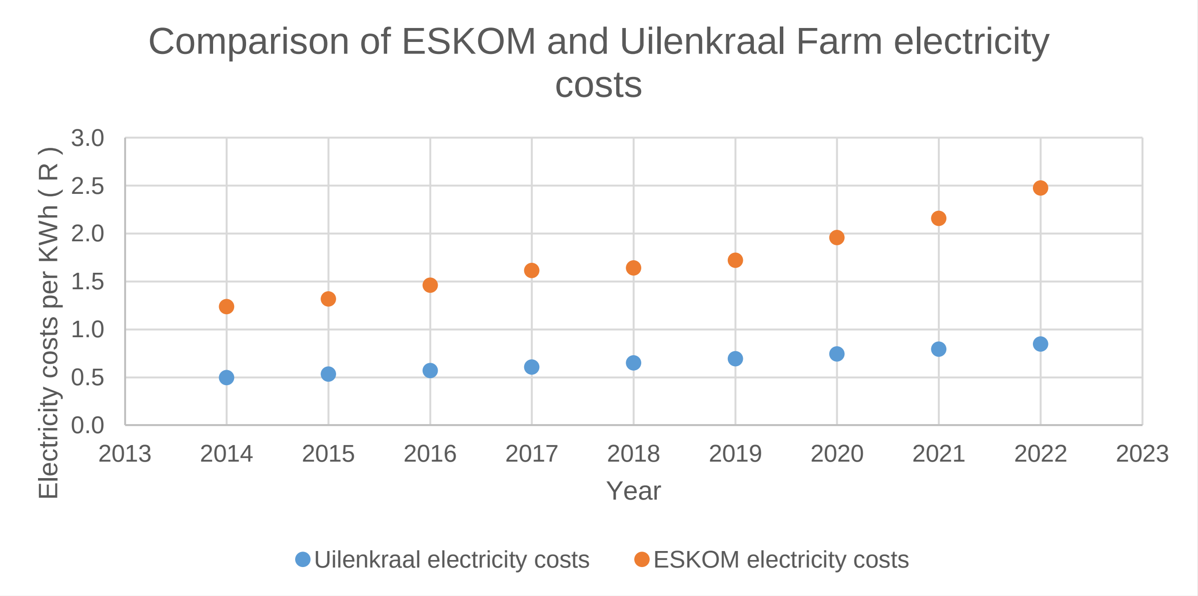

Figure 5.5: Comparison of ESKOM and Uilenkraal Dairy electricity costs

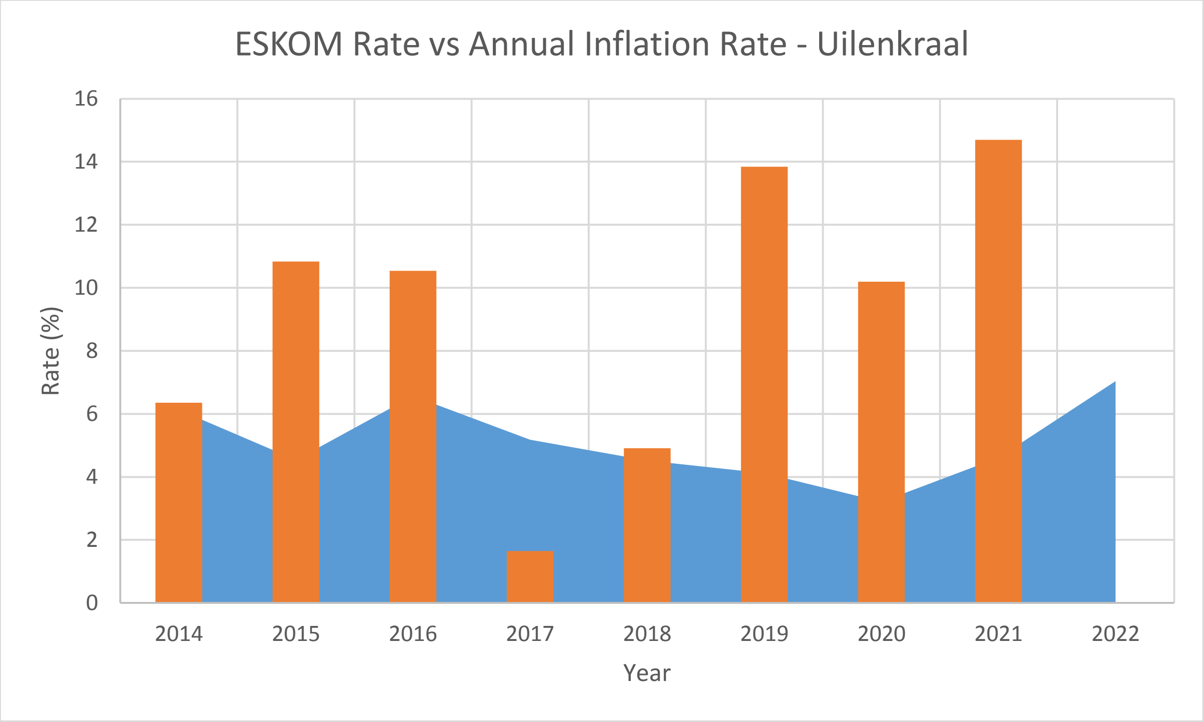

Figure 5.6: ESKOM annual increase vs. the annual inflation rate – Uilenkraal

Table 5.5 gives an overview of the total electricity costs since commissioning. A report published by GreenCape (2017) reported a monthly electricity cost reduction from R160,000 to only R12,000 when both CHP units are in operation. This means that within six years of operation, the CAPEX costs could be paid back with only electricity cost savings, as shown in Table 5.5 below, assuming 24-hour operation and 365 days of operation per annum at 55% efficiency of the CHP units.

A year after commissioning, *Engineering News* reported an electricity milestone of 1.5 GWh electricity production, stipulating that the farm could have produced more electricity if it had fed daily surplus electricity into the national electricity grid. At the time, the facility produced in excess of 1,000 tonne/year of methane (Engineering News, 2015). The plant is expected to pay itself back within ten years (GreenCape, 2017; NIRAS-LTS et al*.*, 2021). The plant has an expected lifespan of 30 years (Farmers Weekly, 2015).

Table 5.5: Comparison of Uilenkraal Dairy Farm electricity tariffs and ESKOM tariffs

Year	Inflation Rate	Charge per kWh to Uilenkraal Farm (R)	ESKOM charge per kWh (R)		Annual Eskom tariff increase	kWh cost savings (R)	Possible cost savings per annum (R) x10^3	Actual cost savings per annum (R) x10^3		Cumulative cost savings (R)		Savings at 55% efficiency	Cumulative cost savings at 55% efficiency
		Electricity costs (average increase of 6.857% p.a.)	Excl. VAT	Incl. VAT (14% until 2018; 15% after)	Eskom annual % increase in electricity charge	CAE charge - ESKOM charge	Based on Total Production electricity needs of Farm (2593 MWh)	Two CHP units @250 kWh each; 4380 MWh per annum	Max Yield of 1.5 GWh per month	Two CHP units @250 kWh each;	Max Yield of 1.5 GWh per month	Two CHP units @250 kWh each; 4380 MWh per annum	Two CHP units @250 kWh each; 4380 MWh per annum
2014	6.13	0.50	1.09	1.24		-0.74	-1918	-3240	-13316	-3240	-13316	-1782	-1782
2015	4.54	0.53	1.16	1.32	6.35	-0.78	-2034	-3435	-14116	-6675	-27432	-1889	-3671
2016	6.57	0.57	1.28	1.46	10.83	-0.89	-2309	-3900	-16028	-10575	-43460	-2145	-5816
2017	5.18	0.61	1.42	1.62	10.54	-1.01	-1438	-4403	-18096	-14978	-61555	-2422	-8238
2018	4.52	0.65	1.43	1.64	1.65	-0.99	-1416	-4337	-17821	-19315	-79377	-2385	-10623
2019	4.12	0.70	1.50	1.72	4.91	-1.03	-1467	-4494	-18468	-23809	-97844	-2472	-13095
2020	3.21	0.74	1.71	1.96	13.84	-1.22	-1740	-5329	-21899	-29138	-119743	-2931	-16026
2021	4.61	0.80	1.88	2.16	10.20	-1.37	-1953	-5981	-24580	-35119	-144323	-3290	-19315
2022	7.04	0.85	2.16	2.48	14.70	-1.63	-2329	-7133	-29316	-42252	-173639	-3923	-23239

The break-even analysis, using two operational CHP units, is given below (Table 5.6):

Table 5.6: Break-even analysis of Uilenkraal

	Assuming 2 CHP units operating 24 hours a day, 365 days per year	**Assuming 2593 MWh per annum production**
Fixed costs	R11,000,000	R11,000,000
Price per unit of electricity (ESKOM) Averaged from 2016 to 2023	R1.73	R1.73
Price per unit of electricity (CAE) Averaged from 2016 to 2023	R0.6615	R0.6615
Break-even calculation	*BEA = 11,000,000/(1.73-0.6615)* *= 1,0294,805.80 kWh*	*BEA = 11,000,000/(1.73-0.6615)* *= 10,294,805.80 kWh*
Payback period	Payback = 10,294,805.80/(3,504,000*0.55) **Payback period = 5.34 years**	Payback = 10,294,805/(2,593,000*0.55) **Payback period = 7.21 years**

Payback on the CAPEX amount is estimated at between five (5) and 7.5 years, operating full-time at a CHP efficiency of 55%.

Based on the co-ownership agreement, the LCOE was not considered as the farm purchases its electricity at a fixed rand value.

5.4 AD process using seaweed as the feedstock

The mass flow of the feedstock and output block diagram is shown in Figure 5.7. Dried seaweed is transported from the purchasing company to the AD site, where electricity, heat and digestate are produced.

Feedstock costs are comprised of the cost of the seaweed, the transport of the seaweed to the AD process, the cost of the inoculum, as well as the cost of the water required to make up the slurry required. The majority of the costs include the costs of the dried seaweed and the transport costs to have the amount of seaweed required on-site for daily methane production.

Ecklonia Maxima, in its dried form, requires a lot of water to reconstitute it. It is also more expensive as it is dried and milled before transport. In its natural environment, seaweed contains between 80% and 90% water (Sudhakar et al. 2018). The recommended ratio in literature to reconstitute dried kelp is 1:10, using water (Dave et al., 2013).

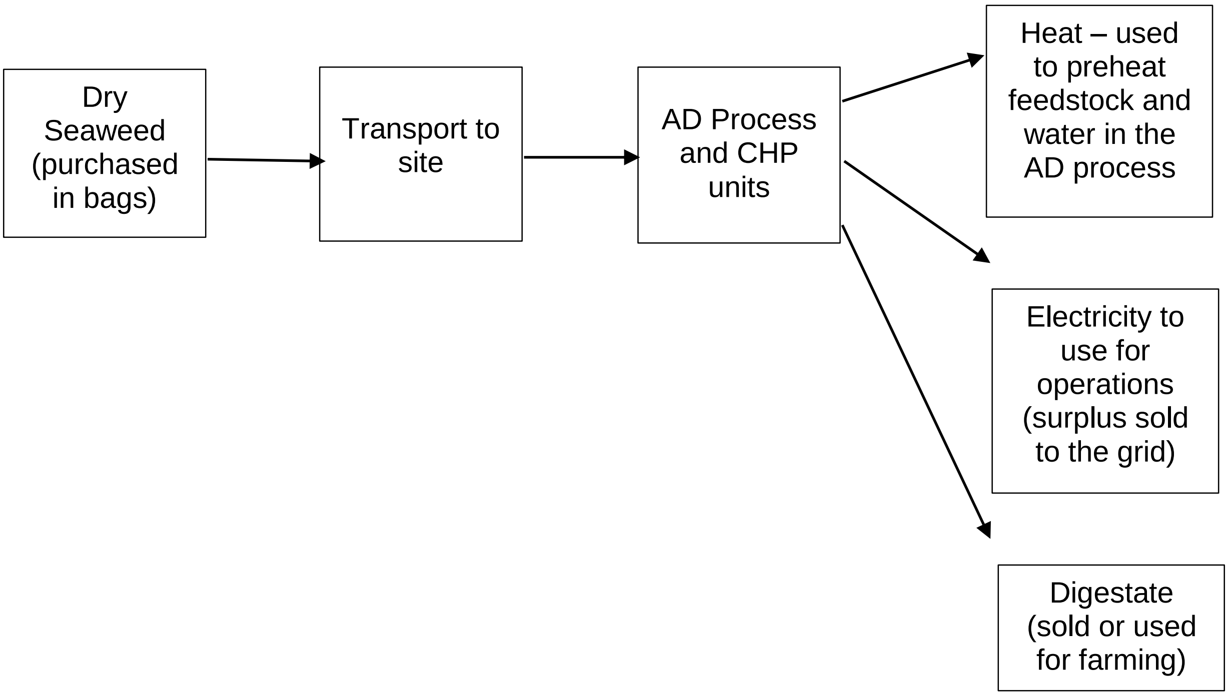

Figure 5.7: Mass flow and electricity production of the AD process

Seaweed was sold wet to abalone farms at a cost of R1,400/tonne delivered in 2013 (Anderson & Rothman, 2013). That price has now increased to R2,037.50 per tonne, excluding delivery (CSIR Enterprise Creation for Development, 2022). As the seaweed is wet, reconstitution is unnecessary before feeding to the AD process. Dry, crushed seaweed is sold in 20 kg bags at R607.20 (Price correct as of 23 August 2023). The dry seaweed would cost R30,360 per tonne, excluding the costs of transporting the seaweed to the site. Considering the water required to reconstitute the seaweed, this dry weight would be equivalent to 10 tonne of wet seaweed, costing R20,375. Based purely on the cost of the seaweed, purchasing the seaweed wet might be cheaper but would have storage and frequency of delivery implications. The deciding factor would be the cost of the transportation based on the quantities required and the storage solutions for the seaweed. Ease of use should also be taken into consideration.

The inoculum utilised in this study is BioGanic fertiliser. The retail cost of the fertiliser is R380/20kg, equivalent to R19/kg. An alternative to this could be animal manure or starter fluid from an existing AD process.

The initial loading of the seaweed to inoculum is 1:1 based on the VS content (Moo-Young et al., 2019). Inoculum is only required once during the start-up of the AD process. Once the culture is established, the AD process should operate without additional inoculum required.

5.4.1 Biogas yield

The annual biogas yield is based on the carbon conversion to methane, as determined in Chapter 4. *Ecklonia Maxima* contains 51.15% C, based on the elemental analysis. Based on calculations using equation 4.2, 49.89% of the available carbon is converted to methane, with the balance going to carbon dioxide. The results obtained showed that 388.413 m^3 CH_4/tonne seaweed is produced. An associated 129.451 m^3/tonne CO_2 is produced.

Table 5.7: Biomethane yield per tonne of *Ecklonia Maxima*

Total carbon (%) (m/m)	51.15
Carbon conversion to methane (%)	49.89
Carbon conversion to carbon dioxide (%)	50.11
Methane yield per tonne (kg/tonne)	255.1874
Density of methane (kg/m^3)	0.657
Methane yield (m^3/tonne seaweed)	**388.413**
Carbon dioxide produced (kg/tonne)	256.3127
Density of carbon dioxide (kg/m^3)	1.98
Carbon dioxide produced (m^3/tonne seaweed)	129.4508

5.4.2 Electricity generated from *Ecklonia Maxima*

Electricity generated is based on the biomethane yield, the energy content in the biomethane, and the efficiency of the CHP generator.

The equation used to estimate the electricity generated is (Garcia, 2014):

$$E\left(\frac{kWh}{annum}\right) = m_{biogas} \times Energy\ content_{biogas} \times Efficiency_{CHP} \qquad (5.5)$$

CHP units have a 35% electricity conversion efficiency and a 50% heat conversion efficiency (Biogas World).

The calorific value of biomethane biogas is between 20 and 26 MJ/m^3. Using the lower value (converted to kJ/m^3 of 30441.4) and substituting this into the equation to estimate the electricity generated, it is found that 755.25 kWh of electricity/tonne of seaweed is possible. We know that one tonne of seaweed produces 255.1874kg of biogas. Substituting these values into equation 5.5 shows:

$$E = 255.1874 \times 30441.4 \times 0.35/3600 = 755.2475$$

Table 5.8: Electricity produced per tonne of *Ecklonia Maxima*

Biogas mass (kg)	255.1874
LCV of biomethane (MJ/m^3)	20
LCV of biomethane (kJ/kg)	30441.4
Efficiency CHP (%)	0.35
E (kWh/tonne seaweed)	755.2475

The calculated electricity generated per tonne of seaweed is 755.25 kWh, based on seaweed producing 255.1874kg of biogas per tonne loaded to the reactor.

5.5 Case study one – Zandam Farm

Case study one compares replacing the cow manure feedstock at Zandam Farm with seaweed in an identical AD process to determine if a similar plant would be economically feasible if erected elsewhere. The IBERT/Zandam agreement is taken as the base case for comparison. Still, the seaweed study assumes that the IBERT contract does not come into effect and that the entire costs of the AD plant would be carried by the operational company where the AD is installed so that actual electricity produced can be considered. As designed, a single CHP unit is utilised, and the seaweed load is based on the amount required to produce 75 kWh of electricity, operating 24 hours a day, 365 days a year. The CHP efficiency is assumed to be between 55% and 80%. It is assumed that the CAPEX costs will be the same as for the initial installation, considering the present-day value of that investment. In contrast, the operating costs depend on the costs of the feedstock, transport, and utilities. The electricity savings are based on the ESKOM tariffs for small enterprises and the calculated LCOE for the AD plant.

5.5.1 CAPEX costs

The CAPEX cost for Zandam Farm was R9,200,000 in 2016. Based on the average inflation rate of 4.86% per annum in South Africa between 2016 and 2023, the CAPEX amount would equate to R12,822,300 in 2023. According to the co-ownership agreement, this would equate to an initial immovable equipment investment of R4,103,140. The initial movable property would be R8,720,000, including the single CHP unit. This is a cumulative price increase of 39.37% over the 2016 investment and does not include linking the AD process to upload surplus electricity to the electricity grid if required. This connection amounts to R11,000 for installing a bi-directional meter, which is a requirement (MyBroadband, 2023). However, a monthly fee is associated with the meter usage, which would add to OPEX costs.

5.5.2 OPEX costs

The AD plant produces 75 kWh of electricity from one CHP unit, operating 24 hours a day and 365 days a week. The efficiency of the CHP unit is assumed to be between 55% and 80%. The initial feedstock costs include loading inoculum, seaweed, and water. Daily feeds include only seaweed and water once the AD process is fully operational. The equivalent costs of the electricity produced by the CHP unit are determined using the ESKOM commercial tariff for small power users, tier 2, along with the associated daily charge of R5.42 excluding Value Added Tax (VAT). The kWh cost is R3.1262, excluding VAT. The values, including VAT, are R6.233 and R3.595, respectively.

Table 5.9 gives an overview of the electricity requirements, the associated biogas requirement and possible electricity cost savings based on ESKOM charges assuming 100% conversion to electricity in the CHP units.

Table 5.9: Case study to replace the feedstock using *Ecklonia Maxima* – Zandam Farm

Scenario – producing enough electricity to run 1 CHP unit operating at 75 kWh			
		Unit Costs (R)	**Total Costs (R)**
Electricity Requirements			
CHP unit (kWh)	75		
Daily electricity yield (kWh) @ 55% efficiency	1,800		
Annual electricity yield (kWh) ESKOM	657,000	3.595	**-2,361,915.00**
Basic daily charge electricity R6.233/day ESKOM		6.233	-2,275.05
Biogas Requirements based on one CHP unit			
Annual biogas required (kg/annum)	27,193.887		
Daily biogas required (kg/day)	74.5038		
Feedstock requirements			
Seaweed load required (kg/day)	291.957262	12% solids	8,863.82
Water load (kg/day)	2,141.019921		153.51
Inoculum load required (kg/day)	291.957262		5,547.19
Start-up costs – feedstock (kg/day)			14,564.52
Daily costs, excluding inoculum			9,017.33
Annual costs			**3,296,873.95**
Additional costs of running 1 CHP unit at total capacity			
Feedstock costs less electricity recovery costs (ESKOM)			**932,683.908**

The annual electricity yield is 657 MWh. The required feedstock to produce the electricity was estimated, considering that each tonne of seaweed produces 255.1874 kg of biogas. Approximately 27 tonne of biogas would be required to operate a single CHP unit at total capacity. Feed to the reactor consists of the inoculum initially required at start-up and the seaweed slurry fed to the reactor daily (consisting of seaweed and water containing 12% solids). The assumption in the case study is that water is bought in from the municipality in totality. The feed costs are R3,290,000 per annum based on online standard costs.

At 55% CHP efficiency, the electricity produced reduces to 361.350 MWh. The feedstock costs would still be R3,290,000 as 27 tonne of biogas is still fed to the CHP unit. Only 55% of the biogas is converted to electricity. The equivalent return on investment electricity costs of R1,299,000, based on the ESKOM tariff for the electricity yield of 361.356 MWh, is much less than the R2,360.000 expected at 100% efficiency. There is a loss of approximately 12 tonne of biogas throughout the year. Assuming that the water required is available via catchment dams on the farms, the annual costs for the feedstock reduce slightly to R3,240,000. There is a slight cost saving of R56,032.46, a 1.699% saving of the total feedstock costs.

Figure 5.8 shows the electricity requirements for CHP efficiencies ranging between 55% and 80%. It also indicates the feedstock costs.

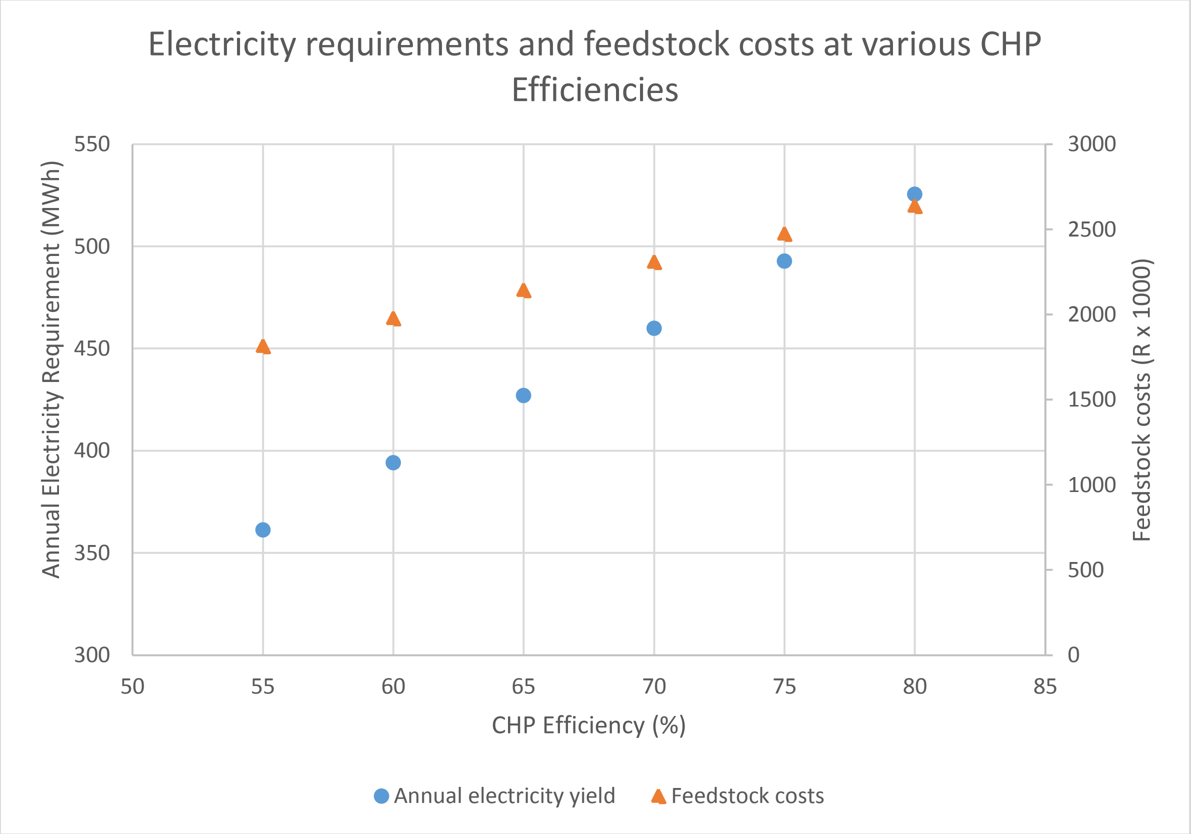

Figure 5.8: Electricity requirements and feedstock costs at various CHP efficiencies

There is a linear relationship between the increased CHP efficiency and output, which is expected. A linear upward relationship exists between the increase in CHP efficiency and feedstock costs. At 80% efficiency, the electricity requirement is 526 MWh, and the Feedstock costs are R2,640,000. This is still only 37% of the required 1,430 MWh. A single CHP unit is, therefore, incapable of providing the production electricity needs of the farm. Should the farm wish to operate independently of ESKOM, a consideration for up-scaling the AD process and installing at least two additional CHP units should be considered.

To determine the actual costs of producing the required electricity, the LCOE calculation needs to be completed when using the seaweed as feedstock. Table 5.10 gives an overview of the data used to determine the LCOE.

The calculated LCOE identified by equation 5.2 is R5.37/kWh at 100% efficiency. This is more than double the ESKOM amount of R2.48/kWh and almost four times the base rate of R1.35 currently charged by IBERT (Figure 5.8). For the AD process utilising seaweed to be feasible, the feedstock cost needs to be reduced significantly.

The seaweed can, therefore, not be purchased dry or bagged. It might be possible to negotiate with the supplier to deliver bulk amounts not bagged for a negotiated bulk price to reduce the costs. If the feedstock costs could be halved by using wet seaweed, which would be cheaper, the LCOE would reduce from R5.35/kWh to R2.68/kWh, which would be comparable to the ESKOM rate currently being charged.

Table 5.10: TLCOE parameters – Zandam case study

	CHP Efficiency (%)						
	100	80	75	70	65	60	55
[a] Discount Rate – based on the Central Bank discount rate for South Africa	5.75%	5.75%	5.75%	5.75%	5.75%	5.75%	5.75%
[b] Growth Rate – based on the Consumer Price Index for South Africa	4.10%	4.10%	4.10%	4.10%	4.10%	4.10%	4.10%
Energy Consumed per annum (kWh x10^3)	657	526.6	492.75	459.9	427.1	394.2	361.35
Annual Inflation Rate (2023)	7.04%	7.04%	7.04%	7.04%	7.04%	7.04%	7.04%
Annual Feedstock costs (Rm)	R3.29	R3.29	R3.29	R3.29	R3.29	R3.29	R3.29
LCOE (R/kWh)	R5.37	R6.71	R7.16	R7.67	R8.26	R8.95	R9.77

The optimum operation of the CHP unit would be approximately 80% conversion, with a LCOE at R6.71.

Zandam Farm requires 1,430 MWh in annual electricity usage. A single CHP unit will only provide between 361.35 MWh and 526.6 MWh, 25 % to 37% of the required electricity for operational needs per annum. The balance would have to be bought in from the municipality at an estimated R3,840,000 per annum, with the CHP unit operating at the bare minimum of 55%.

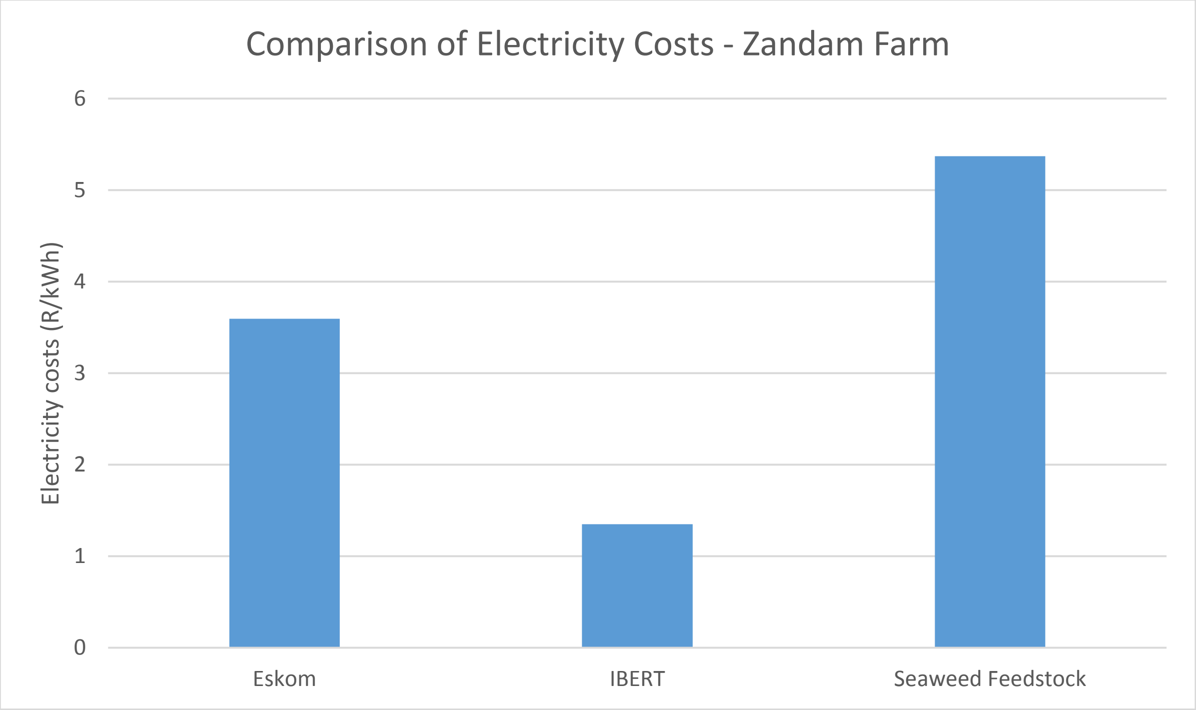

Figure 5.9: Comparison of electricity costs for case study one

The LCOE increases with a decrease in CHP efficiency, as seen in Figure 5.10. Should it be expected that all the required electricity be produced using the AD bioreactor with seaweed as a feedstock, the annual cost of electricity would increase exponentially as additional CHP units would have to be installed, increasing the OPEX as well as CAPEX costs.

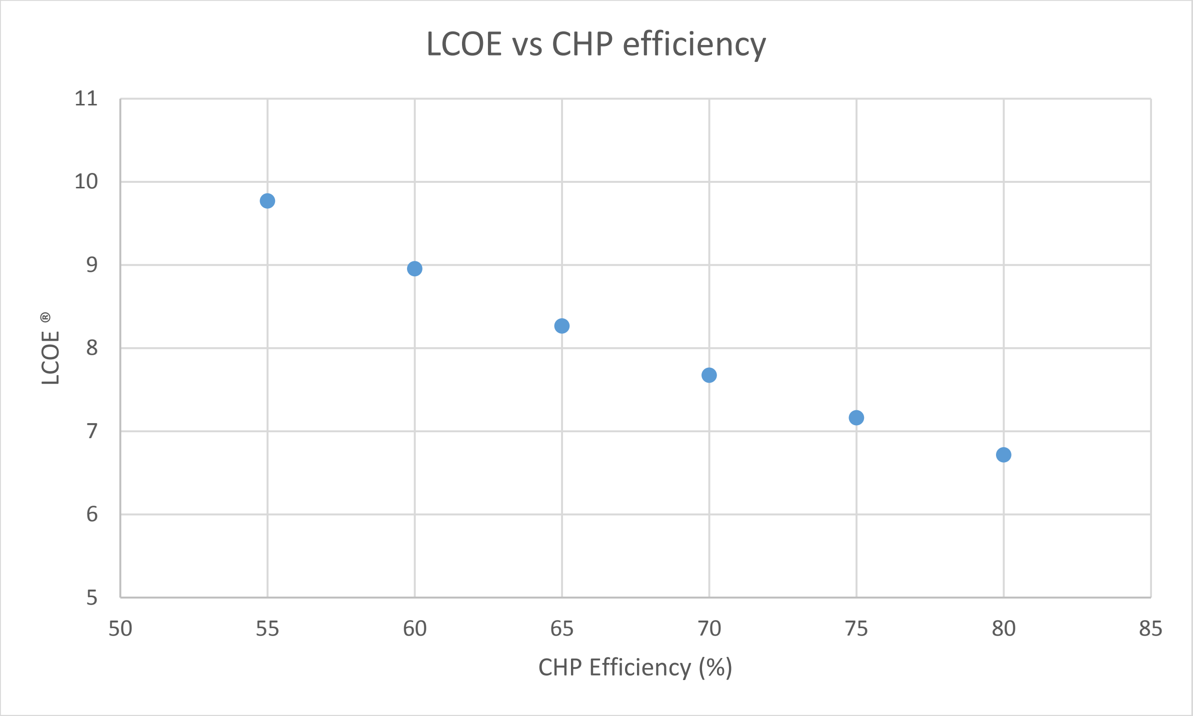

Figure 5.10: LCOE vs CHP efficiency – Zandam Farm

5.6 Case study two – Uilenkraal Dairy

Case study two compares replacing the cow manure feedstock at Uilenkraal Farm with seaweed feedstock using the same design AD process. As with case study one, the electricity supply agreement between Uilenkraal and AEC is taken as the base scenario for comparison. This is compared to purchasing directly from ESKOM and replacing the existing feedstock with seaweed to produce the electricity required independently of ESKOM and AEC. Two operational CHP units will be considered, each with 250 kWh electricity output, operating 24 hours a day and 365 days per annum between 55% and 80% efficiency. It is assumed that the CAPEX amount is the present value of the plant costs on start-up in 2014. The operating costs take into consideration the feedstock, transport, and utilities. The electricity savings are based on the ESKOM tariffs for small enterprises and the calculated LCOE for the AD plant.

5.6.1 CAPEX costs

The CAPEX costs for Uilenkraal Farm were R11,000,000 in 2014. This amount is equivalent to R17,080,000 in 2023, based on an annual average inflation rate of 5.01% between 2014 and 2023. This is a cumulative increase of 55.28% over the 2014 investment, considerably higher than for the Zandam Farm scenario, which was only 39.37%. It does not consider linking the AD process to upload surplus electricity to the electricity grid, which would be an additional R11,000 for the bi-directional meter required, plus the associated monthly administration fee.

5.6.2 OPEX costs

The OPEX costs consist mainly of the feedstock costs. This includes the seaweed costs and the initial inoculum costs required during the start-up phase of the AD reactor. The equivalent electricity costs of the electricity using two CHP units, each producing 250 kWh, is determined using the ESKOM commercial tariff for small power users, tier 2. This cost amounts to R5.42 excluding VAT and R6.233 including VAT. ESKOM's daily electricity availability charge is R3.1262, excluding VAT and R3.595, including VAT.

Table 5.11 overviews the economics of using seaweed as a replacement feedstock, assuming 100% conversion to electricity in the CHP units.

Table 5.11: Case study to replace the feedstock using *Ecklonia Maxima* – Uilenkraal Farm

Scenario - producing electricity to run 2 CHP units operating at 250 kWh each			
		Unit Costs (R)	**Costs (R)**
Electricity Requirements			
CHP unit (kWh)	500		
Daily electricity yield (kWh)	12,000		
Annual electricity yield (kWh) ESKOM	4,380,000	3.595	**-15,746,100.00**
Basic daily charge electricity R6.233/day ESKOM		6.233	-2,275.05
Biogas Requirements based on two CHP units			
Annual biogas required (kg/annum)	181,292.58		
Daily biogas required (kg/day)	496.692		
Feedstock requirements			
Seaweed load required (kg/day)	1,946.382	12% solids	59,092.15
Water load (kg/day)	14,273.467		1,023.41
Inoculum load required (kg/day)	1,946.382		36,981.25
Start-up costs – feedstock (kg/day)			97,096.81
Daily costs, excluding inoculum			60,115.56
Annual costs			**21,979,159.69**
Additional costs of running 2 CHP units at 250 kWh each			
Feedstock costs less electricity recovery costs (ESKOM)			**6,230,784.64**

The annual possible electricity yield is 4,380 MWh, with an associated feedstock cost of almost R22,000,000. This assumes that both CHP units operate full-time and at 100% efficiency. This is obviously impossible to achieve practically. On average, a daily biogas production of 496.7 kg biogas is required to run both CHP units. With the conversion somewhere between 55% and 80%, the amount of electricity will vary, as seen in Figure 5.11. Losses incurred in the CHP unit would negatively affect the electricity production and essentially waste the biogas produced at an elevated feedstock cost. In Figure 5.11 it is assumed that only the biogas required to produce a certain amount of electricity is used, and the feedstock costs are determined for that amount.

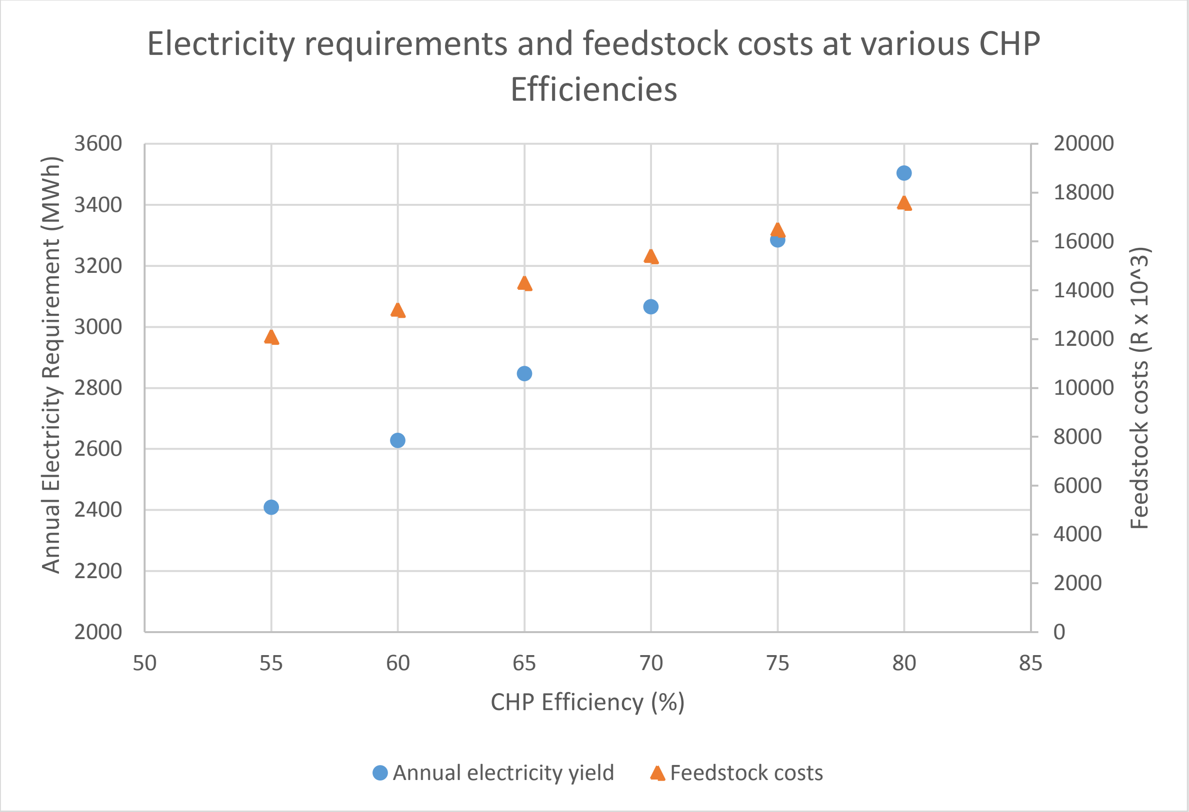

Figure 5.11: Electricity requirements and feedstock costs at various CHP efficiencies

Therefore, a linear cost increase indicates the steepness of the loss being made compared to the 100% efficiency scenario.

Since the same feedstock is utilised at Zandam Farm, the LCOE is expected to range between R5.37/kWh at 100% efficiency and R9.77/kWh at 55% efficiency. This is much higher than the costs currently paid by Uilenkraal, which is R1.35. It is also much higher than the ESKOM electricity tariff of R3.595/kWh, as seen in Figure 5.12 below. The seaweed costs are linked to the LCOE and are based on 100% CHP efficiency. The LCOE would increase as the efficiency of the CHP unit decreases. With the LCOE already almost four times that of the Uilenkraal Farm and 1.5 times that of the ESKOM tariff, it is essential that the CHP units operate at maximum efficiency to keep the costs of the feedstock down.

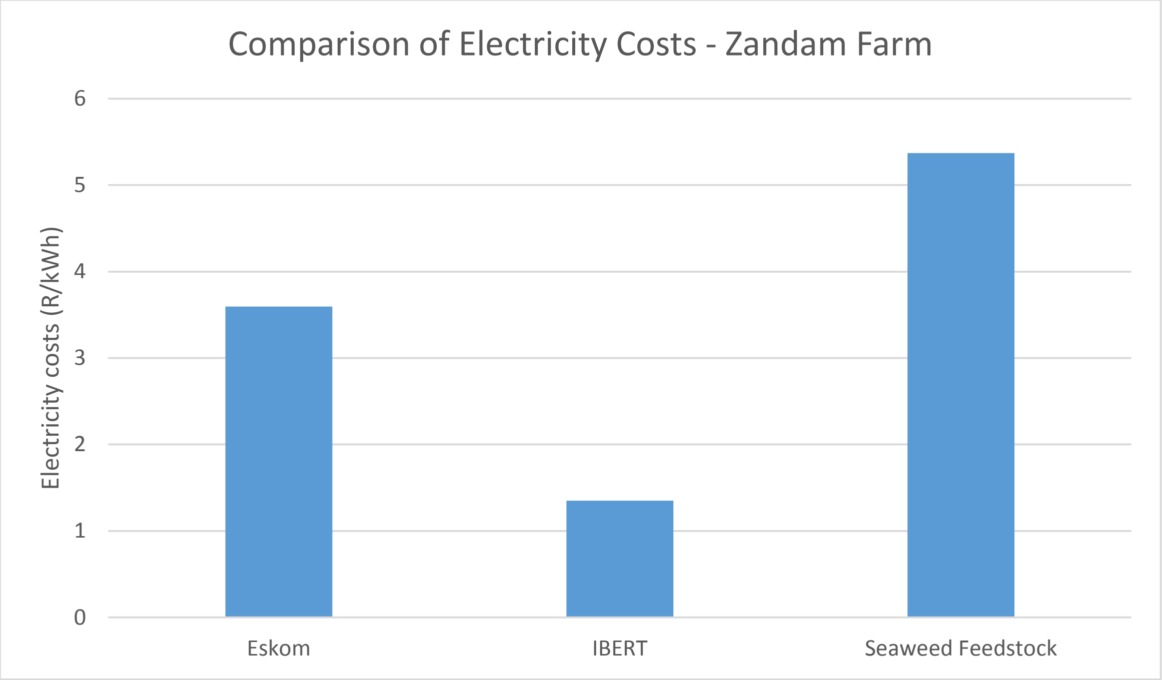

Figure 5.12: Comparison of electricity costs for case study two

5.7 Conclusion

Seaweed feedstock is capable of producing the required biomass in the AD process. Currently, the cost of purchasing dry seaweed in bags is prohibitory as it is costly and makes the AD process unsustainable. Investigations into bulk purchases or self-harvesting could be investigated to reduce the feedstock costs.

The case studies assumed literature values of 35% of biogas being converted to mechanical electricity and then 55% of that electricity converted to the electrical efficiency of the CHP unit. Some CHP units can operate at 90%, and then biogas conversions are higher. Operating at maximum efficiencies could bring the LCOE within range of the ESKOM rates charged and make Zandam Farm viable by using seaweed as a feedstock. The same could be done for Uilenkraal Farm.

Suppose the feedstock costs can be reduced, along with the optimisation and high performance of the CHP units. In that case, the LCOE will also reduce as the feedstock is the most significant contributor to the annual expenses for both farms. The LCOE needs to be competitive with the ESKOM rates rather than with the base costs described, as most companies would not have the same co-ownership agreements as Zandam Farm and Uilenkraal Farm .

It is recommended that a pilot plant study be performed to gauge the actual electricity production, which would give a clearer understanding of the system parameters for electricity generation.

CHAPTER SIX: CONCLUSION

6.1 Introduction

Ecklonia Maxima contains 51.15% carbon based on the laboratory analysis. Approximately 47.78% of the carbon is theoretically converted to methane. The theoretical BMP was determined at 499.64 L/kg VS. The lignin content was determined experimentally as 29%. This value is higher than the range (6-12.9%) suggested in the literature for brown seaweed. Ecklonia Maxima has a reasonably high associated ash content of 24.3%, which might need a pre-treatment step before the AD process. Ecklonia Maxima can produce biomethane via the AD process. The amount of biomethane produced fluctuated depending on the inoculum used. At mesophilic temperatures, the BioGanic/Ecklonia Maxima combination performed best in terms of methane production.

The optimum operating pH for the AD system for both mesophilic and thermophilic temperature ranges is between 6.8 and 7.4. Pre-treatment processes were applied to the BioGanic/Ecklonia Maxima combination with mixed results on the biomethane yield. Using a smaller size distribution (< 1.7mm) for the Ecklonia Maxima yielded higher biomethane yields at mesophilic conditions. All pre-treatment processes at mesophilic conditions increased the methane yield (34.59 ml/g VS – 126.157 ml/g VS) compared to raw seaweed (31.54 ml/g VS). However, the increase was not as significant as the increase (126.157 ml/g VS) observed using the smaller size distribution for the mesophilic range. The opposite was seen for the thermophilic temperature range, where all pre-treatment processes decreased the methane yield.

Statistical analysis shows that temperature influences the biogas yield as the p – value was less than 0.05 at mesophilic conditions (p =0.0205). However, size distribution does not affect the biogas yield at thermophilic conditions (p – value = 0.13). At thermophilic conditions, the increased temperature increases the hydrolysis rate, leading to faster degradation of the carbohydrates and increased biomethane yield. Statistical analysis showed that neither acid nor base pre-treatment methods of the biomaterial influence the biogas yield. The operating temperature is more significant than the pre-treatment methods used.

The AD process was described by first-order kinetics. The modified Gompertz equation was fit for purpose for both the mesophilic and thermophilic temperature ranges. It allows for the determination of lag phase, maximum methane potential, and rate of the chemical reaction.

Using seaweed as a proposed feedstock in the two-dairy cases – studies found that seaweed can produce 255.1874 kg methane per ton of seaweed, with an associated 755.2475 kWh of electricity. The LCOE decreases with an increase in CHP efficiency. The cost of the seaweed biomass is the overriding factor in the feasibility of the process. Reducing the costs would therefore be a priority.

6.2 Future work

It is recommended that a pilot plant be constructed using the optimum conditions for both seaweed size (< 1.7mm), operating temperature (37oF), and pH (7.0 ±0.2) as identified in objectives 1 and 2. The methane produced should be converted to electricity using a CHP unit. The pilot plant will clearly indicate the yields obtainable, giving a better representation of the LCOE. The most essential element is to reduce the cost of the feedstock.

It is recommended that:

i. The seaweed is harvested as cast seaweed to reduce the costs.
ii. The seaweed is used wet to reduce the costs.
iii. A batch of seaweed is collected through all four seasons, and an average chemical analysis is used incorporating all seasons to negate the composition variances linked to when the seaweed is collected.

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APPENDIX 1: OBJECTIVE 1 DATA

Mesophilic data

Mesophilic Data - Based on VS of Seaweed

Temperature: 37⁰C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 42 days

Seaweed	VS	0.7237 g/g	Mass	20 g
Vitaboost	VS	0.287 g/g	Mass	28 g
BioGanic	VS	0.72 g/g	Mass	20 g
Manure	VS	0.66 g/g	Mass	80 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
Vitaboost Run 1	1	7.160			14.476		14.476														
	8	5.700	7.180	220.000	1.954	0.934	15.496	0.000	4.700	16.700	0.079	78.522	0.000	10.340	36.740	0.173	172.747	0.000	0.000	0.000	0.000
	12	7.300	7.110	255.000	1.954	0.762	16.689	1.600	0.600	17.100	0.000	80.700	4.080	1.530	43.605	0.000	205.785	0.263	0.263	0.232	0.232
	19	7.120	7.050	160.000	1.954	0.775	17.868	3.000	0.600	15.100	0.003	81.297	4.800	0.960	24.160	0.005	130.075	0.288	0.551	0.253	0.485
	22	7.510	7.120	95.000	1.954	0.534	19.288	0.900	0.600	12.600	0.004	85.896	0.855	0.570	11.970	0.003	81.602	0.048	0.599	0.042	0.527
	29	7.360	7.140	280.000	1.954	0.373	20.869	2.100	0.800	14.100	0.011	82.989	5.880	2.240	39.480	0.031	232.369	0.305	0.904	0.268	0.796
	33	7.120	7.050	290.000	1.954	0.267	22.557	6.600	11.700	7.100	0.100	74.500	19.140	33.930	20.590	0.290	216.050	0.917	1.821	0.808	1.603
	36	7.080	7.080	590.000	1.954	0.477	24.034	18.600	3.800	11.000	0.014	66.586	109.740	22.420	64.900	0.080	392.860	4.865	6.686	4.284	5.888
	40	7.390	7.190	650.000	1.954	0.256	25.732	40.200	3.600	10.800	0.004	45.396	261.300	23.400	70.200	0.023	295.077	10.872	17.558	9.574	15.462
	43	7.610		450.000			25.732	15.900	2.000	10.400	0.000	71.700	71.550	9.000	46.800	0.001	322.649	2.781	20.338	2.449	17.911
Bio-Ocean Run 2	1	7.090	0.000	0.000	14.476	0.000	14.476														
	8	5.650	7.030	470.000	1.954	0.283	16.147	0.000	6.600	10.800	1.000	81.600	0.000	31.020	50.760	4.700	383.520	0.000	0.000	0.000	0.000
	12	7.190	7.090	1140.000	1.954	1.197	16.905	37.900	5.300	11.200	0.000	45.600	432.060	60.420	127.680	0.000	519.840	26.758	26.758	23.564	23.564
	19	0.000	0.000	460.000	1.954	0.849	18.009	36.700	3.200	14.700	0.001	45.399	168.820	14.720	67.620	0.006	208.834	9.987	36.744	8.795	32.359
	22	7.230	7.110	610.000	1.954	0.534	19.429	14.000	1.800	13.800	0.001	70.399	85.400	10.980	84.180	0.008	429.432	4.742	41.486	4.176	36.535
	29	7.460	7.140	705.000	1.954	0.688	20.695	22.700	1.900	11.700	0.000	63.700	160.035	13.395	82.485	0.000	449.085	8.237	49.723	7.254	43.789
	33	7.410	7.080	410.000	1.954	0.211	23.768	8.100	0.900	13.500	0.000	77.500	33.210	3.690	55.350	0.001	317.749	1.508	54.971	1.328	48.410
	36	7.260	7.080	350.000	1.954	0.320	25.402	4.000	0.400	14.700	0.010	80.890	14.000	1.400	51.450	0.034	283.116	0.589	55.560	0.519	48.929
	40	6.990	7.050	280.000	1.954	0.576	26.780	4.400	4.400	13.600	0.033	77.567	12.320	12.320	38.080	0.094	217.186	0.485	56.045	0.427	49.356
	43	7.040	7.020	150.000	1.954	0.331	28.402	2.300	0.500	16.400	0.001	80.799	3.450	0.750	24.600	0.002	121.198	0.129	56.174	0.113	49.469
Co	1	7.080	0.000	0.000	14.476	0.000	14.476														

	8	6.280	7.090	0.000	1.954	0.748	15.682	0.000	0.000	0.000	0.000	100.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	12	6.950	6.970	370.000	1.954	1.035	16.601	4.100	0.200	18.100	0.000	77.600	15.170	0.740	66.970	0.000	287.120	0.967	0.967	0.852	0.852
	19	7.010	7.050	180.000	1.954	0.499	18.056	9.800	0.400	17.600	0.002	72.198	17.640	0.720	31.680	0.003	129.957	1.063	2.030	0.936	1.788
	22	7.350	7.060	420.000	1.954	0.431	19.579	9.000	0.500	14.300	0.000	76.200	37.800	2.100	60.060	0.000	320.040	2.094	4.123	1.844	3.631
	29	7.540	7.070	440.000	1.954	0.968	21.890	8.800	0.400	14.500	0.000	76.300	38.720	1.760	63.800	0.000	335.720	1.852	8.019	1.631	7.062
	33	7.160	7.120	445.000	1.954	0.237	23.608	11.500	0.700	12.700	0.008	75.092	51.175	3.115	56.515	0.036	334.159	2.338	10.357	2.059	9.120
	36	7.220	7.170	435.000	1.954	0.339	25.223	10.900	1.000	13.100	0.003	74.998	47.415	4.350	56.985	0.011	326.239	2.008	12.365	1.769	10.889
	40	7.130	7.100	180.000	1.954	0.217	26.960	15.200	0.800	12.200	0.000	71.800	27.360	1.440	21.960	0.001	129.239	1.085	13.450	0.955	11.844
	43	7.410	0.000	320.000		0.000	26.960	6.700	0.600	12.900	0.000	79.800	21.440	1.920	41.280	0.000	255.360	0.795	14.245	0.700	12.545
BioGanic Run 2	1	7.14	0	0	14.476	0.000	14.476														
	8	5.2	7.06	190	1.954	0.270	16.160	0.000	7.100	11.300	0.242	81.358	0.000	13.490	21.470	0.459	154.581	0.000	0.000	0.000	0.000
	12	7.19	7.14	1020	1.954	0.534	17.580	27.900	2.900	14.500	0.000	54.700	284.580	29.580	147.900	0.000	557.940	17.610	17.610	15.508	15.508
	19	7.29	6.99	630	1.954	0.431	20.437	18.200	1.300	13.200	0.012	67.288	114.660	8.190	83.160	0.074	423.916	6.062	34.915	5.339	30.748
	22	7.21	7.18	200	1.954	0.533	21.858	1.700	0.500	13.500	0.000	84.300	3.400	1.000	27.000	0.000	168.600	0.166	35.081	0.147	30.894
	29	7.18	7.17	250	1.954	0.344	24.389	2.900	0.600	15.300	0.004	81.196	7.250	1.500	38.250	0.011	202.989	0.318	35.811	0.280	31.537
	33	7.21	7.15	340	1.954	0.467	25.876	19.800	2.000	14.500	0.007	63.693	67.320	6.800	49.300	0.024	216.556	2.760	38.572	2.431	33.968
	36	7.17	7.17	765	1.954	0.484	27.346	21.500	2.100	13.400	0.000	63.000	164.475	16.065	102.510	0.000	481.950	6.356	44.928	5.598	39.566
	40	7.17	7.13	200	1.954	0.345	28.955	12.300	1.900	14.700	0.026	71.074	24.600	3.800	29.400	0.052	142.148	0.900	45.828	0.792	40.358
	43	7.36	0	180		0.000	28.955	2.200	0.400	18.200	0.001	79.199	3.960	0.720	32.760	0.002	142.558	0.137	45.964	0.120	40.478

Thermophilic data

Thermophilic Data - Based on VS of Seaweed

Temperature: 52^{0}C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 42 days

Seaweed	VS	0.7237 g/g	Mass	20 g
Vitaboost	VS	0.287 g/g	Mass	28 g
BioGanic	VS	0.72 g/g	Mass	20 g
Manure	VS	0.66 g/g	Mass	80 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
Vitaboost Run 1	1	7.000			14.476		14.476														
	8	6.530	7.110	190.000	1.954	0.416	16.014	0.000	3.200	4.700	0.058	92.04	0	6.08	8.93	0.1106	174.879	0.000	0.000	0.000	0.000
	12	6.360	6.990	885.000	1.954	0.174	17.794	13.600	36.400	4.900	0.257	44.84	120.36	322.14	43.365	2.2736	396.861	7.516	7.516	6.619	6.619
	15	6.560	7.000	270.000	1.954	0.351	19.397	8.400	2.900	9.200	0.001	79.49	22.68	7.83	24.84	0.0024	214.648	1.275	8.790	1.122	7.741
	19	6.150	6.980	310.000	1.954	0.558	20.794	5.900	4.100	9.800	0.000	80.20	18.29	12.71	30.38	0.0009	248.619	0.943	9.733	0.830	8.572
	22	6.430	7.010	210.000	1.954	0.425	22.323	4.400	2.900	6.500	0.004	86.19	9.24	6.09	13.65	0.0074	181.013	0.444	10.178	0.391	8.963
	26	6.730	7.060	360.000	1.954	0.096	24.181	11.600	2.900	11.20	0.014	74.28	41.76	10.44	40.32	0.0500	267.43	1.871	12.048	1.647	10.610
	29	7.290	7.240	1080.000	1.954	0.473	25.662	37.400	9.600	7.300	0.000	45.70	403.92	103.68	78.84	0.0022	493.558	16.704	28.752	14.710	25.321
	33	7.640	7.230	1760.000	1.954	0.383	27.234	46.600	11.500	5.800	0.000	36.10	820.16	202.4	102.08	0.0035	635.356	31.960	60.712	28.145	53.466
	36	7.150	7.150	1570.000	1.954	0.449	28.739	50.400	10.300	5.800	0.000	33.50	791.28	161.71	91.06	0.0047	525.945	29.055	89.767	25.587	79.053
	40	7.310	7.250	650.000	1.954	0.506	30.187	35.700	6.600	9.800	0.000	47.90	232.05	42.9	63.7	0	311.35	8.074	97.842	7.111	86.164
	43	7.260	0.000	520.000	1.954	0.000	32.141	25.500	5.300	10.60	0.000	58.60	132.6	27.56	55.12	0.0005	304.719	4.393	102.234	3.868	90.032
Bio-Ocean Run 2	1	7.01			14.476		14.476														
	8	6.53	7.1	200	1.954	0.301	16.129	0.2	4.4	3.6	0.113	91.68	0.4	8.8	7.2	0.226	183.374	0.028	0.028	0.024	0.024
	12	6.41	7.2	640	1.954	0.227	17.856	18.2	24.2	4.9	0.058	52.64	116.48	154.88	31.36	0.372	336.908	7.222	7.249	6.360	6.384
	15	6.7	7.05	410	1.954	0.369	19.441	6.7	7.5	8	0.001	77.79	27.47	30.75	32.8	0.003	318.977	1.538	8.788	1.355	7.739
	19	6.21	6.98	370	1.954	0.393	21.003	6.6	8.3	9.4	0.000	75.70	24.42	30.71	34.78	0.001	280.089	1.256	10.044	1.106	8.845
	22	6.46	7.01	355	1.954	0.368	22.589	3.2	2.7	9.3	0.004	84.79	11.36	9.585	33.015	0.012	301.028	0.541	10.585	0.476	9.321
	26	6.68	7.11	725	1.954	0.430	24.113	18.7	7	9.9	0.070	64.33	135.575	50.75	71.775	0.504	466.396	6.002	16.587	5.285	14.607
	29	7.38	7.15	1320	1.954	0.265	25.802	42.4	14.3	6.5	0.000	36.80	559.68	188.76	85.8	0.000	485.760	23.211	39.797	20.440	35.047
	33	7.7	7.14	1570	1.954	0.238	27.519	51.8	14.3	4.1	0.000	29.80	813.26	224.51	64.37	0.000	467.860	31.519	71.316	27.757	62.804

	36	7.18	7.16	1510	1.954	0.483	28.990	54.7	11.6	5.1	0.000	28.60	825.97	175.16	77.01	0.003	431.857	30.015	101.331	26.433	89.237
	40	7.18	7.17	470	1.954	0.503	30.441	42.1	11.1	9	0.000	37.80	197.87	52.17	42.3	0.001	177.659	6.825	108.156	6.011	95.247
	43	7.23	0	930	1.954	0.000	32.395	34.9	12.1	5.7	0.000	47.30	324.57	112.53	53.01	0.001	439.889	10.662	118.819	9.390	104.637
Cow Manure Run 2	1	7.01			14.476		14.476														
	8	6.25	7	0	1.954	0.379	16.051	0	0	0	0	100.0	0.0	0.0	0.0	0.0	0.0	0.000	0.000	0.000	0.000
	12	6.45	7.1	0	1.954	0.401	17.604	0	0	0	0	100.0	0.0	0.0	0.0	0.0	0.0	0.000	0.000	0.000	0.000
	15	6.75	6.92	180	1.954	0.313	19.245	4.7	4.1	9.5	0.0016	81.69	8.5	7.4	17.1	0.0	147.1	0.440	0.440	0.387	0.387
	19	6.56	6.95	275	1.954	0.324	20.876	7.6	5.1	9.4	0.0003	77.90	20.9	14.0	25.9	0.0	214.2	1.001	1.441	0.882	1.269
	22	6.61	6.93	175	1.954	0.366	22.464	3.4	3.7	9.1	0.0037	83.79	6.0	6.5	15.9	0.0	146.6	0.265	1.706	0.233	1.502
	26	6.68	6.91	250	1.954	0.341	24.077	5.8	3.9	9.7	0.0125	80.58	14.5	9.8	24.3	0.0	201.5	0.602	2.308	0.530	2.032
	29	7.08	7.02	400	1.954	0.333	25.698	19.8	4.7	7.9	0.0001	67.60	79.2	18.8	31.6	0.0	270.4	3.082	5.390	2.714	4.746
	33	7.42	7.23	980	1.954	0.497	27.156	37.6	8.8	6	0.0002	47.60	368.5	86.2	58.8	0.0	466.5	13.569	18.959	11.950	16.696
	36	6.97	6.99	1110	1.954	0.325	28.785	36.5	10.9	5.8	0	46.80	405.2	121.0	64.4	0.0	519.5	14.075	33.034	12.395	29.091
	40	7.12	7.11	610	1.954	0.432	30.307	22	5.5	7.2	0.0001	65.30	134.2	33.6	43.9	0.0	398.3	4.428	37.462	3.900	32.991
	43	7.13	0	740	1.954	0.000	32.261	21.6	7.1	6.6	0.0002	64.70	159.8	52.5	48.8	0.0	478.8	4.955	42.417	4.363	37.354
BioGanic Run 1	1	7.07	0.00	0.00	14.476		14.476														
	8	6.05	7.12	916.67	1.954	0.627	15.803	19.133	57.967	4.333	1.000	17.56	175.389	531.361	39.7222	9.16575	161.029	12.116	12.116	10.670	10.670
	12	6.87	7.17	2563.33	1.954	0.580	17.177	59.667	38.333	1.717	0.502	0.218	1529.46	982.611	44.0039	12.8602	5.59747	96.784	108.900	85.232	95.902
	15	7.55	7.24	2295.00	1.954	0.479	18.652	43.967	29.167	1.783	1.000	24.08	1009.04	669.375	40.9275	22.9477	552.715	58.744	167.644	51.733	147.635
	19	7.56	7.19	2126.67	1.954	0.816	19.791	54.267	42.400	6.333	0.350	3.350	1154.07	901.706	134.689	7.4327	71.2327	61.873	229.517	54.488	202.123
	22	7.55	7.14	778.33	1.954	0.387	21.358	25.567	18.933	4.483	0.254	50.76	198.994	147.364	34.8953	1.97697	395.103	10.055	239.572	8.855	210.978
	26	7.52	7.20	571.67	1.954	0.377	22.934	22.900	16.800	5.217	0.046	55.03	130.912	96.04	29.8219	0.26125	314.632	6.130	245.701	5.398	216.376
	29	7.33	7.12	614.17	1.954	0.420	24.469	26.800	20.950	5.417	0.098	46.73	164.597	128.667	33.2674	0.59881	287.036	7.177	252.878	6.320	222.696
	33	7.50	7.15	855.83	1.954	0.518	25.905	36.167	25.383	5.450	0.221	32.77	309.526	217.239	46.6429	1.89139	280.534	12.650	265.528	11.140	233.836
	36	7.47	7.13	614.17	1.954	0.388	27.471	25.283	16.417	2.500	0.197	55.60	155.282	100.825	15.3542	1.20991	341.495	5.994	271.522	5.279	239.115
	40	7.55	7.14	807.50	1.954	0.510	28.916	35.417	30.650	4.733	0.000	29.20	285.99	247.498	38.2217	0.00323	235.787	10.411	281.933	9.168	248.283
	43	7.24	0.00	610.00	1.954	0.000	30.870	34.433	29.700	9.567	0.002	26.29	210.043	181.17	58.3567	0.01037	160.42	7.264	289.197	6.397	254.680

APPENDIX 2: OBJECTIVE 2 DATA

Size distribution: Mesophilic data

Mesophilic Data - Based on VS of Seaweed

Temperature: 37^0C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	1.7 – 3mm																				
Run 1	1	7.170			14.480		14.480														
	8	5.610	7.060	455.000	1.955	0.290	16.145	0.300	41.300	4.300	1.000	53.10	1.365	187.915	19.565	4.549	241.605	0.094	0.094	0.083	0.083
	11	5.790	7.050	320.000	1.955	0.690	17.410	5.100	11.500	4.500	0.486	78.41	16.32	36.8	14.4	1.5536	250.926	1.011	1.105	0.890	0.973
	15	6.390	7.070	490.000	1.955	0.410	27.556	11.000	19.800	5.200	0.077	63.92	53.9	97.02	25.48	0.37877	313.221	3.096	4.201	2.726	3.700
	18	7.390	7.200	1240.000	1.955	0.570	28.941	50.100	24.600	1.900	0.214	23.18	621.24	305.04	23.56	2.65608	287.503	22.544	26.745	19.854	23.553
	22	7.470	7.150	1205.000	1.955	0.630	32.143	49.900	19.500	3.800	0.001	26.78	601.295	234.975	45.79	0.01205	322.928	20.776	47.522	18.297	41.850
	25	7.330	7.200	410.000	1.955	0.510	33.588	40.400	29.900	5.200	0.000	24.50	165.64	122.59	21.32	0	100.45	5.153	52.675	4.538	46.388
	29	7.350		570.000			33.588	42.500	33.100	2.200	0.001	22.19	242.25	188.67	12.54	0.0057	126.534	7.212	59.888	6.352	52.740
Run 2	1	7.190			14.480		14.480														
	8	5.480	7.200	230.000	1.955	0.510	15.925	0.100	6.700	9.500	1.000	82.70	0.23	15.41	21.85	2.29977	190.210	0.016	0.016	0.014	0.014
	11	5.860	7.160	220.000	1.955	0.400	17.480	1.800	2.900	9.300	1.000	85.00	3.96	6.38	20.46	2.19978	187.000	0.249	0.265	0.219	0.233
	15	6.340	7.130	240.000	1.955	0.410	29.663	5.900	2.500	9.300	0.019	82.28	14.16	6	22.32	0.0456	197.474	0.810	1.075	0.713	0.946
	18	7.280	7.190	810.000	1.955	0.330	31.288	40.000	6.200	8.100	0.021	45.67	324	50.22	65.61	0.1701	369.999	10.923	11.997	9.619	10.565
	22	7.330	7.110	1590.000	1.955	0.590	33.453	49.700	9.400	7.000	0.003	33.89	790.23	149.46	111.3	0.04929	538.960	25.257	37.254	22.242	32.808
	25	7.360	7.180	780.000	1.955	0.760	34.648	32.400	7.100	10.00	0.000	50.50	252.72	55.38	78	0	393.9	7.554	44.809	6.653	39.461
	29	7.300		480.000			34.648	22.600	3.800	11.00	0.001	62.59	108.48	18.24	52.8	0.00288	300.477	3.131	47.940	2.757	42.218
Average	1	7.180	0.000	0.000	14.480	0.000	14.480														
	8	5.545	7.130	342.500	1.955	0.400	16.035	0.200	24.000	6.900	1.000	67.90	0.798	101.663	20.708	3.425	215.908	0.055	0.055	0.049	0.049
	11	5.825	7.105	270.000	1.955	0.545	17.445	3.450	7.200	6.900	0.743	81.70	10.140	21.590	17.430	1.877	218.963	0.630	0.685	0.555	0.603

	15	6.365	7.100	365.000	1.955	0.410	28.610	8.450	11.150	7.250	0.048	73.10	34.030	51.510	23.900	0.212	255.348	1.953	2.638	1.720	2.323
	18	7.335	7.195	1025.000	1.955	0.450	30.114	45.050	15.400	5.000	0.118	34.43	472.620	177.630	44.585	1.413	328.752	16.734	19.371	14.736	17.059
	22	7.400	7.130	1397.500	1.955	0.610	32.798	49.800	14.450	5.400	0.002	30.34	695.763	192.218	78.545	0.031	430.944	23.017	42.388	20.270	37.329
	25	7.345	7.190	595.000	1.955	0.635	34.118	36.400	18.500	7.600	0.000	37.50	209.180	88.985	49.660	0.000	247.175	6.354	48.742	5.595	42.924
	29	7.325	0.000	525.000	0.000	0.000	34.118	32.550	18.450	6.600	0.001	42.39	175.365	103.455	32.670	0.004	213.506	5.172	53.914	4.554	47.479
	<1.7mm																				
Run 1	1	7.130			14.480		14.480														
	8	5.720	7.180	530.000	1.955	0.590	15.845	0.200	45.700	3.100	1.000	50.00	1.06	242.21	16.43	5.29947	265.000	0.073	0.073	0.064	0.064
	11	5.940	7.200	220.000	1.955	0.760	17.040	1.800	28.100	3.300	0.225	66.57	3.96	61.82	7.26	0.49456	146.465	0.250	0.323	0.220	0.285
	15	6.510	7.120	615.000	1.955	0.790	18.204	17.100	21.800	4.100	0.059	56.94	105.165	134.07	25.215	0.36223	350.187	6.172	6.495	5.435	5.720
	18	7.600	7.180	1850.000	1.955	0.470	19.689	54.800	23.300	1.600	0.132	20.17	1013.8	431.05	29.6	2.4494	373.100	55.690	62.185	49.043	54.763
	22	7.400	7.110	1485.000	1.955	0.360	21.284	44.200	25.800	3.600	0.278	26.12	656.37	383.13	53.46	4.13424	387.905	33.337	95.521	29.358	84.120
	25	7.390	7.100	1360.000	1.955	0.350	22.889	43.000	29.300	3.100	0.191	24.40	584.8	398.48	42.16	2.59216	331.967	27.476	122.997	24.197	108.317
	29	7.260		1120.000			22.889	41.400	29.000	3.900	0.003	25.69	463.68	324.8	43.68	0.03472	287.805	20.258	143.255	17.840	126.157
Run 2	1	7.160			14.480		14.480														
	8	5.400	7.200	240.000	1.955	0.500	15.935	0.100	5.400	11.60	1.000	81.90	0.24	12.96	27.84	2.39976	196.560	0.017	0.017	0.015	0.015
	11	5.910	7.120	600.000	1.955	0.550	17.340	20.800	6.700	7.500	0.480	64.21	124.8	40.2	45	2.877	387.123	7.832	7.848	6.897	6.912
	15	6.950	7.110	1020.000	1.955	0.370	18.924	37.300	8.500	7.000	0.017	47.18	380.46	86.7	71.4	0.1683	481.271	21.942	29.790	19.323	26.235
	18	7.270	7.140	950.000	1.955	0.240	20.639	40.400	6.100	7.300	0.018	46.18	383.8	57.95	69.35	0.17195	438.728	20.281	50.071	17.860	44.095
	22	7.220	7.100	630.000	1.955	0.360	22.234	23.400	4.600	9.400	0.007	62.59	147.42	28.98	59.22	0.04095	394.339	7.143	57.214	6.290	50.385
	25	7.400	7.110	930.000	1.955	0.960	23.229	34.000	5.500	10.00	0.006	50.49	316.2	51.15	93	0.0558	469.594	14.221	71.435	12.524	62.909
	29	7.290		590.000			23.229	22.400	2.700	13.50	0.001	61.39	132.16	15.93	79.65	0.00708	362.252	5.689	77.125	5.010	67.919
Average	1	7.145	0.000	0.000	14.480	0.000	14.480														
	8	5.560	7.190	385.000	1.955	0.545	15.890	0.150	25.550	7.350	1.000	65.95	0.650	127.585	22.135	3.850	230.780	0.045	0.045	0.040	0.040
	11	5.925	7.160	410.000	1.955	0.655	17.190	11.300	17.400	5.400	0.352	65.54	64.380	51.010	26.130	1.686	266.794	4.041	4.086	3.559	3.598
	15	6.730	7.115	817.500	1.955	0.580	18.564	27.200	15.150	5.550	0.038	52.06	242.813	110.385	48.308	0.265	415.730	14.057	18.143	12.379	15.977
	18	7.435	7.160	1400.000	1.955	0.355	20.164	47.600	14.700	4.450	0.075	33.17	698.800	244.500	49.475	1.311	405.914	37.985	56.128	33.452	49.429
	22	7.310	7.105	1057.500	1.955	0.360	21.759	33.800	15.200	6.500	0.142	44.35	401.895	206.055	56.340	2.088	391.122	20.240	76.367	17.824	67.253
	25	7.395	7.105	1145.000	1.955	0.655	23.059	38.500	17.400	6.550	0.098	37.45	450.500	224.815	67.580	1.324	400.781	20.849	97.216	18.360	85.613
	29	7.275	0.000	855.000	0.000	0.000	23.059	31.900	15.850	8.700	0.002	43.54	297.920	170.365	61.665	0.021	325.029	12.974	110.190	11.425	97.038

Size distribution: Thermophilic data

Thermophilic Data - Based on VS of Seaweed																					
Temperature: 52^0C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days																					
Seaweed			VS	0.7237 g/g		Mass	20 g														
BioGanic			VS	0.72 g/g		Mass	20 g														
	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	1.7 – 3mm																				
Run 1	1	7.200			14.480		14.480														
	8	6.160	7.200	370.000	1.955	0.290	16.145	2.200	3.200	11.20	1.000	82.40	8.14	11.84	41.44	3.69963	304.88	0.562	0.562	0.495	0.495
	11	6.270	7.180	600.000	1.955	0.700	17.400	11.100	14.200	6.100	0.456	68.14	66.6	85.2	36.6	2.7348	408.87	4.125	4.687	3.633	4.128
	15	7.330	7.190	1340.000	1.955	0.290	27.556	31.700	33.200	2.400	0.186	32.51	424.78	444.88	32.16	2.4857	435.69	24.413	29.101	21.499	25.627
	18	7.330	7.200	3300.000	1.955	0.390	29.121	52.500	31.900	3.100	0.234	12.26	1732.5	1052.7	102.3	7.722	404.78	62.871	91.972	55.367	80.994
	22	7.550	7.180	1520.000	1.955	0.420	32.143	41.000	34.300	4.500	0.237	19.96	623.2	521.36	68.4	3.60088	303.44	21.400	113.372	18.846	99.840
	25	7.440	7.150	590.000	1.955	0.190	33.908	29.200	20.000	6.000	0.003	44.79	172.28	118	35.4	0.01829	264.3	5.360	118.732	4.720	104.560
	29	7.320		685.000			33.908	33.900	25.100	5.400	0.007	35.59	232.215	171.935	36.99	0.04452	243.82	6.848	125.580	6.031	110.591
Run 2	1	7.110			14.480		14.480														
	8	5.890	7.170	550.000	1.955	0.440	15.995	8.500	32.500	4.800	1.000	53.20	46.75	178.75	26.4	5.49945	292.6	3.229	3.229	2.843	2.843
	11	6.380	7.090	710.000	1.955	0.520	17.430	13.800	18.100	4.000	0.285	63.82	97.98	128.51	28.4	2.0235	453.09	6.126	9.354	5.395	8.238
	15	7.420	7.210	1470.000	1.955	0.310	29.663	28.800	12.700	3.200	0.005	55.29	423.36	186.69	47.04	0.07203	812.84	24.290	33.644	21.391	29.628
	18	7.490	7.190	1710.000	1.955	0.370	31.248	42.900	14.200	7.200	0.027	35.67	733.59	242.82	123.12	0.45828	610.01	24.731	58.375	21.779	51.408
	22	7.660	7.160	805.000	1.955	0.270	33.453	26.900	7.700	8.900	0.024	56.48	216.545	61.985	71.645	0.18917	454.64	6.930	65.305	6.103	57.511
	25	7.520	7.200	320.000	1.955	0.470	34.938	11.800	3.600	12.00	0.003	72.59	37.76	11.52	38.4	0.00928	232.31	1.129	66.434	0.994	58.505
	29	7.440		310.000			34.938	17.900	2.200	10.00	0.001	69.89	55.49	6.82	31	0.00434	216.69	1.588	68.022	1.399	59.903
Average	1	7.155	0.000	0.000	14.480	0.000	14.480														
	8	6.025	7.185	460.000	1.955	0.365	16.070	5.350	17.850	8.000	1.000	67.80	27.445	95.295	33.920	4.600	298.740	1.895	1.895	1.669	1.669
	11	6.325	7.135	655.000	1.955	0.610	17.415	12.450	16.150	5.050	0.370	65.98	82.290	106.855	32.500	2.379	430.976	5.125	7.021	4.514	6.183
	15	7.375	7.200	1405.000	1.955	0.300	28.610	30.250	22.950	2.800	0.095	43.90	424.070	315.785	39.600	1.279	624.266	24.351	31.372	21.445	27.628
	18	7.410	7.195	2505.000	1.955	0.380	30.184	47.700	23.050	5.150	0.130	23.97	1233.04	647.760	112.710	4.090	507.395	43.801	75.173	38.573	66.201
	22	7.605	7.170	1162.500	1.955	0.345	32.798	33.950	21.000	6.700	0.130	38.22	419.873	291.673	70.023	1.895	379.037	14.165	89.338	12.474	78.675

	25	7.480	7.175	455.000	1.955	0.330	34.423	20.500	11.800	9.000	0.003	58.69	105.020	64.760	36.900	0.014	248.306	3.244	92.583	2.857	81.533
	29	7.380	0.000	497.500	0.000	0.000	34.423	25.900	13.650	7.700	0.004	52.74	143.853	89.378	33.995	0.024	230.251	4.218	96.801	3.715	85.247
	<1.7mm																				
Run 1	1	7.190			14.480		14.480														
	8	6.090	7.180	295.000	1.955	0.260	16.175	1.300	2.900	15.00	0.470	80.33	3.835	8.555	44.25	1.38502	236.97	0.265	0.265	0.233	0.233
	11	6.260	7.130	100.000	1.955	0.580	17.550	1.500	1.800	12.10	0.106	84.49	1.5	1.8	12.1	0.1055	84.495	0.093	0.358	0.082	0.315
	15	7.210	7.070	0.000	1.955	0.340	19.164	0.000			0.000	100	0	0	0	0	0	0.000	0.358	0.000	0.315
	18	7.490	7.140	2050.000	1.955	0.160	20.959	47.600	22.400	6.700	0.076	23.22	975.8	459.2	137.35	1.54775	476.1	50.917	51.275	44.840	45.155
	22	7.700	7.190	835.000	1.955	0.480	22.434	30.100	7.000	9.900	0.019	52.98	251.335	58.45	82.665	0.15447	442.4	11.992	63.267	10.560	55.715
	25	7.540	7.180	400.000	1.955	0.510	23.879	14.800	2.800	12.70	0.002	69.70	59.2	11.2	50.8	0.0092	278.79	2.639	65.905	2.324	58.039
	29	7.350		425.000			23.879	11.000	1.800	14.80	0.000	72.40	46.75	7.65	62.9	0.00042	307.7	1.958	67.863	1.724	59.763
Run 2	1	7.200			14.480		14.480														
	8	5.980	7.170	400.000	1.955	0.540	15.895	5.400	30.300	6.900	1.000	56.40	21.6	121.2	27.6	3.9996	225.6	1.492	1.492	1.314	1.314
	11	6.720	7.200	320.000	1.955	0.430	17.420	6.100	28.000	6.200	0.450	59.25	19.52	89.6	19.84	1.44	189.6	1.228	2.720	1.081	2.395
	15	7.300	7.200	1440.000	1.955	0.410	18.964	29.000	12.100	5.600	0.048	53.25	417.6	174.24	80.64	0.6912	766.83	23.973	26.693	21.112	23.507
	18	7.480	7.190	1245.000	1.955	0.560	20.359	41.100	29.800	5.400	0.976	22.72	511.695	371.01	67.23	12.1512	282.91	26.982	53.675	23.761	47.268
	22	7.530	7.200	1020.000	1.955	0.320	21.994	33.600	25.800	6.300	0.187	34.11	342.72	263.16	64.26	1.90944	347.95	16.834	70.508	14.824	62.093
	25	7.410	7.120	675.000	1.955	0.540	23.409	28.500	22.100	6.700	0.083	42.61	192.375	149.175	45.225	0.56227	287.66	8.747	79.255	7.703	69.796
	29	7.290		390.000			23.409	31.900	25.400	6.900	0.071	35.72	124.41	99.06	26.91	0.27612	139.34	5.315	84.570	4.680	74.476
Average	1	7.195	0.000	0.000	14.480	0.000	14.480														
	8	6.035	7.175	347.500	1.955	0.400	16.035	3.350	16.600	10.95	0.735	68.36	12.718	64.878	35.925	2.692	231.288	0.878	0.878	0.773	0.773
	11	6.490	7.165	210.000	1.955	0.505	17.485	3.800	14.900	9.150	0.278	71.87	10.510	45.700	15.970	0.773	137.047	0.660	1.539	0.582	1.355
	15	7.255	7.135	720.000	1.955	0.375	19.064	14.500	6.050	2.800	0.024	76.62	208.800	87.120	40.320	0.346	383.414	11.986	13.525	10.556	11.911
	18	7.485	7.165	1647.500	1.955	0.360	20.659	44.350	26.100	6.050	0.526	22.97	743.748	415.105	102.290	6.849	379.508	38.950	52.475	34.301	46.212
	22	7.615	7.195	927.500	1.955	0.400	22.214	31.850	16.400	8.100	0.103	43.54	297.028	160.805	73.463	1.032	395.173	14.413	66.887	12.692	58.904
	25	7.475	7.150	537.500	1.955	0.525	23.644	21.650	12.450	9.700	0.043	56.15	125.788	80.188	48.013	0.286	283.227	5.693	72.580	5.013	63.917
	29	7.320	0.000	407.500	0.000	0.000	23.644	21.450	13.600	10.85	0.035	54.06	85.580	53.355	44.905	0.138	223.522	3.636	76.216	3.202	67.120

Chemical pre-treatment: Acid – mesophilic data

Mesophilic Data - Based on VS of Seaweed

Temperature: 37⁰C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH4/VS (ml/g)	Cum CH4/VS (ml/g)	Stand CH4/VS (ml/g)	Stand Cum CH4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	0.15M HCl																				
Run 1	1	7.120			28.880		28.880														
	8	5.650	7.190	910.000	1.955	0.520	30.315	0.300	43.000	2.600	0.000	54.10	2.73	391.3	23.66	0	492.31	0.095	0.095	0.083	0.083
	11	6.230	7.090	395.000	1.955	0.500	31.770	9.300	36.600	3.600	0.198	50.30	36.735	144.57	14.22	0.78249	198.692	1.212	1.306	1.067	1.150
	15	7.030	7.110	1205.000	1.955	0.570	27.556	44.300	31.200	2.500	0.000	22.00	533.815	375.96	30.125	0	265.1	16.803	18.109	14.797	15.948
	18	7.250	7.080	1380.000	1.955	0.850	28.661	43.500	24.200	2.000	0.831	29.46	600.3	333.96	27.6	11.4678	406.672	21.784	39.893	19.184	35.132
	22	7.140	7.150	1440.000	1.955	0.270	32.143	40.100	26.800	1.900	0.466	30.73	577.44	385.92	27.36	6.7104	442.569	20.147	60.041	17.742	52.874
	25	7.170	7.040	575.000	1.955	0.650	33.448	32.000	27.400	4.900	0.125	35.57	184	157.55	28.175	0.71875	204.556	5.724	65.765	5.041	57.916
	29	7.370		440.000			33.448	20.800	8.700	6.800	0.048	63.65	91.52	38.28	29.92	0.2112	280.068	2.736	68.501	2.410	60.325
Run 2	1	7.190			28.880		28.880														
	8	5.750	7.110	400.000	1.955	0.400	30.435	0.300	12.000	7.500	0.000	80.20	1.	48	30	0	320.8	0.042	0.042	0.037	0.037
	11	6.240	7.070	440.000	1.955	0.410	31.980	15.600	3.700	7.700	0.081	72.91	68.64	16.28	33.88	0.35816	320.841	2.255	2.297	1.986	2.023
	15	7.090	7.200	810.000	1.955	0.680	29.663	38.200	6.200	6.900	0.021	48.67	309.42	50.22	55.89	0.16686	394.303	9.676	11.972	8.521	10.543
	18	7.450	7.200	1005.000	1.955	0.670	30.948	36.600	6.100	7.200	0.019	50.08	367.83	61.305	72.36	0.19296	503.312	12.400	24.373	10.920	21.464
	22	7.170	7.170	560.000	1.955	0.530	33.453	23.400	4.000	8.400	0.012	64.18	131.04	22.4	47.04	0.06944	359.450	4.234	28.607	3.729	25.193
	25	7.320	7.070	490.000	1.955	0.570	34.838	15.600	3.800	11.40	0.009	69.19	76.44	18.62	55.86	0.04361	339.036	2.285	30.892	2.012	27.205
	29	7.240		415.000			34.838	27.100	14.200	5.200	0.027	53.47	112.465	58.93	21.58	0.11246	221.912	3.228	34.120	2.843	30.048
Average	1	7.155	0.000	0.000	28.880	0.000	28.880														
	8	5.700	7.150	655.000	1.955	0.460	30.375	0.300	27.500	5.050	0.000	67.15	1.965	219.650	26.830	0.000	406.555	0.068	0.068	0.060	0.060
	11	6.235	7.080	417.500	1.955	0.455	31.875	12.450	20.150	5.650	0.140	61.61	52.688	80.425	24.050	0.570	259.767	1.734	1.802	1.527	1.587
	15	7.060	7.155	1007.500	1.955	0.625	28.610	41.250	18.700	4.700	0.010	35.34	421.618	213.090	43.008	0.083	329.702	13.239	15.041	11.659	13.246
	18	7.350	7.140	1192.500	1.955	0.760	29.804	40.050	15.150	4.600	0.425	39.77	484.065	197.633	49.980	5.830	454.992	17.092	32.133	15.052	28.298
	22	7.155	7.160	1000.000	1.955	0.400	32.798	31.750	15.400	5.150	0.239	47.46	354.240	204.160	37.200	3.390	401.010	12.191	44.324	10.736	39.034

	25	7.245	7.055	532.500	1.955	0.610	34.143	23.800	15.600	8.150	0.067	52.38	130.220	88.085	42.018	0.381	271.796	4.005	48.328	3.527	42.560
	29	7.305	0.000	427.500	0.000	0.000	34.143	23.950	11.450	6.000	0.038	58.56	101.993	48.605	25.750	0.162	250.991	2.982	51.311	2.626	45.187
	0.3M HCl																				
Run 1	1	7.060			28.880		28.880														
	8	5.300	7.080	570.000	1.955	0.570	30.265	0.100	39.900	3.000	0.000	57.00	0.57	227.43	17.1	0	324.9	0.020	0.020	0.017	0.017
	11	5.850	7.170	280.000	1.955	0.360	31.860	0.700	2.500	11.00	0.036	85.76	1.96	7	30.8	0.10052	240.139	0.065	0.084	0.057	0.074
	15	6.560	7.050	320.000	1.955	0.680	33.134	5.100	1.500	10.40	0.006	82.99	16.32	4.8	33.28	0.01984	265.580	0.512	0.597	0.451	0.526
	18	7.230	7.160	660.000	1.955	0.600	34.489	23.100	2.600	8.400	0.017	65.88	152.46	17.16	55.44	0.11154	434.828	4.601	5.198	4.052	4.578
	22	7.280	7.200	1210.000	1.955	0.410	36.034	39.600	4.700	7.200	0.009	48.49	479.16	56.87	87.12	0.10648	586.743	13.893	19.091	12.235	16.812
	25	7.470	7.140	510.000	1.955	0.770	37.219	30.200	4.600	9.300	0.008	55.89	154.02	23.46	47.43	0.03927	285.050	4.274	23.365	3.764	20.577
	29	7.340		470.000			37.219	29.800	4.200	6.800	0.006	59.19	140.06	19.74	31.96	0.02867	278.211	3.763	27.128	3.314	23.891
Run 2	1	7.050			28.880		28.880														
	8	5.320	7.240	220.000	1.955	0.690	30.145	0.500	23.800	7.800	0.000	67.90	1.1	52.36	17.16	0	149.38	0.038	0.038	0.034	0.034
	11	5.770	7.200	320.000	1.955	0.310	31.790	5.100	23.700	5.000	0.136	66.06	16.32	75.84	16	0.43392	211.406	0.541	0.579	0.477	0.510
	15	6.540	7.140	640.000	1.955	0.480	33.264	31.600	35.000	2.000		31.40	202.24	224	12.8	0	200.96	6.362	6.941	5.603	6.113
	18	7.160	7.180	980.000	1.955	0.420	34.799	37.000	22.600	1.800		38.60	362.6	221.48	17.64	0	378.28	10.901	17.842	9.600	15.712
	22	7.160	7.140	1280.000	1.955	0.840	35.914	44.800	23.000	2.500		29.70	573.44	294.4	32	0	380.16	16.479	34.320	14.512	30.224
	25	7.240	7.150	640.000	1.955	0.420	37.449	37.900	22.900	4.500	0.214	34.48	242.56	146.56	28.8	1.37088	220.709	6.754	41.074	5.948	36.172
	29	7.220		580.000			37.449	37.600	15.900	2.200	0.014	44.28	218.08	92.22	12.76	0.08294	256.857	5.823	46.898	5.128	41.300
Average	1	7.055	0.000	0.000	28.880	0.000	28.880														
	8	5.310	7.160	395.000	1.955	0.630	30.205	0.300	31.850	5.400	0.000	62.45	0.835	139.895	17.130	0.000	237.140	0.029	0.029	0.025	0.025
	11	5.810	7.185	300.000	1.955	0.335	31.825	2.900	13.100	8.000	0.086	75.91	9.140	41.420	23.400	0.267	225.773	0.303	0.332	0.267	0.292
	15	6.550	7.095	480.000	1.955	0.580	33.199	18.350	18.250	6.200	0.003	57.19	109.280	114.400	23.040	0.010	233.270	3.437	3.769	3.027	3.319
	18	7.195	7.170	820.000	1.955	0.510	34.644	30.050	12.600	5.100	0.008	52.24	257.530	119.320	36.540	0.056	406.554	7.751	11.520	6.826	10.145
	22	7.220	7.170	1245.000	1.955	0.625	35.974	42.200	13.850	4.850	0.004	39.09	526.300	175.635	59.560	0.053	483.452	15.186	26.706	13.373	23.518
	25	7.355	7.145	575.000	1.955	0.595	37.334	34.050	13.750	6.900	0.111	45.18	198.290	85.010	38.115	0.705	252.880	5.514	32.220	4.856	28.374
	29	7.280	0.000	525.000	0.000	0.000	37.334	33.700	10.050	4.500	0.010	51.74	179.070	55.980	22.360	0.056	267.534	4.793	37.013	4.221	32.595

Chemical pre-treatment: Acid – thermophilic data

Thermophilic Data - Based on VS of Seaweed

Temperature: 52⁰C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	0.15M HCl																				
Run 1	1	7.200			28.880		28.880														
	8	5.870	7.060	350.000	1.955	0.390	30.445	8.300	37.400	3.800	0.000	50.50	29.05	130.9	13.3	0	176.75	1.006	1.006	0.886	0.886
	11	6.090	7.100	380.000	1.955	0.530	31.870	21.000	40.200	5.300	0.274	33.22	79.8	152.76	20.14	1.04044	126.259	2.621	3.627	2.308	3.194
	15	6.650	7.150	1120.000	1.955	0.550	27.556	37.000	37.300	2.800	0.000	22.90	414.4	417.76	31.36	0	256.48	13.003	16.630	11.451	14.645
	18	7.610	7.200	1310.000	1.955	0.570	28.941	42.300	23.800	4.000	0.043	29.85	554.13	311.78	52.4	0.56068	391.129	20.109	36.739	17.709	32.354
	22	7.370	7.220	1340.000	1.955	0.740	32.143	43.700	20.700	5.500	0.009	30.09	585.58	277.38	73.7	0.11792	403.222	20.233	56.972	17.818	50.172
	25	7.400	7.180	570.000	1.955	0.340	33.758	34.600	10.200	8.800	0.004	46.39	197.22	58.14	50.16	0.02394	264.456	6.136	63.108	5.403	55.576
	29	7.350		440.000			33.758	21.000	4.800	4.800	0.019	69.38	92.4	21.12	21.12	0.0814	305.278	2.737	65.845	2.410	57.986
Run 2	1	7.060			28.880		28.880														
	8	5.780	7.120	160.000	1.955	0.750	30.085	1.600	16.000	7.900	0.000	74.50	2.56	25.6	12.64	0	119.2	0.089	0.089	0.078	0.078
	11	6.110	7.050	130.000	1.955	0.910	31.130	8.900	11.700	7.000	0.000	72.40	11.57	15.21	9.1	0	94.12	0.385	0.473	0.339	0.417
	15	6.660	7.040	480.000	1.955	0.380	29.663	24.900	9.500	8.400	0.024	57.17	119.52	45.6	40.32	0.11424	274.445	3.839	4.313	3.381	3.798
	18	7.470	7.200	1480.000	1.955	0.560	31.058	45.100	28.000	3.800	0.053	23.04	667.48	414.4	56.24	0.78588	341.094	22.502	26.815	19.817	23.614
	22	7.260	7.200	2220.000	1.955	0.440	33.453	50.100	37.900	2.000	0.478	9.522	1112.22	841.38	44.4	10.6160	211.384	35.812	62.627	31.537	55.152
	25	7.370	7.170	190.000	1.955	0.510	34.898	22.900	21.100	9.200	0.004	46.79	43.51	40.09	17.48	0.00779	88.9122	1.301	63.927	1.145	56.297
	29	7.280		505.000			34.898	19.900	5.400	10.90	0.004	63.79	100.495	27.27	55.045	0.01767	322.172	2.880	66.807	2.536	58.833
Average	1	7.130	0.000	0.000	28.880	0.000	28.880														
	8	5.825	7.090	255.000	1.955	0.570	30.265	4.950	26.700	5.850	0.000	62.50	15.805	78.250	12.970	0.000	147.975	0.547	0.547	0.482	0.482
	11	6.100	7.075	255.000	1.955	0.720	31.500	14.950	25.950	6.150	0.137	52.81	45.685	83.985	14.620	0.520	110.190	1.503	2.050	1.323	1.805
	15	6.655	7.095	800.000	1.955	0.465	28.610	30.950	23.400	5.600	0.012	40.03	266.960	231.680	35.840	0.057	265.463	8.421	10.471	7.416	9.222
	18	7.540	7.200	1395.000	1.955	0.565	29.999	43.700	25.900	3.900	0.048	26.45	610.805	363.090	54.320	0.673	366.112	21.306	31.777	18.763	27.984
	22	7.315	7.210	1780.000	1.955	0.590	32.798	46.900	29.300	3.750	0.244	19.80	848.900	559.380	59.050	5.367	307.303	28.023	59.799	24.678	52.662

	25	7.385	7.175	380.000	1.955	0.425	34.328	28.750	15.650	9.000	0.004	46.59	120.365	49.115	33.820	0.016	176.684	3.718	63.518	3.274	55.937
	29	7.315	0.000	472.500	0.000	0.000	34.328	20.450	5.100	7.850	0.011	66.58	96.448	24.195	38.083	0.050	313.725	2.808	66.326	2.473	58.410
	0.3M HCl																				
Run 1	1	7.170			28.880		28.880														
	8	6.010	7.070	190.000	1.955	0.550	30.285	1.500	20.500	5.600	0.000	72.48	2.85	38.95	10.64	0	137.56	0.099	0.099	0.087	0.087
	11	6.020	7.180	290.000	1.955	0.320	31.920	14.800	28.900	8.300	0.213	47.78	42.92	83.81	24.07	0.61683	138.583	1.417	1.516	1.248	1.335
	15	7.110	7.120	1190.000	1.955	0.340	33.534	39.700	34.200	3.200	0.482	22.41	472.43	406.98	38.08	5.73818	266.771	14.801	16.317	13.034	14.369
	18	7.460	7.140	1090.000	1.955	0.700	34.789	42.900	26.700	2.800	0.258	27.34	467.61	291.03	30.52	2.81656	298.023	13.944	30.261	12.280	26.649
	22	7.340	7.200	1610.000	1.955	0.410	36.334	56.100	25.700	3.300	0.045	14.85	903.21	413.77	53.13	0.7245	239.165	25.962	56.223	22.864	49.513
	25	7.340	7.200	680.000	1.955	0.400	37.889	37.100	18.300	6.600	0.004	37.99	252.28	124.44	44.88	0.02516	258.374	6.943	63.166	6.115	55.627
	29	7.210		430.000			37.889	31.500	15.600	5.400	0.034	47.46	135.45	67.08	23.22	0.14792	204.102	3.575	66.741	3.148	58.775
Run 2	1	7.050			28.880		28.880														
	8	5.780	7.210	120.000	1.955	0.510	30.325	2.300	9.200	2.100	0.763	85.63	2.76	11.04	2.52	0.91512	102.764	0.096	0.096	0.084	0.084
	11	5.700	7.080	400.000	1.955	0.270	32.010	15.700	19.700	8.600	0.199	55.80	62.8	78.8	34.4	0.7968	223.203	2.071	2.166	1.824	1.908
	15	6.550	7.180	710.000	1.955	0.520	33.444	28.300	27.900	3.400	0.342	40.05	200.93	198.09	24.14	2.42678	284.413	6.277	8.444	5.528	7.436
	18	7.410	7.190	1240.000	1.955	0.670	34.729	46.100	29.100	1.000	0.639	23.16	571.64	360.84	12.4	7.9174	287.202	17.092	25.536	15.052	22.488
	22	7.270	7.190	1450.000	1.955	0.910	35.774	55.600	39.400	0.800	0.000	4.200	806.2	571.3	11.6	0	60.9	23.214	48.750	20.443	42.931
	25	7.340	7.180	1515.000	1.955	0.470	37.259	38.900	41.500	3.200	0.159	16.24	589.335	628.725	48.48	2.41036	246.049	16.474	65.224	14.508	57.439
	29	7.170	7.180	1295.000			37.259	42.100	36.700	1.200	0.249	19.75	545.195	475.265	15.54	3.22455	255.775	14.633	79.856	12.886	70.325
Average	1	7.110	0.000	0.000	28.880	0.000	28.880														
	8	5.895	7.140	155.000	1.955	0.530	30.305	1.900	14.850	3.850	0.381	79.01	2.805	24.995	6.580	0.458	120.162	0.097	0.097	0.086	0.086
	11	5.860	7.130	345.000	1.955	0.295	31.965	15.250	24.300	8.450	0.206	51.79	52.860	81.305	29.235	0.707	180.893	1.744	1.841	1.536	1.621
	15	6.830	7.150	950.000	1.955	0.430	33.489	34.000	31.050	3.300	0.412	31.23	336.680	302.535	31.110	4.082	275.593	10.539	12.380	9.281	10.902
	18	7.435	7.165	1165.000	1.955	0.685	34.759	44.500	27.900	1.900	0.448	25.25	519.625	325.935	21.460	5.367	292.613	15.518	27.898	13.666	24.569
	22	7.305	7.195	1530.000	1.955	0.660	36.054	55.850	32.550	2.050	0.023	9.528	854.705	492.535	32.365	0.362	150.033	24.588	52.486	21.653	46.222
	25	7.340	7.190	1097.500	1.955	0.435	37.574	38.000	29.900	4.900	0.081	27.11	420.808	376.583	46.680	1.218	252.212	11.709	64.195	10.311	56.533
	29	7.190	3.590	862.500	0.000	0.000	37.574	36.800	26.150	3.300	0.142	33.60	340.323	271.173	19.380	1.686	229.939	9.104	73.299	8.017	64.550

Chemical pre-treatment: Alkaline – mesophilic data

Mesophilic Data - Based on VS of Seaweed																					
Temperature: 37⁰C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days																					
Seaweed			VS	0.7237 g/g		Mass	20 g														
BioGanic			VS	0.72 g/g		Mass	20 g														
	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	0.15M NaOH																				
Run 1	1	7.150			28.880		28.880														
	8	5.650	7.070	450.000	1.955	0.700	30.135	0.200	37.600	7.200	1.000	54.00	0.9	169.2	32.4	4.49955	243.000	0.031	0.031	0.027	0.027
	11	6.300	7.050	0.000	1.955	0.470	31.620					100.0	0	0	0	0	0	0.000	0.031	0.000	0.027
	15	7.040	7.160	0.000	1.955	0.490	27.556					100.0	0	0	0	0	0	0.000	0.031	0.000	0.027
	18	7.500	7.200	1510.000	1.955	0.570	28.941	43.600	8.000	5.500	0.001	42.89	658.36	120.8	83.05	0.01208	647.777	23.891	23.923	21.040	21.067
	22	7.310	7.220	1010.000	1.955	0.610	32.143	23.100	4.600	9.900	0.000	62.40	233.31	46.46	99.99	0.00464	630.235	8.062	31.984	7.099	28.167
	25	7.300	7.120	385.000	1.955	0.270	33.828	17.100	3.600	11.50	0.000	67.80	65.835	13.86	44.275	0.00138	261.028	2.048	34.032	1.804	29.970
	29	7.390		590.000			33.828	30.100	4.100	8.900	0.000	56.90	177.59	24.19	52.51	0.00241	335.707	5.250	39.282	4.623	34.594
Run 2	1	7.090			28.880		28.880														
	8	5.820	7.090	0.000	1.955	0.550	30.285	0	0	0	0	100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	11	6.550	7.130	0.000	1.955	0.420	31.820	0	0	0	0	100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	15	7.330	7.200	0.000	1.955	0.420	29.663	0	0	0	0	100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	18	7.210	7.160	445.000	1.955	0.400	31.218	7.600	3.400	10.80	0.007	78.19	33.82	15.13	48.06	0.03159	347.958	1.140	1.140	1.004	1.004
	22	7.160	7.090	140.000	1.955	0.670	33.453	14.700	3.700	12.20	0.000	69.40	20.58	5.18	17.08	0.00014	97.1598	0.659	1.799	0.581	1.585
	25	7.480	7.140	0.000	1.955	0.390	35.018	0	0	0	0	100.0	0	0	0	0	0	0.000	1.799	0.000	1.585
	29	7.370		1080.000			35.018	43.800	23.500	2.200	0.142	30.35	473.04	253.8	23.76	1.53036	327.869	13.508	15.308	11.896	13.481
Average	1	7.120	0.000	0.000	28.880	0.000	28.880														
	8	5.735	7.080	225.000	1.955	0.625	30.210	0.100	18.800	3.600	0.500	77.00	0.450	84.600	16.200	2.250	121.500	0.016	0.016	0.014	0.014
	11	6.425	7.090	0.000	1.955	0.445	31.720	0.000	0.000	0.000	0.000	100.0	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.014
	15	7.185	7.180	0.000	1.955	0.455	28.610	0.000	0.000	0.000	0.000	100.0	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.014
	18	7.355	7.180	977.500	1.955	0.485	30.079	25.600	5.700	8.150	0.004	60.54	346.090	67.965	65.555	0.022	497.868	12.516	12.531	11.022	11.036
	22	7.235	7.155	575.000	1.955	0.640	32.798	18.900	4.150	11.05	0.000	65.90	126.945	25.820	58.535	0.002	363.698	4.360	16.892	3.840	14.876

	25	7.390	7.130	192.500	1.955	0.330	34.423	8.550	1.800	5.750	0.000	83.90	32.918	6.930	22.138	0.001	130.514	1.024	17.916	0.902	15.777
	29	7.380	0.000	835.000	0.000	0.000	34.423	36.950	13.800	5.550	0.071	43.62	325.315	138.995	38.135	0.766	331.789	9.379	27.295	8.260	24.037
	0.3M NaOH																				
Run 1	1	7.200			28.880		28.880														
	8	5.750	7.020	140.000	1.955	0.470	30.365	0.300	40.200	7.500	0.785	51.21	0.42	56.28	10.5	1.09886	71.7011	0.015	0.015	0.013	0.013
	11	6.730	7.180	260.000	1.955	0.370	31.950	27.900	25.500	4.800	0.405	41.39	72.54	66.3	12.48	1.05274	107.627	2.389	2.403	2.104	2.117
	15	7.430	7.190	1220.000	1.955	0.310	33.594	50.700	22.000	3.000	0.456	23.84	618.54	268.4	36.6	5.56076	290.899	19.360	21.763	17.049	19.166
	18	7.340	7.190	590.000	1.955	0.780	34.769	26.200	16.000	3.000	0.315	54.48	154.58	94.4	17.7	1.86027	321.459	4.601	26.365	4.052	23.218
	22	7.520	7.110	1390.000	1.955	0.310	36.414	45.800	20.100	3.100	0.419	30.58	636.62	279.39	43.09	5.81715	425.082	18.310	44.675	16.125	39.342
	25	7.320	7.150	460.000	1.955	0.310	38.059	23.500	12.500	8.800	0.088	55.11	108.1	57.5	40.48	0.40434	253.515	2.969	47.643	2.614	41.957
	29	7.270		480.000			38.059	21.800	16.200	6.000	0.150	55.85	104.64	77.76	28.8	0.71952	268.080	2.749	50.393	2.421	44.378
Run 2	1	7.180			28.880		28.880														
	8	5.910	7.040	520.000	1.955	0.680	30.155			42.90	1.000	56.10	0.2	54.3	22.3	5.19948	291.720	0.007	0.007	0.006	0.006
	11	6.650	7.150	190.000	1.955	0.600	31.510	13.400	27.000	4.800	0.230	54.57	25.46	51.3	9.12	0.43643	103.683	0.844	0.851	0.744	0.750
	15	7.230	7.170	410.000	1.955	0.570	32.894	35.400	19.600	7.000	0.389	37.61	145.14	80.36	28.7	1.59367	154.206	4.606	5.457	4.056	4.806
	18	7.480	7.200	1220.000	1.955	0.210	34.639	46.300	17.400	1.700	0.273	34.32	564.86	212.28	20.74	3.32816	418.791	17.172	22.629	15.122	19.928
	22	7.450	7.050	1250.000	1.955	0.400	36.194	41.500	23.400	4.000	0.242	30.85	518.75	292.5	50	3.0275	385.722	14.976	37.605	13.188	33.117
	25	7.450	7.150	1190.000	1.955	0.400	37.749	41.700	25.100	4.000	0.343	28.85	496.23	298.69	47.6	4.08527	343.394	13.710	51.315	12.074	45.191
	29	7.520		1105.000			37.749	44.000	20.300	1.700	0.034	33.96	486.2	224.315	18.785	0.37680	375.323	12.880	64.195	11.343	56.533
Average	1	7.190	0.000	0.000	28.880	0.000	28.880														
	8	5.830	7.030	330.000	1.955	0.575	30.260	0.150	20.100	25.20	0.892	53.65	0.310	55.290	16.400	3.149	181.711	0.011	0.011	0.009	0.009
	11	6.690	7.165	225.000	1.955	0.485	31.730	20.650	26.250	4.800	0.317	47.98	49.000	58.800	10.800	0.745	105.655	1.617	1.627	1.424	1.433
	15	7.330	7.180	815.000	1.955	0.440	33.244	43.050	20.800	5.000	0.422	30.72	381.840	174.380	32.650	3.577	222.553	11.983	13.610	10.553	11.986
	18	7.410	7.195	905.000	1.955	0.495	34.704	36.250	16.700	2.350	0.294	44.40	359.720	153.340	19.220	2.594	370.126	10.887	24.497	9.587	21.573
	22	7.485	7.080	1320.000	1.955	0.355	36.304	43.650	21.750	3.550	0.330	30.72	577.685	285.945	46.545	4.422	405.403	16.643	41.140	14.656	36.230
	25	7.385	7.150	825.000	1.955	0.355	37.904	32.600	18.800	6.400	0.216	41.98	302.165	178.095	44.040	2.245	298.455	8.339	49.479	7.344	43.574
	29	7.395	0.000	792.500	0.000	0.000	37.904	32.900	18.250	3.850	0.092	44.90	295.420	151.038	23.793	0.548	321.702	7.815	57.294	6.882	50.456

Chemical pre-treatment: Alkaline – thermophilic data

Thermophilic Data - Based on VS of Seaweed

Temperature: 52⁰C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	0.15M NaOH																				
Run 1	1	7.050			28.880		28.880														
	8	6.400	7.200	900.000	1.955	0.940	29.895	4.800	24.300	7.200	0.000	63.70	43.2	218.7	64.8	0	573.3	1.496	1.496	1.317	1.317
	11	7.350	7.200	1360.000	1.955	0.690	31.160	49.700	23.000	6.100	0.084	21.11	675.92	312.8	82.96	1.1356	287.184	22.610	24.106	19.911	21.229
	15	7.480	7.210	1580.000	1.955	0.570	27.556	48.300	20.500	5.300	0.063	25.83	763.14	323.9	83.74	0.99856	408.221	24.491	48.597	21.568	42.797
	18	7.690	7.170	880.000	1.955	0.450	29.061	27.600	9.200	8.500	0.021	54.67	242.88	80.96	74.8	0.18832	481.171	8.814	57.411	7.762	50.559
	22	7.500	7.030	420.000	1.955	0.700	32.143	24.900	6.900	8.700	0.025	59.47	104.58	28.98	36.54	0.10542	249.794	3.599	61.010	3.169	53.728
	25	7.510	7.190	345.000	1.955	0.310	33.788	18.800	6.900	10.90	0.018	63.38	64.86	23.805	37.605	0.06175	218.668	2.018	63.028	1.777	55.505
	29	7.550		195.000			33.788	10.700	1.700	11.90	0.002	75.69	20.865	3.315	23.205	0.00351	147.611	0.618	63.645	0.544	56.049
Run 2	1	7.080			28.880		28.880														
	8	6.250	7.100	430.000	1.955	0.490	30.345	0.500	16.000	8.800	1.000	73.70	2.15	68.8	37.84	4.29957	316.910	0.074	0.074	0.066	0.066
	11	7.260	7.180	875.000	1.955	0.900	31.400	61.800	18.300	6.000	0.062	13.83	540.75	160.125	52.5	0.54075	121.084	17.820	17.895	15.693	15.759
	15	7.520	7.190	2215.000	1.955	0.610	29.663	46.200	15.900	7.400	0.049	30.45	1023.33	352.185	163.91	1.08756	674.487	32.591	50.485	28.701	44.460
	18	7.660	7.200	620.000	1.955	0.370	31.248	26.800	7.000	9.400	0.032	56.76	166.16	43.4	58.28	0.19778	351.962	5.602	56.087	4.933	49.393
	22	7.530	7.050	380.000	1.955	0.780	33.453	19.700	4.100	10.70	0.015	65.48	74.86	15.58	40.66	0.05814	248.841	2.396	58.483	2.110	51.502
	25	7.560	7.200	310.000	1.955	0.250	35.158	15.900	3.300	13.20	0.013	67.58	49.29	10.23	40.92	0.04092	209.519	1.473	59.956	1.298	52.800
	29	7.470		350.000			35.158	12.600	1.800	11.50	0.002	74.09	44.1	6.3	40.25	0.0077	259.342	1.254	61.210	1.105	53.905
Average	1	7.065	0.000	0.000	28.880	0.000	28.880														
	8	6.325	7.150	665.000	1.955	0.715	30.120	2.650	20.150	8.000	0.500	68.70	22.675	143.750	51.320	2.150	445.105	0.785	0.785	0.691	0.691
	11	7.305	7.190	1117.500	1.955	0.795	31.280	55.750	20.650	6.050	0.073	17.47	608.335	236.463	67.730	0.838	204.134	20.215	21.000	17.802	18.494
	15	7.500	7.200	1897.500	1.955	0.590	28.610	47.250	18.200	6.350	0.056	28.14	893.235	338.043	123.825	1.043	541.354	28.541	49.541	25.134	43.628
	18	7.675	7.185	750.000	1.955	0.410	30.154	27.200	8.100	8.950	0.027	55.72	204.520	62.180	66.540	0.193	416.567	7.208	56.749	6.347	49.976
	22	7.515	7.040	400.000	1.955	0.740	32.798	22.300	5.500	9.700	0.020	62.48	89.720	22.280	38.600	0.082	249.318	2.997	59.746	2.639	52.615

	25	7.535	7.195	327.500	1.955	0.280	34.473	17.350	5.100	12.05	0.016	65.48	57.075	17.018	39.263	0.051	214.094	1.746	61.492	1.537	54.152
	29	7.510	0.000	272.500	0.000	0.000	34.473	11.650	1.750	11.70	0.002	74.89	32.483	4.808	31.728	0.006	203.477	0.936	62.428	0.824	54.977
	0.3M NaOH																				
Run 1	1	7.110			28.880		28.880														
	8	6.780	7.060	2630.000	1.955	0.910	29.925	1.400	57.300	1.500		39.80	36.82	1506.99	39.45	0	1046.74	1.275	1.275	1.123	1.123
	11	7.570	7.200	2420.000	1.955	0.380	31.500	68.800	28.100	1.200		1.900	1664.96	680.02	29.04	0	45.98	55.638	56.913	48.997	50.120
	15	7.620	7.200	1625.000	1.955	0.590	32.864	45.400	24.000	5.000	0.211	25.38	737.75	390	81.25	3.4255	412.574	23.421	80.334	20.626	70.746
	18	7.780	7.200	540.000	1.955	0.320	34.499	15.300	3.400	10.20	0.024	71.07	82.62	18.36	55.08	0.13176	383.808	2.514	82.848	2.214	72.960
	22	7.470	7.070	330.000	1.955	0.670	35.784	11.400	1.900	12.00	0.005	74.69	37.62	6.27	39.6	0.01617	246.493	1.090	83.938	0.960	73.920
	25	7.560	7.190	470.000	1.955	0.280	37.459	19.000	1.500	10.70	0.004	68.79	89.3	7.05	50.29	0.01739	323.342	2.496	86.434	2.198	76.118
	29	7.540		320.000			37.459	9.200	1.600	12.20	0.002	76.99	29.44	5.12	39.04	0.00576	246.394	0.786	87.220	0.692	76.810
Run 2	1	7.200			28.880		28.880														
	8	6.640	7.210	760.000	1.955	0.370	30.465	0.500	24.100	5.500	0.000	69.90	3.8	183.16	41.8	0	531.24	0.132	0.132	0.116	0.116
	11	7.420	7.200	1010.000	1.955	0.560	31.860	43.500	38.300	3.300	0.000	14.90	439.35	386.83	33.33	0	150.49	14.422	14.553	12.700	12.816
	15	7.310	7.180	1350.000	1.955	0.740	33.074	48.100	12.600	6.900	0.112	32.28	649.35	170.1	93.15	1.50525	435.894	20.382	34.935	17.949	30.765
	18	7.770	7.200	2310.000	1.955	0.720	34.309	50.200	24.200	2.900	0.350	22.35	1159.62	559.02	66.99	8.09424	516.275	35.061	69.996	30.876	61.641
	22	7.600	7.180	120.000	1.955	0.460	35.804	12.400	4.900	10.40	0.014	72.28	14.88	5.88	12.48	0.01644	86.7435	0.434	70.429	0.382	62.023
	25	7.600	7.180	720.000	1.955	0.270	37.489	33.500	20.100	6.300	0.015	40.08	241.2	144.72	45.36	0.10728	288.612	6.737	77.166	5.933	67.956
	29	7.440		540.000			37.489	35.800	28.200	2.000	0.058	33.94	193.32	152.28	10.8	0.31374	183.286	5.157	82.323	4.541	72.497
Average	1	7.155	0.000	0.000	28.880	0.000	28.880														
	8	6.710	7.135	1695.000	1.955	0.640	30.195	0.950	40.700	3.500	0.000	54.85	20.310	845.075	40.625	0.000	788.990	0.703	0.703	0.619	0.619
	11	7.495	7.200	1715.000	1.955	0.470	31.680	56.150	33.200	2.250	0.000	8.400	1052.15	533.425	31.185	0.000	98.235	35.030	35.733	30.849	31.468
	15	7.465	7.190	1487.500	1.955	0.665	32.969	46.750	18.300	5.950	0.161	28.83	693.550	280.050	87.200	2.465	424.235	21.901	57.634	19.287	50.755
	18	7.775	7.200	1425.000	1.955	0.520	34.404	32.750	13.800	6.550	0.187	46.71	621.120	288.690	61.035	4.113	450.042	18.787	76.422	16.545	67.301
	22	7.535	7.125	225.000	1.955	0.565	35.794	11.900	3.400	11.20	0.009	73.49	26.250	6.075	26.040	0.016	166.619	0.762	77.184	0.671	67.972
	25	7.580	7.185	595.000	1.955	0.275	37.474	26.250	10.800	8.500	0.009	54.44	165.250	75.885	47.825	0.062	305.978	4.616	81.800	4.065	72.037
	29	7.490	0.000	430.000	0.000	0.000	37.474	22.500	14.900	7.100	0.030	55.47	111.380	78.700	24.920	0.160	214.840	2.971	84.771	2.617	74.653

Chemical pre-treatment: Microwave – mesophilic data

Mesophilic Data - Based on VS of Seaweed

Temperature: 37^{0}C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH_4/VS (ml/g)	Cum CH_4/VS (ml/g)	Stand CH_4/VS (ml/g)	Stand Cum CH_4/VS (ml/g)
					Added	Removed	Total	CH_4	CO_2	O_2	H_2S	Other	CH_4	CO_2	O_2	H_2S	Other				
	30 Seconds																				
Run 1	1	7.180			28.880		28.880														
	8	5.790	7.110	0.000	1.955	0.390	30.445					100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	11	6.140	7.080	0.000	1.955	0.490	31.910					100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	15	7.040	7.190	90.000	1.955	0.740	27.556	4.100	0.800	11.10	0.081	83.91	3.69	0.72	9.99	0.0729	75.5271	0.116	0.116	0.102	0.102
	18	7.450	7.180	270.000	1.955	0.480	29.031	16.400	1.400	10.60	0.016	71.58	44.28	3.78	28.62	0.04428	193.275	1.607	1.723	1.415	1.517
	22	7.240	7.200	480.000	1.955	0.990	32.143	36.900	8.400	9.600	0.027	45.07	177.12	40.32	46.08	0.13152	216.348	6.101	7.824	5.373	6.890
	25	7.220	7.120	495.000	1.955	0.930	33.168	25.800	5.200	10.60	0.017	58.38	127.71	25.74	52.47	0.08464	288.995	3.973	11.797	3.499	10.389
	29	7.140		270.000			33.168	14.400	3.700	12.50	0.002	69.39	38.88	9.99	33.75	0.00486	187.375	1.172	12.969	1.032	11.421
Run 2	1	7.150			28.880		28.880														
	8	6.180	7.160		1.955	0.380	30.455	0	0	0	0	100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	11	6.130	7.190		1.955	0.390	32.020	0	0	0	0	100.0	0	0	0	0	0	0.000	0.000	0.000	0.000
	15	7.190	7.080	120.000	1.955	0.670	29.663	2.600	3.000	8.900	0.113	85.38	3.12	3.6	10.68	0.135	102.465	0.097	0.097	0.086	0.086
	18	7.430	7.200	390.000	1.955	0.580	31.038	42.300	7.100	7.500	0.018	43.08	164.97	27.69	29.25	0.06942	168.020	5.562	5.659	4.898	4.984
	22	7.230	7.200	840.000	1.955	0.500	33.453	35.500	9.200	8.800	0.004	46.49	298.2	77.28	73.92	0.03696	390.563	9.608	15.267	8.461	13.445
	25	7.190	7.160	550.000	1.955	0.920	34.488	21.700	8.000	9.500	0.005	60.79	119.35	44	52.25	0.02585	334.374	3.568	18.834	3.142	16.586
	29	7.240		380.000			34.488	20.700	5.900	12.00	0.003	61.39	78.66	22.42	45.6	0.01178	233.308	2.281	21.115	2.009	18.595
Average	1	7.165	0.000	0.000	28.880	0.000	28.880														
	8	5.985	7.135	0.000	1.955	0.385	30.450	0.000	0.000	0.000	0.000	100.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	11	6.135	7.135	0.000	1.955	0.440	31.965	0.000	0.000	0.000	0.000	100.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	15	7.115	7.135	105.000	1.955	0.705	28.610	3.350	1.900	10.00	0.097	84.65	3.405	2.160	10.335	0.104	88.996	0.107	0.107	0.094	0.094
	18	7.440	7.190	330.000	1.955	0.530	30.034	29.350	4.250	9.050	0.017	57.33	104.625	15.735	28.935	0.057	180.648	3.584	3.691	3.156	3.250
	22	7.235	7.200	660.000	1.955	0.745	32.798	36.200	8.800	9.200	0.016	45.78	237.660	58.800	60.000	0.084	303.456	7.854	11.545	6.917	10.167

	25	7.205	7.140	522.500	1.955	0.925	33.828	23.750	6.600	10.05	0.011	59.58	123.530	34.870	52.360	0.055	311.685	3.770	15.316	3.320	13.488
	29	7.190	0.000	325.000	0.000	0.000	33.828	17.550	4.800	12.25	0.002	65.39	58.770	16.205	39.675	0.008	210.342	1.727	17.042	1.520	15.008
	60 seconds																				
Run 1	1	7.190			28.880		28.880														
	8	5.590	7.160	85.000	1.955	0.460	30.375	0.300	40.300	6.000	1.000	52.40	0.255	34.255	5.1	0.84991	44.5400	0.009	0.009	0.008	0.008
	11	5.900	7.120	120.000	1.955	0.690	31.640	1.100	37.500	4.220	0.541	56.64	1.32	45	5.064	0.6486	67.9674	0.043	0.052	0.038	0.046
	15	7.050	7.170	860.000	1.955	0.440	33.154	31.700	22.300	3.000	0.395	42.60	272.62	191.78	25.8	3.3927	366.407	8.616	8.669	7.588	7.634
	18	7.690	7.140	790.000	1.955	0.460	34.649	65.800	23.300	1.600	0.101	9.199	519.82	184.07	12.64	0.79632	72.6736	15.679	24.347	13.807	21.441
	22	7.550	7.160	1465.000	1.955	0.920	35.684	45.100	26.700	3.300	0.142	24.75	660.715	391.155	48.345	2.08616	362.698	19.069	43.416	16.793	38.234
	25	7.360	7.180	470.000	1.955	0.920	36.719	29.400	14.200	6.100	0.020	50.28	138.18	66.74	28.67	0.09212	236.317	3.872	47.288	3.410	41.644
	29	7.380		350.000			36.719	14.500	3.000	12.00	0.001	70.49	50.75	10.5	42	0.0028	246.747	1.382	48.671	1.217	42.862
Run 2	1	7.070			28.880		28.880														
	8	5.590	7.050	325.000	1.955	0.540	30.295	0.100	0.200	10.30	1.000	88.40	0.325	0.65	33.475	3.24967	287.300	0.011	0.011	0.010	0.010
	11	6.050	7.190	380.000	1.955	0.920	31.330	1.100	1.800	10.52	0.168	86.41	4.18	6.84	39.976	0.6365	328.367	0.138	0.149	0.122	0.131
	15	6.640	7.080	195.000	1.955	0.400	32.884	1.600	2.000	11.30	0.049	85.05	3.12	3.9	22.035	0.09555	165.849	0.100	0.249	0.088	0.219
	18	7.280	7.120	610.000	1.955	0.990	33.849	37.400	11.200	9.200	0.058	42.14	228.14	68.32	56.12	0.35075	257.069	6.938	7.186	6.110	6.329
	22	7.400	7.110	1430.000	1.955	0.600	35.204	45.200	16.100	4.100	0.046	34.55	646.36	230.23	58.63	0.6578	494.122	19.095	26.282	16.816	23.145
	25	7.240	7.140	510.000	1.955	0.540	36.619	41.900	28.400	3.500	0.132	26.06	213.69	144.84	17.85	0.67065	132.949	6.070	32.352	5.346	28.490
	29	7.240		700.000			36.619	40.800	32.400	3.400	0.044	23.35	285.6	226.8	23.8	0.3066	163.493	7.799	40.151	6.868	35.359
Average	1	7.130	0.000	0.000	28.880	0.000	28.880														
	8	5.590	7.105	205.000	1.955	0.500	30.335	0.200	20.250	8.150	1.000	70.40	0.290	17.453	19.288	2.050	165.920	0.010	0.010	0.009	0.009
	11	5.975	7.155	250.000	1.955	0.805	31.485	1.100	19.650	7.370	0.354	71.52	2.750	25.920	22.520	0.643	198.167	0.091	0.101	0.080	0.089
	15	6.845	7.125	527.500	1.955	0.420	33.019	16.650	12.150	7.150	0.222	63.82	137.870	97.840	23.918	1.744	266.128	4.358	4.459	3.838	3.927
	18	7.485	7.130	700.000	1.955	0.725	34.249	51.600	17.250	5.400	0.079	25.67	373.980	126.195	34.380	0.574	164.871	11.308	15.767	9.959	13.885
	22	7.475	7.135	1447.500	1.955	0.760	35.444	45.150	21.400	3.700	0.094	29.65	653.538	310.693	53.488	1.372	428.411	19.082	34.849	16.804	30.690
	25	7.300	7.160	490.000	1.955	0.730	36.669	35.650	21.300	4.800	0.076	38.17	175.935	105.790	23.260	0.381	184.634	4.971	39.820	4.378	35.067
	29	7.310	0.000	525.000	0.000	0.000	36.669	27.650	17.700	7.700	0.022	46.92	168.175	118.650	32.900	0.155	205.120	4.591	44.411	4.043	39.110
	120 seconds																				
Run 1	1	7.140			28.880		28.880														
	8	5.690	7.100	490.000	1.955	0.320	30.515	0.100	36.000	5.100	1.000	57.80	0.49	176.4	24.99	4.89951	283.220	0.017	0.017	0.015	0.015
	11	5.970	7.050	280.000	1.955	0.540	31.930	4.200	22.500	4.900	0.279	68.12	11.76	63	13.72	0.7812	190.738	0.385	0.402	0.339	0.354
	15	6.690	7.040	520.000	1.955	0.650	33.234	13.900	20.200	4.600	0.063	61.23	72.28	105.04	23.92	0.32708	318.432	2.264	2.666	1.994	2.348

	18	7.560	7.200	1185.000	1.955	0.370	34.819	50.700	23.700	2.200	0.408	22.99	600.795	280.845	26.07	4.83598	272.454	18.078	20.744	15.920	18.268
	22	7.400	7.180	1570.000	1.955	0.540	36.234	47.300	25.600	2.600	0.292	24.20	742.61	401.92	40.82	4.58126	380.068	21.328	42.071	18.782	37.050
	25	7.290	7.100	1190.000	1.955	0.370	37.819	39.400	29.200	4.900	0.037	26.46	468.86	347.48	58.31	0.43792	314.912	12.940	55.011	11.395	48.445
	29	7.260		940.000			37.819	42.700	35.700	3.000	0.003	18.59	401.38	335.58	28.2	0.03008	174.809	10.613	65.624	9.346	57.792
Run 2	1	7.130			28.880		28.880														
	8	5.620	7.080	220.000	1.955	0.670	30.165	0.700	2.800	9.100	1.000	86.40	1.54	6.16	20.02	2.19978	190.080	0.053	0.053	0.047	0.047
	11	5.980	7.120	100.000	1.955	0.490	31.630	1.300	3.600	9.200	0.156	85.74	1.3	3.6	9.2	0.1564	85.7436	0.043	0.096	0.038	0.085
	15	6.590	7.130	335.000	1.955	0.400	33.184	3.800	41.000	10.40	0.009	44.79	12.73	137.35	34.84	0.03015	150.049	0.402	0.499	0.354	0.439
	18	7.590	7.210	890.000	1.955	0.420	34.719	36.400	2.300	10.50	0.012	50.78	323.96	20.47	93.45	0.10769	452.012	9.762	10.261	8.597	9.037
	22	7.380	7.200	1185.000	1.955	0.720	35.954	47.200	26.000	9.100	0.015	17.68	559.32	308.1	107.835	0.17182	209.573	16.110	26.371	14.187	23.224
	25	7.370	7.150	580.000	1.955	0.730	37.179	13.100	1.900	13.50	0.005	71.49	75.98	11.02	78.3	0.02842	414.671	2.113	28.484	1.861	25.085
	29	7.300		400.000			37.179	11.800	0.900	14.90	0.001	72.39	47.2	3.6	59.6	0.0052	289.594	1.270	29.754	1.118	26.203
Average	1	7.135	0.000	0.000	28.880	0.000	28.880														
	8	5.655	7.090	355.000	1.955	0.495	30.340	0.400	19.400	7.100	1.000	72.10	1.015	91.280	22.505	3.550	236.650	0.035	0.035	0.031	0.031
	11	5.975	7.085	190.000	1.955	0.515	31.780	2.750	13.050	7.050	0.218	76.93	6.530	33.300	11.460	0.469	138.241	0.214	0.249	0.189	0.220
	15	6.640	7.085	427.500	1.955	0.525	33.209	8.850	30.600	7.500	0.036	53.01	42.505	121.195	29.380	0.179	234.241	1.333	1.582	1.174	1.394
	18	7.575	7.205	1037.500	1.955	0.395	34.769	43.550	13.000	6.350	0.210	36.89	462.378	150.658	59.760	2.472	362.233	13.920	15.502	12.259	13.652
	22	7.390	7.190	1377.500	1.955	0.630	36.094	47.250	25.800	5.850	0.153	20.94	650.965	355.010	74.328	2.377	294.821	18.719	34.221	16.485	30.137
	25	7.330	7.125	885.000	1.955	0.550	37.499	26.250	15.550	9.200	0.021	48.97	272.420	179.250	68.305	0.233	364.792	7.527	41.748	6.628	36.765
	29	7.280	0.000	670.000	0.000	0.000	37.499	27.250	18.300	8.950	0.002	45.49	224.290	169.590	43.900	0.018	232.202	5.941	47.689	5.232	41.997

Chemical pre-treatment: Microwave – thermophilic data

Thermophilic Data - Based on VS of Seaweed

Temperature: 52^{0}C ; Adjust pH to 7 with each feed; Measure DO and purge with nitrogen till a reading of 1 is obtained; Duration 28 days

Seaweed	VS	0.7237 g/g	Mass	20 g
BioGanic	VS	0.72 g/g	Mass	20 g

	Day	pH	Adj pH	Volume Gas (ml)	Volatile Solids (g/g)			Gas Produced (%)					Gas Produced (ml)					CH$_4$/VS (ml/g)	Cum CH$_4$/VS (ml/g)	Stand CH$_4$/VS (ml/g)	Stand Cum CH$_4$/VS (ml/g)
					Added	Removed	Total	CH$_4$	CO$_2$	O$_2$	H$_2$S	Other	CH$_4$	CO$_2$	O$_2$	H$_2$S	Other				
	30 Seconds																				
Run 1	1	7.130			28.880		28.880														
	8	5.820	7.180	110.000	1.955	0.490	30.345	0.400	0.800	10.50	1.000	87.30	0.44	0.88	11.55	1.09989	96.0301	0.015	0.015	0.013	0.013
	11	6.160	7.200	240.000	1.955	0.560	31.740	8.500	6.100	6.800	0.583	78.01	20.4	14.64	16.32	1.398	187.242	0.672	0.688	0.592	0.605
	15	7.200	7.130	660.000	1.955	0.500	27.556	62.900	24.700	3.000	0.150	9.251	415.14	163.02	19.8	0.9867	61.0533	13.080	13.767	11.518	12.124
	18	7.490	7.120	1610.000	1.955	0.710	28.801	48.100	15.900	7.800	0.070	28.13	774.41	255.99	125.58	1.11895	452.901	28.103	41.870	24.749	36.872
	22	7.610	7.140	625.000	1.955	0.520	32.143	49.100	31.500	9.900	0.006	9.494	306.875	196.875	61.875	0.04	59.335	10.655	52.525	9.383	46.256
	25	7.360	7.170	420.000	1.955	0.590	33.508	19.400	5.600	11.50	0.005	63.49	81.48	23.52	48.3	0.02226	266.677	2.535	55.060	2.232	48.488
	29	7.380		270.000			33.508	19.100	6.500	13.20	0.003	61.19	51.57	17.55	35.64	0.00756	165.232	1.539	56.599	1.355	49.843
Run 2	1	7.080			28.880		28.880														
	8	6.120	7.140	250.000	1.955	0.570	30.265	1.600	3.800	11.40	0.875	82.32	4	9.5	28.5	2.1875	205.812	0.139	0.139	0.122	0.122
	11	6.160	7.170	405.000	1.955	0.390	31.830	6.500	10.200	10.50	0.224	72.57	26.325	41.31	42.525	0.9072	293.932	0.870	1.008	0.766	0.888
	15	7.360	7.190	1380.000	1.955	0.300	29.663	40.000	8.200	8.500	0.029	43.27	552	113.16	117.3	0.4002	597.139	17.342	18.351	15.272	16.160
	18	7.380	7.140	1245.000	1.955	0.570	31.048	37.200	5.900	8.200	0.022	48.67	463.14	73.455	102.09	0.27763	606.037	15.614	33.964	13.750	29.910
	22	7.540	7.190	495.000	1.955	0.800	33.453	16.200	2.800	12.30	0.003	68.69	80.19	13.86	60.885	0.01534	340.049	2.583	36.547	2.275	32.185
	25	7.290	7.190	400.000	1.955	1.070	34.338	13.600	1.500	14.50	0.001	70.39	54.4	6	58	0.0048	281.595	1.626	38.173	1.432	33.617
	29	7.360		320.000			34.338	9.800	1.000	14.90	0.003	74.29	31.36	3.2	47.68	0.00896	237.751	0.913	39.086	0.804	34.421
Average	1	7.105	0.000	0.000	28.880	0.000	28.880														
	8	5.970	7.160	180.000	1.955	0.530	30.305	1.000	2.300	10.95	0.937	84.81	2.220	5.190	20.025	1.644	150.921	0.077	0.077	0.068	0.068
	11	6.160	7.185	322.500	1.955	0.475	31.785	7.500	8.150	8.650	0.403	75.29	23.363	27.975	29.423	1.153	240.587	0.771	0.848	0.679	0.747
	15	7.280	7.160	1020.000	1.955	0.400	28.610	51.450	16.450	5.750	0.089	26.26	483.570	138.090	68.550	0.693	329.097	15.211	16.059	13.395	14.142
	18	7.435	7.130	1427.500	1.955	0.640	29.924	42.650	10.900	8.000	0.046	38.40	618.775	164.723	113.835	0.698	529.469	21.858	37.917	19.249	33.391
	22	7.575	7.165	560.000	1.955	0.660	32.798	32.650	17.150	11.10	0.005	39.09	193.533	105.368	61.380	0.028	199.692	6.619	44.536	5.829	39.220

	25	7.325	7.180	410.000	1.955	0.830	33.923	16.500	3.550	13.00	0.003	66.94	67.940	14.760	53.150	0.014	274.136	2.081	46.616	1.832	41.053
	29	7.370	0.000	295.000	0.000	0.000	33.923	14.450	3.750	14.05	0.003	67.74	41.465	10.375	41.660	0.008	201.492	1.226	47.843	1.080	42.132
	60 seconds																				
Run 1	1	7.070			28.880		28.880														
	8	5.990	7.180	555.000	1.955	0.550	30.285	12.200	41.600	2.600	1.000	42.60	67.71	230.88	14.43	5.54944	236.430	2.345	2.345	2.065	2.065
	11	6.440	7.150	610.000	1.955	0.750	31.490	19.200	35.000	3.100	0.423	42.27	117.12	213.5	18.91	2.57725	257.892	3.867	6.212	3.406	5.470
	15	7.370	7.190	1210.000	1.955	0.700	32.744	42.900	27.900	4.400	0.018	24.78	519.09	337.59	53.24	0.2178	299.862	16.484	22.696	14.517	19.987
	18	7.450	7.170	1845.000	1.955	0.370	34.329	45.500	31.700	4.100	0.193	18.50	839.475	584.865	75.645	3.56085	341.454	25.637	48.334	22.577	42.565
	22	7.480	7.180	970.000	1.955	0.320	35.964	33.300	29.400	6.400	0.088	30.81	323.01	285.18	62.08	0.84972	298.880	9.409	57.743	8.286	50.851
	25	7.370	7.200	615.000	1.955	0.710	37.209	29.300	27.100	5.700	0.083	37.81	180.195	166.665	35.055	0.51229	232.572	5.010	62.753	4.412	55.263
	29	7.390		300.000			37.209	10.300	8.300	11.60	0.015	69.78	30.9	24.9	34.8	0.0459	209.354	0.830	63.584	0.731	55.995
Run 2	1	7.110			28.880		28.880														
	8	6.090	7.200	330.000	1.955	0.550	30.285	1.600	2.800	10.60	1.000	84.00	5.28	9.24	34.98	3.29967	277.200	0.183	0.183	0.161	0.161
	11	6.230	7.180	550.000	1.955	0.270	31.970	21.800	8.900	10.30	0.295	58.70	119.9	48.95	56.65	1.6225	322.877	3.959	4.142	3.487	3.648
	15	7.300	7.170	1280.000	1.955	0.260	33.664	38.400	10.100	10.10	0.045	41.35	491.52	129.28	129.28	0.576	529.344	15.375	19.517	13.540	17.187
	18	7.490	7.200	1930.000	1.955	0.690	34.929	46.500	11.500	803.0	0.018	761.0	897.45	221.95	15497.9	0.34354	14687.6	26.659	46.175	23.477	40.664
	22	7.580	7.160	485.000	1.955	0.570	36.314	18.100	4.100	13.40	0.008	64.39	87.785	19.885	64.99	0.03783	312.302	2.513	48.688	2.213	42.877
	25	7.430	7.190	305.000	1.955	0.440	37.829	10.700	3.100	15.90	0.008	70.29	32.635	9.455	48.495	0.02379	214.391	0.899	49.587	0.791	43.669
	29	7.390		380.000			37.829	9.400	2.000	12.30	0.002	76.29	35.72	7.6	46.74	0.00646	289.933	0.944	50.531	0.832	44.500
Average	1	7.090	0.000	0.000	28.880	0.000	28.880														
	8	6.040	7.190	442.500	1.955	0.550	30.285	6.900	22.200	6.600	1.000	63.30	36.495	120.060	24.705	4.425	256.815	1.264	1.264	1.113	1.113
	11	6.335	7.165	580.000	1.955	0.510	31.730	20.500	21.950	6.700	0.359	50.49	118.510	131.225	37.780	2.100	290.385	3.913	5.177	3.446	4.559
	15	7.335	7.180	1245.000	1.955	0.480	33.204	40.650	19.000	7.250	0.032	33.06	505.305	233.435	91.260	0.397	414.603	15.930	21.106	14.028	18.587
	18	7.470	7.185	1887.500	1.955	0.530	34.629	46.000	21.600	403.5	0.105	371.2	868.463	403.408	7786.77	1.952	7173.09	26.148	47.254	23.027	41.614
	22	7.530	7.170	727.500	1.955	0.445	36.139	25.700	16.750	9.900	0.048	47.60	205.398	152.533	63.535	0.444	305.591	5.961	53.216	5.250	46.864
	25	7.400	7.195	460.000	1.955	0.575	37.519	20.000	15.100	10.80	0.046	54.05	106.415	88.060	41.775	0.268	223.482	2.955	56.170	2.602	49.466
	29	7.390	0.000	340.000	0.000	0.000	37.519	9.850	5.150	11.95	0.009	73.04	33.310	16.250	40.770	0.026	249.644	0.887	57.057	0.781	50.247
	120 seconds																				
Run 1	1	7.200			28.880		28.880														
	8	6.120	7.200	90.000	1.955	0.300	30.535	7.000	9.100	10.80	0.328	72.77	6.3	8.19	9.72	0.29484	65.4951	0.218	0.218	0.192	0.192
	11	6.450	7.200	130.000	1.955	0.560	31.930	11.400	15.100	7.600	0.229	65.67	14.82	19.63	9.88	0.29705	85.3729	0.485	0.703	0.427	0.620
	15	7.410	7.130	895.000	1.955	0.260	33.624	42.600	233.100	5.500	0.183	181.3	381.27	2086.24	49.225	1.63606	1623.38	11.941	12.644	10.516	11.135

	18	7.460	7.180	1620.000	1.955	0.570	35.009	49.000	22.000	5.600	0.122	23.27	793.8	356.4	90.72	1.9683	377.111	23.608	36.252	20.790	31.925
	22	7.560	7.190	820.000	1.955	0.920	36.044	36.200	19.100	8.500	0.008	36.19	296.84	156.62	69.7	0.06642	296.773	8.479	44.731	7.467	39.392
	25	7.590	7.170	510.000	1.955	0.780	37.219	29.100	16.200	6.100	0.017	48.58	148.41	82.62	31.11	0.08415	247.775	4.117	48.849	3.626	43.018
	29	7.300		530.000			37.219	33.200	17.700	6.500	0.006	42.59	175.96	93.81	34.45	0.03286	225.747	4.728	53.576	4.163	47.182
Run 2	1	7.110			28.880		28.880														
	8	6.250	7.180	85.000	1.955	0.560	30.275	0.600	2.800	12.70	0.179	83.72	0.51	2.38	10.795	0.15232	71.1626	0.018	0.018	0.016	0.016
	11	6.680	7.180	200.000	1.955	0.850	31.380	15.600	16.900	7.600	0.088	59.81	31.2	33.8	15.2	0.176	119.624	1.031	1.048	0.908	0.923
	15	7.330	7.110	1525.000	1.955	0.650	32.684	59.300	30.300	2.100	0.009	8.292	904.325	462.075	32.025	0.12962	126.445	28.819	29.867	25.379	26.302
	18	7.460	7.150	1190.000	1.955	0.380	34.259	39.100	28.400	5.700	0.095	26.70	465.29	337.96	67.83	1.13169	317.788	14.236	44.103	12.537	38.839
	22	7.430	7.200	700.000	1.955	0.320	35.894	31.900	27.800	7.000	0.059	33.24	223.3	194.6	49	0.4123	232.687	6.518	50.621	5.740	44.579
	25	7.450	7.110	685.000	1.955	0.580	37.269	28.700	25.800	5.800	0.049	39.65	196.595	176.73	39.73	0.33359	271.611	5.477	56.098	4.823	49.402
	29	7.240		720.000			37.269	34.800	35.000	3.900	0.003	26.29	250.56	252	28.08	0.02016	189.339	6.723	62.821	5.921	55.323
Average	1	7.155	0.000	0.000	28.880	0.000	28.880														
	8	6.185	7.190	87.500	1.955	0.430	30.405	3.800	5.950	11.75	0.253	78.24	3.405	5.285	10.258	0.224	68.329	0.118	0.118	0.104	0.104
	11	6.565	7.190	165.000	1.955	0.705	31.655	13.500	16.000	7.600	0.158	62.74	23.010	26.715	12.540	0.237	102.498	0.758	0.876	0.667	0.771
	15	7.370	7.120	1210.000	1.955	0.455	33.154	50.950	131.700	3.800	0.096	86.54	642.798	1274.16	40.625	0.883	748.465	20.380	21.256	17.947	18.719
	18	7.460	7.165	1405.000	1.955	0.475	34.634	44.050	25.200	5.650	0.108	24.99	629.545	347.180	79.275	1.550	347.450	18.922	40.178	16.663	35.382
	22	7.495	7.195	760.000	1.955	0.620	35.969	34.050	23.450	7.750	0.034	34.71	260.070	175.610	59.350	0.239	264.731	7.498	47.676	6.603	41.986
	25	7.520	7.140	597.500	1.955	0.680	37.244	28.900	21.000	5.950	0.033	44.11	172.503	129.675	35.420	0.209	259.694	4.797	52.473	4.225	46.210
	29	7.270	0.000	625.000	0.000	0.000	37.244	34.000	26.350	5.200	0.005	34.44	213.260	172.905	31.265	0.027	207.543	5.725	58.199	5.042	51.252

APPENDIX 3: OBJECTIVE 3 DATA

BioGanic overview

Days	**Mesophilic**			
	Raw Seaweed Cumulative Methane ml/g VS	**Modified Gompertz Cumulative Methane ml/g VS**	**Residual**	**Residual square**
1	0.000	0.002	-0.002	0.000
8	0.000	3.468	-3.468	12.024
12	15.508	13.133	2.375	5.641
19	30.748	29.266	1.482	2.197
22	30.894	32.848	-1.954	3.817
29	31.537	36.443	-4.906	24.066
33	33.968	37.114	-3.146	9.895
36	39.566	37.360	2.206	4.866
40	40.358	37.523	2.835	8.036
44	40.478	37.595	2.883	8.311
Pmax	**Rmax**	**ƛ**		**SSR**
37.653	**13.329**	**7.360**		**78.853**

Days	**Thermophilic**			
	Raw Seaweed Cumulative Methane ml/g VS	**Modified Gompertz Cumulative Methane ml/g VS**	**Residual**	**Residual Square**
1	0.000	0.428	-0.428	0.183
8	10.670	28.312	-17.642	311.238
12	95.902	76.739	19.163	367.214
19	147.635	166.490	-18.855	355.530
22	210.978	193.088	17.890	320.050
29	216.376	228.154	-11.779	138.737
33	233.836	237.395	-3.559	12.664
36	239.115	241.533	-2.418	5.847
40	248.283	244.830	3.453	11.925
44	254.680	246.633	8.047	64.760
Pmax	**Rmax**	**ƛ**		**SSR**
248.776	**66.159**	**6.542**		**1587.964**

Size distribution – mesophilic data

1.7–3 mm

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual Square
1	0.000	0.000	0.000	0.000
8	0.083	0.000	0.083	0.007
11	0.973	0.004	0.969	0.939
15	3.700	4.880	-1.180	1.393
18	23.553	22.425	1.128	1.273
22	41.850	42.113	-0.264	0.069
25	46.388	48.293	-1.905	3.629
29	52.740	51.104	1.635	2.674
Pmax	**Rmax**	**ƛ**		**SSR**
52.089	**31.074**	**14.599**		**9.985**

< 1.7 mm

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual Square
1	0	1.51421E-33	0.000	0.000
8	0.064	0.000	0.064	0.004
11	0.285	0.243	0.041	0.002
15	5.720	13.566	-7.846	61.564
18	54.763	45.639	9.123	83.236
22	84.120	89.681	-5.561	30.920
25	108.317	109.953	-1.636	2.676
29	126.157	123.164	2.993	8.956
30		124.951		
35		129.501		
37		130.221		
39		130.655		
42		131.005		
Pmax	**Rmax**	**ƛ**		**SSR**
131.312	**57.977**	**14.293**		**187.358**

Size distribution – thermophilic data

1.7–3 mm

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.495	0.000	0.495	0.245
11	4.128	0.001	4.127	17.029
15	25.627	26.111	-0.484	0.234
18	80.994	79.680	1.314	1.726
22	99.840	103.085	-3.245	10.531
25	104.560	106.093	-1.532	2.348
29	110.591	106.799	3.792	14.382
Pmax	**Rmax**	**ƛ**		**SSR**
106.899	**96.793**	**13.743**		**46.495**

< 1.7 mm

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.0000	0.000	0.000
8	1.314	0.0352	1.278	1.635
11	2.395	2.5536	-0.158	0.025
15	23.507	24.1802	-0.673	0.453
18	47.268	45.6605	1.608	2.585
22	62.093	63.7359	-1.643	2.700
25	69.796	70.0394	-0.244	0.059
29	74.476	73.5921	0.884	0.781
Pmax	**Rmax**	**ƛ**		**SSR**
75.459	**35.636**	**11.806**		**8.238**

Acid pre-treatment – mesophilic data

0.15M HCl

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.083	0.001	0.082	0.007
11	1.150	0.681	0.469	0.220
15	15.948	15.853	0.095	0.009
18	35.132	35.537	-0.405	0.164
22	52.874	52.355	0.519	0.270
25	57.916	57.826	0.090	0.008
29	60.325	60.652	-0.326	0.107
Pmax **61.917**	**Rmax** **32.098**	**ƛ** **12.692**		**SSR** **0.784**

0.3M HCl

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0	1.62966E-20	0.000	0.00000000
8	0.034	0.002	0.032	0.001
11	0.510	0.288	0.223	0.050
15	6.113	5.902	0.211	0.044
18	15.712	16.121	-0.409	0.167
22	30.224	29.738	0.486	0.236
25	36.172	36.455	-0.283	0.080
29	41.300	41.272	0.028	0.001
Pmax **44.859**	**Rmax** **17.720**	**ƛ** **13.716**		**SSR** **0.580**

Acid pre-treatment – thermophilic data

0.15M HCl

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.078	0.000	0.078	0.006
11	0.417	0.000	0.417	0.174
15	3.798	0.630	3.168	10.034
18	23.614	24.408	-0.793	0.629
22	55.152	53.332	1.820	3.312
25	56.297	57.759	-1.462	2.137
29	58.833	58.752	0.081	0.007
Pmax	**Rmax**	**ƛ**		**SSR**
58.879	**55.746**	**15.937**		**16.299**

0.3M HCl

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.084	0.032	0.052	0.003
11	1.908	0.804	1.104	1.219
15	7.436	8.405	-0.969	0.939
18	22.488	21.684	0.804	0.646
22	42.931	43.340	-0.408	0.167
25	57.439	57.322	0.117	0.014
29	70.325	70.314	0.011	0.000
Pmax	**Rmax**	**ƛ**		**SSR**
86.096	**26.012**	**14.108**		**2.987**

Alkaline pre-treatment – mesophilic data

0.15M NaOH

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.027	0.000	0.027	0.001
11	0.027	0.000	0.027	0.001
15	0.027	0.856	-0.828	0.686
18	21.067	20.557	0.510	0.260
22	28.167	30.569	-2.402	5.772
25	29.970	31.225	-1.255	1.575
29	34.594	31.308	3.285	10.793
Pmax	**Rmax**	**ƛ**		**SSR**
31.313	**38.814**	**15.393**		**19.088**

0.3M NaOH

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.006	0.037	-0.031	0.001
11	0.750	0.741	0.009	0.000
15	4.806	6.903	-2.097	4.395
18	19.928	17.308	2.620	6.866
22	33.117	34.314	-1.197	1.433
25	45.191	45.488	-0.297	0.088
29	56.533	56.110	0.423	0.179
Pmax	**Rmax**	**ƛ**		**SSR**
69.743	**20.404**	**14.044**		**12.964**

Alkaline pre-treatment – thermophilic data

0.15M NaOH

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	1.317	2.932	-1.615	2.607
11	21.229	20.124	1.104	1.220
15	42.797	43.402	-0.605	0.366
18	50.559	50.962	-0.403	0.163
22	53.728	54.334	-0.606	0.367
25	55.505	55.066	0.439	0.193
29	56.049	55.361	0.688	0.473
Pmax	**Rmax**	**ƛ**		**SSR**
55.455	**34.102**	**8.221**		**5.388**

0.3M NaOH

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.116	0.845	-0.729	0.531
11	12.816	10.010	2.806	7.875
15	30.765	36.998	-6.233	38.854
18	61.641	53.716	7.926	62.817
22	62.023	65.420	-3.397	11.539
25	67.956	69.203	-1.247	1.556
29	72.497	71.291	1.206	1.454
Pmax	**Rmax**	**ƛ**		**SSR**
72.388	**33.895**	**9.825**		**124.627**

Microwave pre-treatment – mesophilic data

30 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000
15	0.086	0.431	-0.345	0.119
18	4.984	4.862	0.122	0.015
22	13.445	13.423	0.022	0.000
25	16.586	16.794	-0.207	0.043
29	18.595	18.446	0.149	0.022
Pmax	**Rmax**	**ƛ**		**SSR**
19.053	**11.230**	**15.977**		**0.199**

60 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.008	0.000	0.008	0.000
11	0.046	0.036	0.010	0.000
15	7.634	6.936	0.698	0.487
18	21.441	22.510	-1.068	1.141
22	38.234	37.106	1.129	1.274
25	41.644	41.500	0.144	0.021
29	42.862	43.520	-0.658	0.434
Pmax	**Rmax**	**ƛ**		**SSR**
44.255	**25.773**	**13.860**		**3.356**

120 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.015	0.000	0.015	0.000
11	0.354	0.058	0.296	0.088
15	2.348	4.190	-1.842	3.392
18	18.268	16.633	1.635	2.674
22	37.050	37.643	-0.593	0.352
25	48.445	48.994	-0.549	0.301
29	57.792	57.272	0.519	0.270
Pmax	**Rmax**	**ƛ**		**SSR**
63.244	**25.901**	**14.994**		**7.076**

Microwave pre-treatment – thermophilic data

30 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.013	0.000	0.013	0.000
11	0.605	0.001	0.605	0.365
15	12.124	12.260	-0.136	0.019
18	36.872	36.496	0.376	0.142
22	46.256	47.230	-0.975	0.950
25	48.488	48.649	-0.161	0.026
29	49.843	48.990	0.853	0.728
Pmax	**Rmax**	**ƛ**		**SSR**
49.040	**43.782**	**13.693**		**2.229**

60 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	2.065	0.020	2.045	4.181
11	5.470	2.442	3.028	9.170
15	19.987	22.858	-2.871	8.241
18	42.565	39.804	2.761	7.622
22	50.851	51.531	-0.681	0.463
25	55.263	54.940	0.324	0.105
29	55.995	56.602	-0.608	0.370
Pmax	**Rmax**	**ƛ**		**SSR**
57.300	**30.616**	**11.484**		**30.152**

120 Seconds

Days	Cumulative Methane ml/g VS	Modified Gompertz Cumulative Methane ml/g VS	Residual	Residual square
1	0.000	0.000	0.000	0.000
8	0.016	0.044	-0.029	0.001
11	0.923	3.415	-2.492	6.208
15	26.302	24.285	2.018	4.071
18	38.839	38.863	-0.023	0.001
22	44.579	48.051	-3.472	12.055
25	49.402	50.559	-1.156	1.337
29	55.323	51.733	3.590	12.889
Pmax	**Rmax**	**ƛ**		**SSR**
52.198	**28.731**	**11.010**		**36.561**

APPENDIX 4: EDITING CERTIFICATE