

UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES



**THE EFFECTS OF INOCULUM ACCLIMATISATION ON THE
METHANOGENESIS OF ECKLONIA MAXIMA**

BY

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DECLARATIONS

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I hereby declare that this MPhil thesis which is the outcome of my own original research was prepared under strict supervision and in accordance with the University of Ghana's academic regulations and that no part of it has been presented for another degree in this University or elsewhere.

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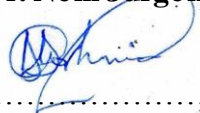
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ABSTRACT

In the present scenario of the over dependence and shortcomings of fossil fuels, integrated biorefinery techniques have been developed to process biodegradable and sustainable feedstock for use as green energy. Brown seaweed biomass, recently has been under the spotlight for the production of biogas via anaerobic digestion.

In this study, the effects of acclimatising inoculum to *Ecklonia maxima*, a brown seaweed type for the production of biogas have been investigated. Biomass of *Ecklonia maxima* were subjected to an acclimatisation period to enable anaerobic microbes cultured on the inoculum adapt to the feedstock. Mixtures of *Ecklonia maxima* with either acclimatised or unacclimatised inoculum were prepared and anaerobically digested for a period of 20 days. The proximate and ultimate results carried on the feedstock confirmed them suitable for the purpose. The carbon, hydrogen, nitrogen and sulphur (CHNS) content analysis result was used to calculate the theoretical yield potential which was 1844.0 ml/gVS for *Ecklonia maxima*. SEM - EDX were conducted on the samples to observe their morphology and chemical compositions. Fourier transform infrared (FTIR) spectroscopy was further conducted to identify the bonds and functional groups where both spectra recorded the presence of carboxylic, hydroxyl, ketones and ethers groups. Samples of biogas produced were analysed using gas chromatography to characterise the percentage of carbon dioxide (CO₂) and methane (CH₄). An optimum amount of ~55% methane was recorded by the mixture of *Ecklonia maxima* and acclimatised inoculum. The cumulative biogas yield for acclimatised sample was recorded as 1571.8 ml/gVS, very similar to 1564.0 ml/gVS predicted as the biogas potential (A) at a production rate (μ) of 296.2 ml/day using the modified Gompertz equation. The volume of biogas generated from the feedstock implies a positive influence of acclimatising inoculum on *E. maxima*

DEDICATION

This work is dedicated to my parents, Mr. Morrison Asare Darko and Mrs. Josephine Darko and my siblings for their resolute support all through my educational journey.

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LIST OF ACRONYMS AND ABBREVIATIONS

AD	Anaerobic Digestion
AMPTS	Automated Methane Potential System
BMP	Biomethane Potential
EDX	Energy Dispersive X-ray
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GHG	Green House Gases
HRT	Hydraulic Retention Time
ISR	Inoculum to substrate Ratio
MC	Moisture Content
OLR	Organic Loading Rate
pH	Potential of Hydrogen
SEM	Scanning Electron Microscopy
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile solids

CHAPTER ONE

INTRODUCTION

1.1 Background

The energy insecurity coupled with the negative impacts associated with the conventional energy sources are among the greatest challenges faced globally in the 21st century. The awareness of the need to resort to renewable alternatives increased over the years due to the continuous increase in the human population and the fast depletion of the existing fossil fuels [1]. Overdependence on these fossil fuels and other eco-unfriendly resources such as firewood and charcoal in our part of the world and their immense impact on the environment ranging from deforestation, air pollution, greenhouse gases (GHG) emissions, global warming and among others cannot be overemphasized [2][3]. Several factors such as environmental friendliness, cost effectiveness and abundance of substitute energy resources have been considered, rendering some unsustainable while a few of the minority have been confirmed promising and fit for the task.

Biofuels such as biogas, bioethanol and biodiesel have been considered as an alternative to current and posterity's energy demand. The gaseous biofuels however, present significant advantages over the liquid biofuels thus the need to effectively exploit these biomasses for biogas production. There already exist infrastructures such as anaerobic digestion (AD) capable of generating renewable gaseous energy from various biomasses such as algae, food wastes, animal manure and municipal solid wastes. Many of such processes were discovered centuries ago and it is at our advantage to efficiently utilize and improve upon them.

The choice of biomass for biogas generation, moreover, is of high importance because there is a vast range of energy crops to choose from. The interest therefore from in recent times has been utilizing and optimizing anaerobic digestion on the third-generation energy biomass.

Kinetic models such as the modified Gompertz equation and Logistic models are often used to predict and fit the cumulative yield data and further predict microbial activities on the biomass. The first and second generations have been explored in past times, yet due to the demerits associated with them, there is the urgency to resort to the much cheaper and efficient alternative.

1.2 Justification/ Motivation

The advocacy for finding alternative energy sources by both renewable energy experts and civil society organization (CSOs) have been on the rise recently. The use of energy undoubtedly cuts across all fields and sectors ranging from agricultural activities through to the transportation industries. The demands are high and yet on the other hand, the production is low and unsustainable. This work therefore employs one of the very old biological processes referred to as Anaerobic Digestion (AD) where microorganisms are grown to convert biomass into green biogas. Anaerobic digestion (AD) is a renewable energy technology that has been in use for centuries and is considered as a significant means of generating renewable biogas. This process aside being a renewable energy option, also contributes to greenhouse gases reduction. The basics of anaerobic digestion have been presented in literature and therefore there is the need to optimize the AD mechanisms on the sustainable third -generation energy crops to help curb the recurrent energy challenges.

1.3 Problem Statement

The overdependence on fossil fuels has over the years served as a menace due to the negative influence they have had on our climate and the world at large. The production of cost effective and eco-friendly bioenergy undoubtedly is a major step to alleviating our current climate and energy challenges. In this work therefore, one of the most promising renewable

energy sources classified as part of the third-generation energy crops has been explored for use as biogas to serve as a perfect substitute for natural gas, firewood and charcoal. Biogas from these sources could be used in lieu of the conventional natural gas for purposes ranging from electricity, heating and car fueling. A couple of AD process factors are explored in relation to biogas yield. The biogas produced is further modeled using the modified Gompertz equation to predict the production potential of the substrates used. The biogas generated would go a long way in serving the current and future generation's energy need.

1.4 Aims and Objectives

This work is aimed to devise and implement a methodology that will exploit the process factors of anaerobic digestion (AD) and determine their effects on biogas yield from brown seaweed (*Ecklonia maxima*). The objectives of this work are:

- To assess the impact process conditions of anaerobic digestion have on biogas yield.
- To identify and optimize the process conditions for *Ecklonia maxima* biogas production.
- To investigate the influence of inoculum acclimatisation on the methanogenesis of *Ecklonia maxima*.
- To utilize the experimental biogas data to model and predict the standard methane quantity required for domestic consumption.

1.5 Hypothesis

- i. Inoculum acclimatized to the same substrate used as feedstock improves biogas yield.
- ii. Using inoculum to substrate ratio (ISR) of 2: 1 is optimum for biogas yield.
- iii. The methanogenesis stage could be a rate determining step.

1.6 Organizational Structure of Report

This report consists of five chapters. The first chapter introduces the research, states the motivation and purpose, objectives and gives the scope of the study. The second chapter reviews macroalgae, describes anaerobic digestion and biogas generation from brown seaweed. The third chapter presents materials and detailed methodology employed to obtain credible results. The fourth chapter discusses the findings of the experiments and models whereas the conclusions and suggestions for further work are presented in chapter five. Additional relevant information is given in the appendices.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background and Overview of Macroalgae

Macroalgae or seaweeds are multicellular plant-like marine organisms that have very high polysaccharide and negligible lignin content[4][5]. Macroalgae are referred to as the third-generation energy biomass. They are regarded to be carbon neutral and consist of structures resembling roots, stems and leaves of other terrestrial plants[6][7]. These multiple cell marine organism are abundant in nature and have a higher photosynthesis efficiency than their terrestrial rivals [1][2][8][9]. They further have an upper hand over other biofuel generations by virtue of requiring non-arable land for cultivation implying zero competition over land and water for agriculture unlike the land-based biomass [2,3][6][8][10-12] acceleration in the growth rate of macroalgae as a result of oceanic eutrophication has however been observed in time past[6][13] leading to the recording of millions of tonnes of harvested seaweed globally per year [14].

The total number of macroalgae species in existence is yet unconfirmed, but there currently are about 12,500 taxonomically classified seaweed species [14-15][4]. They are grouped into three phyla based on their dominant pigmentation and colour of thallus vis: red, green and brown algae[1][16]. The Rhodophyceae, red algae make up majority of the seaweed population and are often found in the neritic and littoral parts of the ocean. They contain the phycoerythrin and phycocyanin pigments responsible for their characteristic red colour. The Chlorophyta are mostly found in the shallow parts of the ocean and owe their green colour to the chlorophyll A and B pigments. The Phaeophyceae are the brown seaweeds and have the fucoxanthin pigment responsible for their dark brown colour [4]. The relative proportions of carbohydrates, lipids and proteins in all these three phyla render them suitable for energy production. However, their composition vary based on species type, season of harvest and

environmental conditions[17][18]. The conversion of these third generation biofuels into gaseous products has been observed to be more fruitful than converting same samples into liquid biofuels[21][2].

Table 2.1: Carbohydrate composition of the three macroalgae phyla [19]

Brown macroalgae	Red macroalgae	Green macroalgae
Cellulose	Cellulose	Cellulose
Laminarin	Carrageenan	Starch
Mannitol	Agar	
Alginate	Lignin	
Fucoidan		

Table 2.2: Lipids, proteins and carbohydrates contents of various seaweed (% dry weight)[20]

Macroalgae	Lipids	Proteins	Carbohydrates
Brown algae			
Ecklonia stolonifera	2.4	13.6	48.6
Laminaria japonica	1.8-2.4	9.4-14.8	51.9-59.7
Unduria pinnatifida	1.8-2.0	15.9-18.3	40.1-52
Sargassum fulvellum	1.4	13	39
Saccharina japonica	0.5	19.9	44.5
Hizikia fusiforme	0.4-1.5	5.9-13.9	28.6-50
Red algae			
Porphyra tenera	4.4	38.7	35.9
Gracilaria verrucosa	3.2	15.6	33.5

Gelidium amansii	0-3.1	15.6-16.3	61-67.3
Green algae			
Ulva lactuca	6.2	20.6	54.3
Enteromorpha linza	1.8	31.6	37.4
Codium fragile	1.8	10.9	32.3

2.2. Anaerobic digestion of brown macroalgae

Anaerobic digestion (AD) is a multistep cost-effective energy generation process favourable for a vast range of materials and waste [2][22-26]. The process is a biological one that takes place in the absence of oxygen and allows growth of bacteria for the microbial decomposition of organic feedstock into biogas [27-30]. This biological process requires the activeness and working together of different microorganism species for biogas production [31]. The biogas produced is mainly composed of ~ 55-65% of methane (CH₄) and ~35-45% carbon dioxide (CO₂) [4]. A sustainable and eco-friendly biomethane is obtained after biogas is purified to remove excess CO₂ which reduces biogas energy value when left unremoved [5][32].

Table 2.3: Theoretical composition and quantity of biogas formed from carbohydrates, protein and fats [33]

Seaweed Composition	Biogas formed (m³/kgVS)	Biogas composition (CH₄:CO₂)
Carbohydrates	0.38	50:50
Fat	1.0	70:30
Protein	0.53	60:40

Anaerobic digestion has exhibited several striking advantages over other energy generation methods. A number of these merits include: very little or no chemical usage, possible energy recovery, employs simple technology with better treatment efficiency, no fossil fuels

requirement for treatment, reduces greenhouse gases emission(GHG) and less energy input [26][24].

The process of anaerobic digestion occurs in four successive stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis[34-35][27-28]. The anaerobic reactor used in the process like any other operates optimally under certain conditions for better yield. The microbes are required to be in sufficient contact with the substrate, have sufficient adaptation to the feedstock, presence of favourable operational conditions and among others [24] [2][21]. All of the four successive stages are required to occur in balanced rates to avoid inhibition of microbes [34][2].

2.2.1. Hydrolysis

Hydrolysis is the first step in anaerobic digestion where hydrolytic bacteria break down complex and insoluble organic macromolecules into smaller components suitable for use by acidogenic bacteria [35][23][27]. In this biological hydrolysis process, hydrolytic bacteria secrete extracellular enzymes that convert carbohydrates, lipids and proteins into sugars, long chain fatty acids (LCFAs) and amino acids respectively[23][31]. Organic waste stabilization does not essentially occur in this stage, the organic feedstock are simply converted into soluble forms[23]. The hydrolysis process moreover, is very sensitive to temperature and produces substrates for the acidogenic bacteria. The products of the hydrolytic breakdown stage then diffuse through the cell membranes of acidogenic bacteria. Hydrolysis is generally considered to be the rate limiting step during anaerobic digestion and is often as a result of the availability of accessible substrate particle surface area and not the lack of enzyme [24].

The hydrolysis process is illustrated by the reaction below:



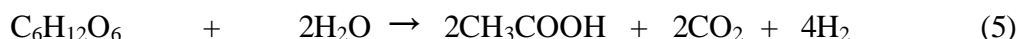
2.2.2. Acidogenesis

The acidogenesis stage is a complex stage that involves acidogenic fermentation [23] and is observed to proceed faster than the other stages of anaerobic digestion thus results in higher bacteria growth rate [24][35]. The soluble monomers from the hydrolysis stage are converted into metabolic products such as hydrogen and volatile fatty acids (VFAs) and alcohols [2][21]. During acidogenesis, the acidogenic microorganisms absorb products of hydrolysis through their cell membranes to produce intermediate volatile fatty acids (VFAs) consisting of groups of organic acids such as acetate and butyrate. The main reactions which occur during acidogenesis [36] are represented in reactions 2-3:



2.2.3. Acetogenesis

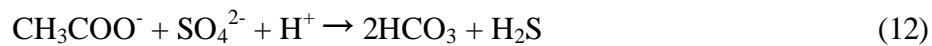
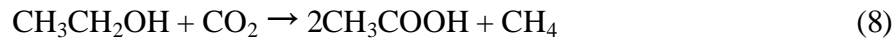
The methanogenic microorganisms directly utilise some of the products formed during the acidogenesis stage. A number of these compounds such as fatty acids and alcohols with more than two carbons, in addition to aromatic and branched chain fatty acids are further degraded into acetic acid, carbon dioxide and hydrogen during the acetogenesis phase [37]. The acetogenesis stage is where higher Volatile Fatty Acids (VFAs) are converted into acetates hydrogen gas, acetic acid and carbon dioxide[2][21]. The prior stages obviously, renders portions of the substrate suitable for acetogenesis[35]. The primary bacteria responsible for acetogenesis are mandatory producers of H_2 and thus live symbiotically with H_2 consumers. The acetogenic bacteria then make higher VFAs accessible to the methanogenic microorganisms [23]. The main reaction that occur during acetogenesis [36]is given in equations (4) to (7) below:



2.2.4. Methanogenesis

This is the final stage of the anaerobic digestion process where methanogens convert end products of preceding stages to methane and carbon dioxide [27][31]. These methanogenic microorganisms are groups of anaerobic archaea that reduce carbon dioxide using hydrogen as electron donor and decarboxylate acetate to form methane (CH_4). These methanogens are mainly grouped into two namely the aceticlastic methanogens (acetate converters) and the hydrogenotrophic methanogens (hydrogen utilizing) [2][38]. Methanogens generally are slow growers, however, the growth rate of the acetate converting are much slower compared to that of the hydrogen utilizing and this results in the doubling times of days [24][31]. The aceticlastic methanogens regardless dominate this final stage using acetate from prior stages as main substrate. In the process of their metabolism, carbon is parted into two for methane and carbon dioxide formation [31]. Methanosarcina and Methanosaeta species are the most common aceticlastic methanogens whereas Methanobacterium, Methanococcus, Methanogenium and Methanobrevibacter are some examples of hydrogen utilizing methanogens [24][31]. These methanogens, moreover, are very sensitive to oxygen and require higher pH than prior stages. Methanogenesis could however be the rate limiting step considering the slow growth rate of methanogens and the rapid occurrence of preceding stages. This eventually leads to increase in volatile fatty-acids (VFAs), decrease in pH and

inhibition of methanogens [2][21][31,34]. The stop in biogas production marks the end of methanogenesis usually within thirty (30) days. The aceticlastic methanogenesis accounts for two-thirds (2/3) of the methane produced whereas the remaining one-third (1/3) is obtained from the hydrogenotrophic methanogenesis [35]. The reactions (8) to (13) represent the methanogenesis stage [36]:



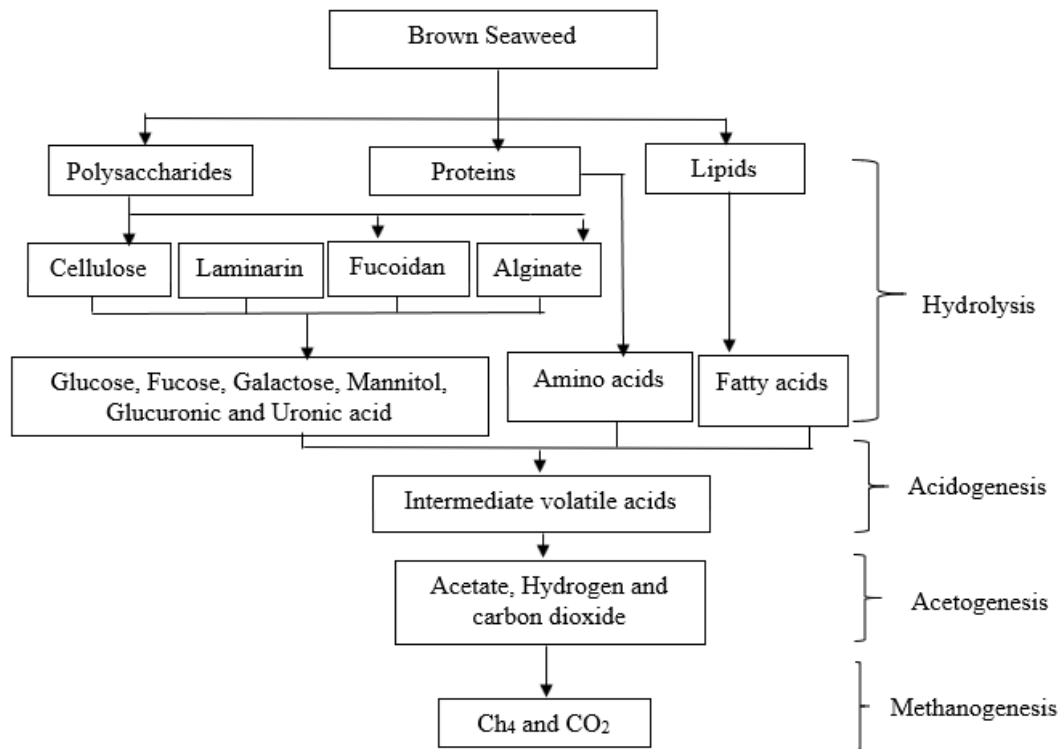


Figure 2.1: The major steps of anaerobically digesting brown seaweed

2.3. Process Parameters of Anaerobic Digestion

Anaerobic digestion is influenced by several physical or chemical operational and environmental factors [10]. Notable among these factors are temperature, pH, inoculum to substrate ratio (ISR), inoculum source, organic loading rate (OLR), hydraulic retention time (HRT) and redox potential [10][23]. These factors significantly influence the production of methane from anaerobic digestion [10].

2.3.1. Temperature

Production of methane from anaerobic digestion is hugely influenced by temperature since anaerobic microorganisms are very sensitive to changes in temperature. The temperature for optimum growth and function of a microorganism is dependent on its original source [31]. It is therefore very important to maintain a very stable temperature for growth and development

of microorganisms[10][22]. The production of biogas can occur over a wide range of temperatures categorized under psychrophilic ($< 20^{\circ}\text{C}$), mesophilic ($20\text{-}45^{\circ}\text{C}$) and thermophilic ($>45^{\circ}\text{C}$) conditions[2][10][34]. The most commonly used temperatures are 35°C - 37°C for the mesophilic or usually 50 - 55 $^{\circ}\text{C}$ for thermophilic conditions[27][34,39]. Thermophilic conditions causes higher hydrolysis and degradation rates. However, the slightest change in thermophilic temperature significantly risks microbial community washout. Most biogas digesters in use currently are however operated under mesophilic condition considering the energy requirement of thermophilic conditions and how less competitive the latter will be, upon improvement of heat supply and direct use of biogas[34]. A decrease in temperature is very likely to increase volatile acid concentration and affect digestion rate of methanogens sometimes cause death of methanogens [10][31].

2.3.2. Effects of pH on Methanogenesis of Seaweed

The pH of an anaerobic digestion system is very important for methane production since the growth of microbes or methanogens is directly influenced by fluctuations in pH. Abrupt changes in the pH eventually interfere with methanogenesis. Methane production often takes place within a very narrow pH range of 6.8 to 7.2. [39]. Extreme increase in alkalinity can lead to microbial granules disintegration and eventually failure of the entire process[40]. The optimum pH for hydrolysis and acidogenesis is given between ~ 5.5 and ~ 6.5 [41] although the optimum pH for methanogenesis is 7.0. Inhibition notwithstanding occurs with severe decrease in pH[31,34][42-43]. Accumulation of volatile fatty acid (VFA) is likely to decrease the pH of the system whereas protein degradation on the contrary caused pH and ammonia increase. The pH determines relative proportions of the ionised and non-ionised forms of these compounds. The presence of non-ionised ammonia and volatile fatty acid is significant at high and lower pH respectively. The presence of non-ionised sulfide on the other hand is also dominant at lower pH. The existence of these in their non-ionised forms renders the

system toxic for methanogenic activities. It is therefore very important to correct the pH conditions for improved yield.

2.3.3. Inoculum to substrate ratio (ISR)

Inoculum refers to a medium usually at a neutral pH and specific temperature that contains incubated anaerobic microorganisms for further substrate degradation [25]. Inoculum could be obtained from varying sources ranging from digested sludge from municipal waste water treatment plants, animal rumens and animal manure. In addition, raw organic substances such as municipal solid waste, fruit and vegetables, grasses, terrestrial crops and aquatic biomass are often introduced as substrates into the inoculum for biogas production [43]. The microorganism content in both inoculum and substrate is very important and so is the ratio of inoculum to substrates. Moreover, the degradability of these organic substrates depends on their composition and ratios relative to the inoculum used. The inoculum to substrate ratio (ISR) is observed to influence the rate of anaerobic biodegradation as well as methane yield [25][29]. For the purpose of harmonizing anaerobic digestion regardless the ISR values omissions in some prior works, an ISR value of 2 on volatile solids (VS) basis has been suggested [25][44]. Moreover, higher ISR has been observed to favour rate and yield of biogas [43].

2.2.4. Organic Loading rate (OLR)

Organic loading rate (OLR) is a term used to represent the mass of organic matter added per unit reactor volume per unit time. The recommended OLR is given as 1.9- 5 kgVS $m^{-3}d^{-1}$ where less than 3 kgVS $m^{-3}d^{-1}$ is suggested for mesophilic systems [31][10]. However, these recommended OLR values vary with different substrates, their chemical composition and digesters [10]. The quantity and rate of the loading are very required to follow a specific pattern and not vary by more than 15% per week. The feedstock is required to be consistent since microorganisms adapt to materials over time.

2.3.5. Hydraulic retention time (HRT)

The hydraulic retention time (HRT) is used to refer to the duration within which organic matter remains in contact with the microbes [10]. HRT is considered an important anaerobic digestion parameter in the sense that, it plays a vital role in determining the level of acidification of an AD process[42]. HRT is required to be sufficient to enable effective degradation of substrates and this is usually between 10 to 25 days [31][2][45]. Low HRT reduces efficiency of substrate degradation whereas longer retention time results in increased specific methane yield (SMY)[2]. The HRT under mesophilic conditions are observed to be longer than under thermophilic conditions since methanogens grow faster at higher temperatures despite high ammonia inhibition and microbes washout [34].

$$\text{Hydraulic retention time (d)} = \frac{\text{Reactor capacity (L)}}{\text{Daily fresh substrate added (L/d)}}$$

2.4. Biomethane Potential (BMP) tests

The biomethane potential (BMP) is a biological test that enables the evaluation of produced biogas or methane [38][44]. These tests are carried out in batches where substrates and inoculum samples are introduced into small closed digestion reactors of volumes ranging from 100 millimetres to 2 litres [46][47]. The tests depending on the homogeneity of the substrate are typically carried out in triplicates to enable accurate statistical analysis. Triplicates are also set for reactors to contain only inoculum for further analysis. The reactors are purged with Nitrogen gas to ensure anaerobic conditions[47]. The quantity and composition of gas produced are monitored daily and cumulatively over a period using different techniques. The cumulative gas produced is recorded until steady state is reached. The specific BMP is expressed as biogas volume per unit mass of feedstock [48]. The specific methane yield (SMY) is used to refer to the result obtained from BMP given as the

amount of biogas produced from a given weighted substrate measured in L CH₄/kg VS [47].

The test proceeds usually up till 30 days or less until no gas yield is recorded.

2.5. Theoretical biogas yield

The biogas potential of a feedstock is calculated via the modified Buswell equation which is based on the major compositions of the substrate. This equation assumes complete conversion of all organic materials into methane, carbon dioxide and ammonia[2]

$$C_c H_h O_o N_n + \frac{1}{4} (4_c - h - 2_o + 3_n) H_2 O$$

$$= \frac{1}{8} (4_c + h - 2_o - 3_n) CH_4 + \frac{1}{8} (4_c - h + 2_o + 3_n) CO_2 + n NH_3$$

The theoretical specific methane yield can then be calculated from

$$\frac{\frac{c}{2} + \frac{h}{8} - \frac{3}{8}o - \frac{n}{4}}{12c + h + 14o + 16n} * 22.4 * 1000 \left(\frac{mL}{gVS} \right)$$

Where 22.4 L, is the volume of 1 mol of gas at standard conditions and 1000 for converting from litres to millilitres. The actual specific methane yield is often lower the theoretical specific methane yield since some organic matter are really not degraded[2].

The methane content (MC) can be estimated as the percentage of methane from the biogas mixture produced as:

$$MC = \frac{4 + h - 3o - 2n}{8c}$$

2.6. Biogas production and composition

Biogas predominantly consists of methane and carbon dioxide, however contains traces of other gases such as O₂, H₂, N₂, H₂S and water vapour. Hydrogen Sulphide is produced in excess when substrates contain large amounts of Sulphur and are very detrimental to the

digesters. Hydrogen sulphide just like carbon dioxide can be scrubbed from biogas produced however, it is an expensive process.

Methane is the most valuable fraction of the biogas produced where it has a heating value of approximately 37MJ/m³. The heating value of biogas is decreased by the presence of carbon dioxide. As the fraction of carbon dioxide increases, the heating value decreases. Biogas with carbon dioxide greater than 30% requires to be supplemented since it will not produce self-sustained burn on its own [49].

Brown seaweed has the potential as a precursor for the production of biogas and significant volumes of methane.

Table 2.4: Comparison of biomethane yield from different seaweed species under different pretreatment methods

Seaweed specie	Pre-treatment	Biogas (ml g/ VS)	CH₄ (ml/gVS)	Reference
Saccharina latissima	Chopping	-	340 ± 48	[50]
Saccharina latissima	Drying and milling	-	335	[51]
S. latissima	maceration	-	333	[52]
Laminariaceae spp.	Mechanical comminution	685 mlg/TS	-	[52]
Laminariaceae spp.	Beating	-	335	[5]
Laminaria digitata	Drying and milling	-	184	[51]
P. canaliculata	Beating	-	340	[50]

2.7. Methanogenesis Inhibition

Inhibitors refer to all substances, conditions and activities that cause extreme shift in microbial growth and activities[2]. Inhibitors enter the process by the degradation of an initially non-inhibiting material or the presence of some contaminated material. Prior works have reported varied inhibition levels of different substances as a result of differences in the

anaerobic digestion process and the methane producers are the most affected. Methanogenesis inhibition is often observed by decrease in methane production and accumulation of volatile fatty acids[32]. The microorganisms responsible methanogenesis are observed to be sensitive thus the slightest change from optimum conditions increases the possibility of inhibition [26]. The effect of the inhibition varies depending on the concentration of inhibitors, temperature, pH and the microorganisms present. The aceticlastic methanogens for instance are very sensitive to ammonia occurring as a result of increased pH and temperature, eventually inhibiting the process [53-54][26]

2.7.1. Inhibition levels

Different levels of inhibition have been observed for anaerobic digestion and these are attributed to the differences in substrates and procedures used[32].Mechanisms such as antagonism, synergism, complex formation and acclimation arise and significantly influence the process.

2.7.1.1. Antagonism

This level of inhibition occurs when the collective combination of inhibition levels is lower than the individual inhibition levels.

2.7.1.2. Synergism

This is when the presence of numerous inhibitors combine to produce an inhibition level greater than their inhibition levels.

2.7.1.3. Complex formation

This inhibition is characterised by the attachment of the inhibitory substances to the similar structures making them invisible to the microorganisms.

2.7.1.4. *Acclimatisation or Adaptation*

The microorganisms at a point adapt to the inhibitors and grow once again, however, it is not same for all microorganisms. Some of these anaerobic microorganisms learn to break down the inhibitors and sometimes form defense systems in tend to make them resistant to the new toxic environment.

2.8. Conditions that influence Methanogenesis Inhibition

2.8.1. *Growth rate of methanogens*

Methanogens as aforementioned are responsible for methane production and are dissimilar from other microorganisms thus are not as vigorous as the others, making them vulnerable to disturbance occurring along the process. They are however very important for the anaerobic digestion thus inhibiting these methanogens disrupts and severely affects the entire process [32][31]. Methanogens generally grow slowly and this often makes methanogenesis the rate limiting stage of the biogas production process. The two known acetoclastic methanogens namely methanosaeta and methanosarcina regardless, have different growth rates where the latter grows faster yet unable to function at very low acetate concentration [34]. The growth rate of methanogens often limits the retention time since very short retention time washes out these methanogens from the process as a result of insufficient growth time [34]. The absence of methanogens implies inhibition of methane production.

Table 2.5: Comparison of acetoclastic methanogens

Methanogens	Doubling time	Lowest acetate concentration
Methanosarcina	1 day	20 mg/L
Methanosaeta	2-12 days	4 mg/L

2.8.2. *Inoculum Acclimation*

Inoculum acclimation is the process of culturing microorganisms on the substrate to get them adapted to the medium. The substrates used for the AD process are often different from the inoculum or inoculum source. Inoculum acclimation is then carried out to increase the affinity of AD microbes to the substrate to be used. These microorganisms are further able to establish biodegradation pathways and likely increased presence of appropriate degraders [25]. Inoculum acclimation is done over a period to enhance microbes adaptation, a complete process and to ensure steady conditions [48]. The biomethane yield highly depends on the metabolic capabilities of the methanogens thus reducing the activities of the methanogens leads to underestimated biogas yield [47].

2.8.3. *Stirring*

The agitation of reactors during anaerobic digestion is one factor that contributes to biogas yield by ensuring efficient organic material transfer to the microbes. Agitation of AD reactors and digester could be done via magnetic stirrers, orbital shakers or manual shaking. The intensity and duration for the stirring influences the mixing and homogeneity process. Stirring is not necessarily required to be continuous, it is only important to stir severally within a day depending on the total solids of the used feedstock[39]. The purpose of the stirring is to enable contact between methanogens or microbes and substrates thus enhancing mixing and biogas production. Insufficient stirring causes settling down or sedimentation of denser substrates thus insufficient microbe-substrate contact, eventually inhibiting biogas yield [25].

CHAPTER THREE

MATERIALS AND METHODS

This section provides details of materials and equipment used for the experimental set-up, as well as the analytical methods and characterisation procedures used in this work.

3.1. Substrate preparation

The substrate utilised was a waste product left over from an enzymatic hydrolysis process that recovered polysaccharides from the South African brown seaweed, *Ecklonia maxima*. Subsequent to enzymatic hydrolysis, the aqueous hydrolysate and solid residues were separated by filtration through a cheese cloth and the residues frozen until further use as feedstock for anaerobic digestion process.

3.2. Inocula preparation and characterisation

The inoculum used for the biomethane potential (BMP) experiments were prepared from cow manure obtained from the Mariendahl experimental farm located on the outskirts of Stellenbosch. The collected cow manure was bagged in 1kg sealed plastic bags and frozen until use. Five kilogram (5 kg) of defrosted cow manure was diluted with 16 litres of water to make up to 70% of the 30 litres anaerobic digester. The digester was operated at 37°C and automatically stirred periodically to ensure digestion of all other substrates. 5 litres of the inoculum, after every 10 days were withdrawn and same amount prepared for re-feeding to ensure culture stability and nourishment. The biogas flow rate of the digester was also checked on daily basis to confirm activeness and growth of the cultured microorganisms.



Figure 3.1 30 L mesophilic anaerobic digester used for inoculum preparation.

3.3. Substrate characterisation

Proximate analyses were done on the different sets of inoculum and residues of *E. maxima*. The total solids (TS), volatile (VS) and pH of the samples were analysed as per the BMP protocol defined by Angelidaki *et al* [47].

The pH for all samples was measured using the Hanna pH meter and neutralised using either HCl or KOH prior to the experimental set-up.

20 ml of gas from all reactors were taken weekly using 50 ml syringes for analysis throughout the entire digestion period.

The gas samples were directly injected into the Compact GC 4.0 Gas chromatography equipment to determine their methane (CH₄) and Carbon dioxide (CO₂) percentage.

The determination of elemental composition of the samples was outsourced to the Stellenbosch Centre for Analytical Facilitation. The elementary composition results were further used in calculating the theoretical methane yields of samples.

The inoculum and residues of *Ecklonia maxima* were subjected to Fourier transform infrared (FTIR) analysis to identify functional groups present in samples.

3.3.1. Total solids (%TS)/Moisture content (MC)

The total solids and moisture content of samples were determined based on the protocol established by A. Sluiter et al [55]. Ceramic crucibles were first weighed and oven dried at 105°C for a minimum of four hours. The samples were thoroughly mixed and ~2g weighed into the pre-dried crucibles. The crucibles containing samples were then oven dried overnight at 105°C, cooled in a desiccator and weighed until a constant weight was achieved. The weights before and after oven-drying were measured, recorded and used for the determination total solids given by equation 3.1 below:

$$\%TS = \frac{\text{Weight}(\text{crucible}+\text{sample})_{\text{ovendried}} - \text{Weight}(\text{crucible})_{\text{ovendried}}}{\text{Weight}(\text{sample})_{\text{wet}}} * 100 \quad (\text{equation 3.1})$$

3.3.2. Volatile Solids (%VS)

The crucibles with oven dried samples were placed in a muffle furnace at ~550°C for a minimum of four hours. The ashed samples were cooled in desiccator and accurately weighed using a mass balance. The masses of the ashed samples were recorded and used to calculate the volatile solids via equation 3.2:

$$\%VS = \frac{\text{Weight}_{\text{ovendried}} - \text{Weight}_{\text{furnace_dried}}}{\text{Weight}_{\text{wet}}(\text{sample})} * 100 \quad (\text{equation 3.2})$$

3.3.3. pH calibration and measurement

The pH of all samples for the experiment runs was measured prior to and at the end of the anaerobic digestion period using the Hanna pH meter. The pH meter was calibrated with standard buffer solutions of pH 4,7 and 9 before use. The pH electrode was thoroughly cleaned with distilled water before measuring different sample sets. The sample volumes were either neutralised with Hydrochloric acid (3M) or potassium hydroxide (1M) depending on initial pH value of samples. The electrode was dipped into all reactors and pH value recorded for all.

3.3.4. Elemental Analysis

Samples of dried *E. maxima* residues, acclimatised and unacclimatised inoculum were submitted to the Central Analytical Facility (CAF) in Stellenbosch University for elemental analysis. The concentrations of carbon, hydrogen oxygen, nitrogen and sulphur were measured for the three different samples using an Elementar Vario EL Cube Elemental Analyzer (Elementar, Langenselbold, Germany). Sample size ranging from 1 mg to 1 g was transferred into tin vessels and loaded into the integrated carousel and helium flushed to remove atmospheric nitrogen. The catalytic combustion afterwards was done at a fixed temperature of 1200°C. The combustion gases on hot copper were reduced in a second furnace in order to keep the analysis gases in the carrier gas system. The gas mixture was then separated into their various components using three different columns and afterwards measured with a thermal conductivity detector (TCD). The EL system was able to identify and calculate the elemental composition using the detector signal and stored calibration curves.

3.4. FTIR and SEM analyses of substrates and Inoculum

3.4.1. FTIR experiments

Fourier transform infrared analysis (FTIR) of the brown seaweed and inoculum was conducted using the PerkinElmer spectra two FTIR machine at the Physics department of the University of Ghana. The analysis was done within the spectra range of 4000 - 400 cm^{-1} mainly to identify the distinct bonds and functional groups present in the seaweed and the inoculum.

3.4.2. SEM with EDX experiments

The Phenom ProXSEM desktop machine located at the Department of Earth Science, University of Ghana was used in conjunction with the EDX equipment to characterise the morphology and the respective quantities of elements in the substrates.

3.5. Automated Methane Potential Test System (AMPTS)

The anaerobic digestion experiment was carried out using the Automated Methane Potential Test System II (AMPTS II) with a software version of ge_2.1 (v1.1711). AMPTS II was designed for automatic real time measurement of biomethane production from any organic biomass. The system comprises of 15 reactors each of 500ml capacity and connected to agitators to ensure homogeneous stirring of substrates and inoculum. These reactors are housed within a temperature adjustable water-bathe and each reactor connected with tubings for gas scrubbing and sample drawing. The produced biogas passes through tubes into alkali solutions for fixation and removal CO_2 and H_2S . This is the stage referred to as scrubbing, after which the remaining gas is transported into the gas flow meter. Each reactor is connected to a wet gas flow meter cell and the data obtained then recorded by the system.



Fig. 3.2. AMPTS II comprising of temperature-controlled water-bath, reactors with overhead stirrers, alkali solutions and flow meter cells.

3.6. Experimental Approach

This research study proceeded in the experimental approach as illustrated in Fig.3.3

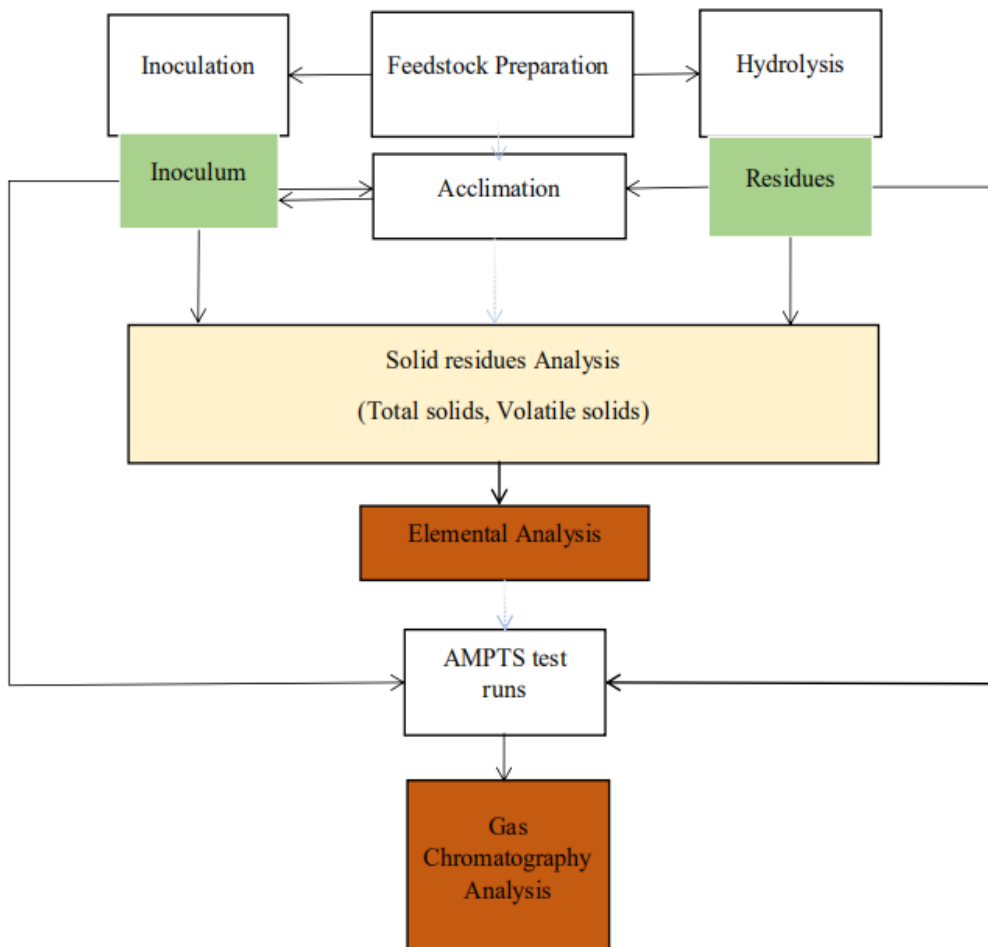


Fig.3.3. Experimental approach to study

3.6.1. Inoculation and Acclimation

The primary objective of the acclimation step was to enable microbe adaptation to the feedstock and observe its influence on methane yield. The acclimation process involved the introduction of residues of *E. maxima* into the inoculum and subjecting the mixture to anaerobic conditions for 14 days. Four (4) litres of inoculum after the first fourteen days of inoculation was collected from the 30L prepared inoculum into 4 litres airtight bottles then mixed with residues of *Ecklonia maxima* at an ISR of 100:1. The acclimation process for this mixture was carried out for 14 days at 37°C and under gentle manual mixing. The seaweed residues were fed once into the inoculum thus no additional feeding during the acclimation process. The experimental study thus employed two different inoculum sets namely the acclimatised inoculum and unacclimatised inoculum. The first set been the new mixture consisting inoculum and seaweed residues labeled as the acclimatised inoculum.

The remaining inoculum within the 30 L anaerobic digester with zero *Ecklonia maxima* was labeled and used as the unacclimatised inoculum. The two inoculum sets were then withdrawn into airtight bottles and incubated for 3 days as advised by Angelidaki et al., 2009 to ensure sufficient degassing before commencement of the main experimental runs.

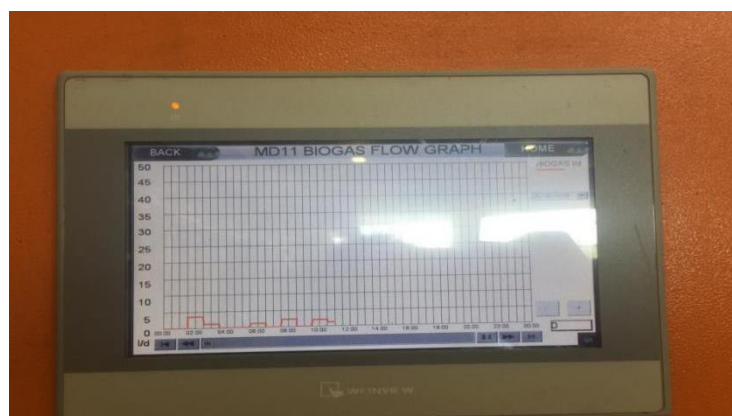


Fig.3.4: Control panel for monitoring biogas flow in the anaerobic digester

3.6.2. Hydrolysis and Feedstock treatment

The *Ecklonia maxima* used as feedstock in this study consisted of its stipes and fronds. The two different parts were milled and mixed with water at a ratio of 1:1:2 and subjected to polysaccharides recovery process. The extraction procedure was conducted using a cellulase enzyme at a pH of 5.5, enzyme to substrate ratio of 0.5 % vol/dry weight, temperature of 55°C and ran for a period of 4 hours. Once the run time was due the residues were separated using a large filtering cloth and the residues used as the feedstock for this study work

3.6.3. Experimental Test runs

The setup for the anaerobic digestion is shown in Table 3.1. The experimental setup consisted of ten reactors each containing substrate amounts automatically predicted by the AMPTS II software depending on their respective total and volatile solids.

Two sets of controls as recommended by Hansen *et al.* 2004 were used in this test and run the same way as all other reactors. One control set contained only inoculum referred to as the Blank and the other labelled as Control contained inoculum and a type of pure cellulose referred to as Avicel® PH-101 cellulose from Sigma Aldrich which is used to quantify the cellulase activity. The Controls as stated were used as positive controls to confirm the effectiveness of the inoculum types. The Blanks on the other hand, were used as reference in determining the background methane produced by only the inoculum.

Duplicate Blanks were prepared for each of the two inoculum sets. The reactor labelled “Blank Accl.” denotes runs containing only 400 ml of acclimatised inoculum whereas “Blank Unaccl.” represents runs with solely 400 ml of unacclimatised inoculum. The “Control Accl.” similarly, contained acclimatised inoculum and Avicel whereas “Control Unacclimatised” contained unacclimatised inoculum and Avicel®, both sets at a 2:1 ratio. “Sample Acclimatised” likewise “Sample Unacclimatised” contains measured amounts (as shown in

table 3.1) of *E. maxima* except “Sample Acclimatised” was mixed with “Acclimatised Inoculum only” and “Sample Unacclimatised” mixed with “Unacclimatised inoculum” only. All test runs were set up at 400 ml working volumes and at neutral pH.

The reactors were all closed with rubber stoppers and flushed with nitrogen gas through the tubing for 2 minutes to ensure anaerobic digestion. The temperature for the water bath within which all reactors were contained was set at 37°C throughout the experiment. Moreover, all reactors were connected to mechanical agitators that stirred continuously for 300 seconds at 40 rpm and off for 600 seconds during the whole experimental period.

The anaerobic digestion of this test proceeded until all easily degradable organic materials completed their degradation cycles which was characterised by the reduced and an almost insignificant methane yield. The AMPTS II software used the water displacement method to collect and record data on the generated biogas for the entire 20-days experimental study.

Table 3.1: Starting volumes of feedstock used in the experimental setup

Sample name	Replications	Acclimated Inoculum (ml)	Unacclimated Inoculum (ml)	Avicel® (g)	<i>E. maxima</i> (g)
Control Accl.	1	394.25	-	5.75	-
Control Unaccl.	1	-	390.23	9.77	-
Blank Accl.	2	400	-	--	-
Blank Unaccl.	2	-	400	-	-
Sample Accl.	2	336.16	-	-	63.84
Sample Unaccl.	2	-	332.45	-	67.55

3.7. Kinetic modeling of biogas production

The kinetics of biogas produced from the samples was modeled using the modified Gompertz equation. The modified Gompertz equation is given in equation 3.3 as follows:

$$y(t) = A \cdot \exp \left\{ -\exp \left[\frac{L\lambda}{A} (\lambda - t) + 1 \right] \right\} \quad (\text{equation 3.3})$$

Where $y(t)$ is the cumulative specific biogas production (ml/gVS), A is biogas production potential (ml/gVS), μ is the maximum biogas production rate, e is euler's constant (2.718182) and is λ the lag phase or minimum time to produce biogas (days)

The kinetic constants were determined using non-linear regression with the help of Microsoft Excel solver software. The coefficient of correlation was also calculated to determine the accuracy the fit in curves.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Substrate Characterisation

4.1.1. Chemical composition of seaweed and inoculum (FTIR analyses)

The FTIR spectra of *Ecklonia maxima* residues and inoculum prepared from cow manure were recorded as shown in Fig. 4.1 and 4.2 respectively. The spectra of the samples recorded bands showing presence of hydroxyl, carboxylic and ethers functional groups. The results are reported as a graph of transmittance against wavenumber.

4.1.1.1. FTIR spectrum of *Ecklonia maxima* residues

The spectrum of *Ecklonia maxima* showed five main distinguished bands in the normal spectra of 4000- 450 cm^{-1} which were identified at 3345 cm^{-1} , 2926 cm^{-1} , 1601 cm^{-1} , 1416 cm^{-1} and 1031 cm^{-1} . Stretches of O-H bonds appeared at 3345 which is similar to that carried out on alginate and *Sargassum filipendula* residues[56]. The peak recorded at 2926 cm^{-1} corresponds to the aliphatic -C-H bonds[57] indicating the presence of alkanes [58]. The O-H and CH stretching vibrations recorded on *Ecklonia maxima* conforms to ranges given by E. Gomez et al [59] on the identification of polysaccharides in brown seaweed. The region between 1800 cm^{-1} and 1450 cm^{-1} show characteristic vibrations for proteins which is mainly due to N-H bending stretch vibrations or to C=O [60]. The bands recorded at 1601 cm^{-1} and 1416 cm^{-1} respectively represents the asymmetric and symmetric stretching vibrations of carboxylic groups present as mannuronate and guluronate units in alginate [61]. The presence of C-S-O and sulphate ester groups which is characteristic of fucoidan [59] could be attributed to the small vibrational stretches recorded between 1350 cm^{-1} and 1220 cm^{-1} . The peak recorded at 1031 cm^{-1} is given as the C-O stretch corresponding to the guluronic parts of alginate whereas bands around 1100 cm^{-1} indicates the presence of mannuronic acid units[61]. The IR bands shown in the spectrum of *E. maxima* residues has

indicated the presence of alginate structures when [59] compared to work by E. Gomez *et al.*

This confirms alginate to be the main polysaccharide content of *Ecklonia maxima*

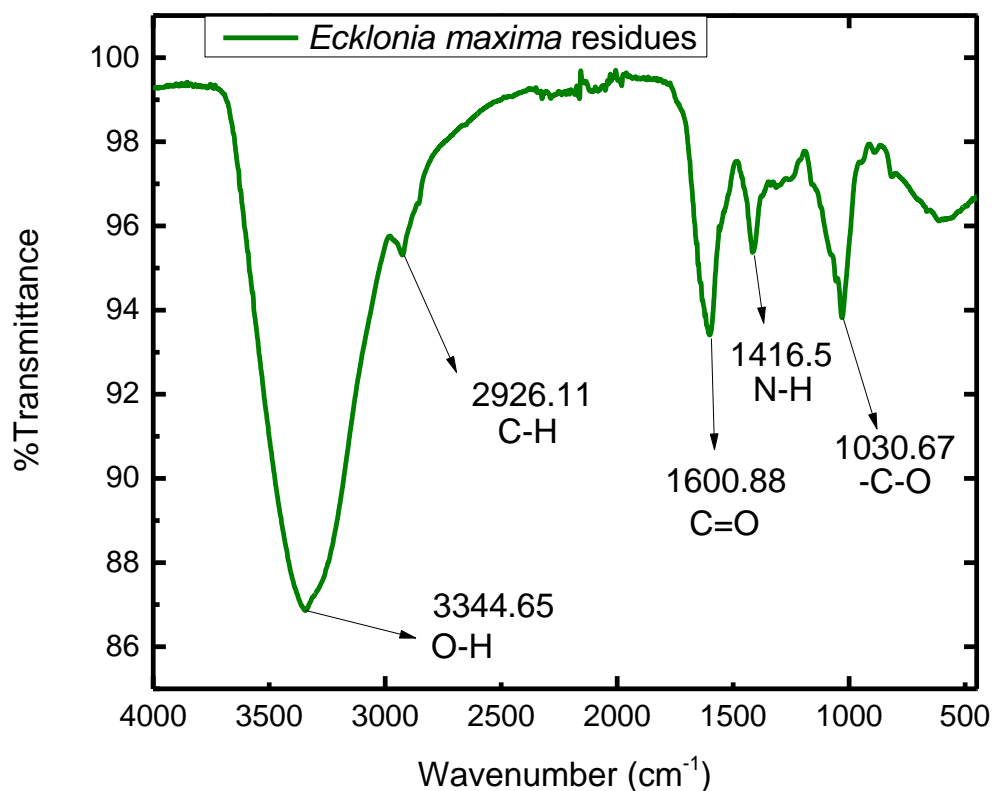


Fig.4.1: FTIR spectroscopy on “*Ecklonia maxima*” residues

4.1.1.2. FTIR spectroscopy of Inoculum

The spectrum recorded for the inoculum identified five distinguished peaks at different frequencies revealing different functional groups within the normal infrared region of 4000 - 450 cm⁻¹. The broad peak observed from 3351 to 3400 corresponds to the alcohols and hydroxyl acids caused by the stretching of O-H bonds [58]. The bands identified at 2919 and 2848 represents the -C-H bond vibrations showing the presence of alkanes.

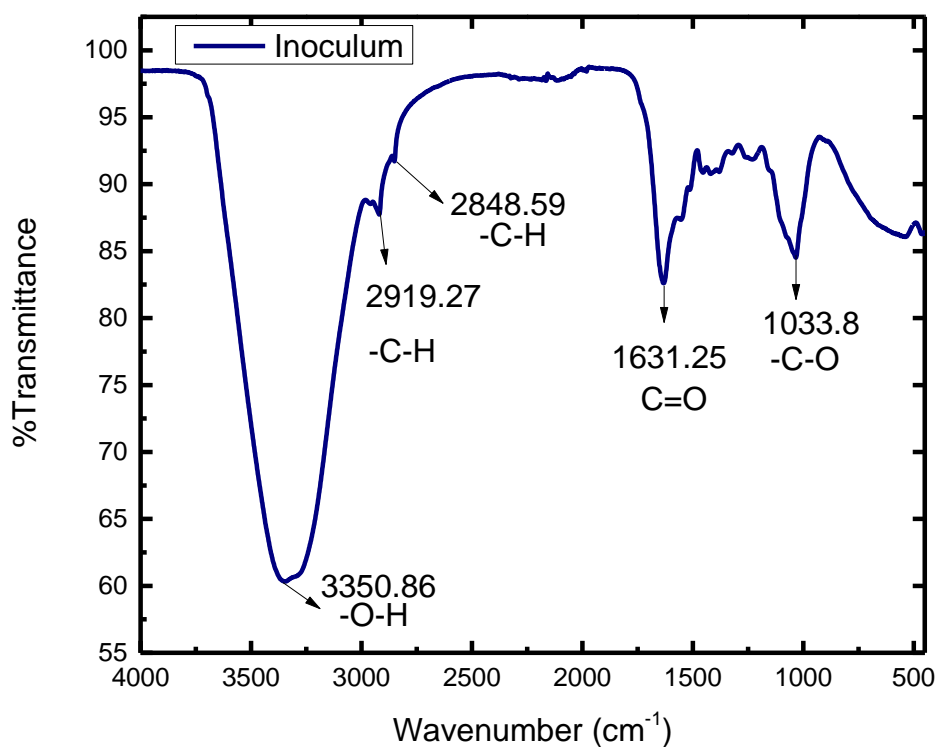


Fig.4.2. FTIR spectrum of inoculum prepared from cow manure

4.1.2. Morphological and Energy Dispersive X-ray spectroscopy analyses

(SEM-EDX Analysis)

Scanning electron microscopy images and results from the energy dispersive x-ray spectroscopy of the seaweed residues and inoculum are illustrated in Fig 4.3 and 4.4 respectively. The SEM photographs for both samples showed rough surfaces with relatively sized pores. SEM image of seaweeds showed a much compact morphology with irregularly scattered micropores. The fibrous nature of the cow manure used for the inoculum is attributed to the lamellar structures observed in the SEM of the inoculum. The dominant elements percent concentration recorded for both samples are calcium, nitrogen, potassium and silicon. The highest carbon content of 43.40% was recorded by the inoculum and 35.94%

from *E. maxima* as shown in table 4.1(a) and (b) respectively. The elements identified to exist in these substrates confirmed their suitability for biogas production.

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
C	51.21	43.40	C	68.95
O	32.83	37.06		
N	7.78	7.68	N	12.21
Si	1.68	3.33	Si	5.29
B	4.35	3.32	B	5.27
Ca	0.52	1.47	Ca	2.33
K	0.42	1.15	K	1.83
Cl	0.31	0.77	Cl	1.22
P	0.31	0.67	P	1.06
Mg	0.29	0.49	Mg	0.79
S	0.16	0.36	S	0.57
Al	0.16	0.30	Al	0.47

Table 4.1(a): Elemental composition and stoichiometric concentration of *E. maxima*

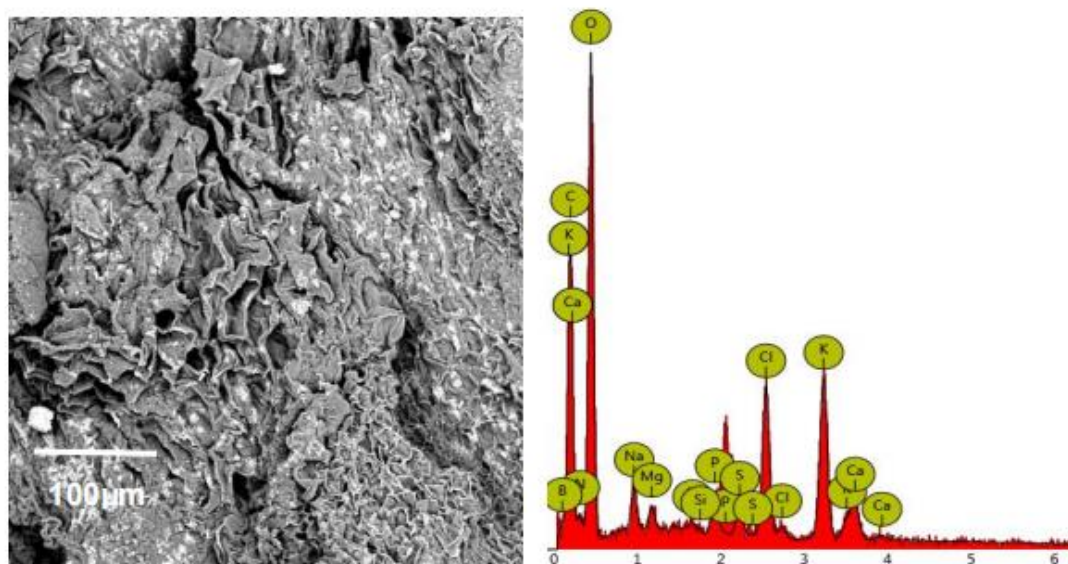


Fig.4.3 (a): SEM- EDX micrograph of “*Ecklonia maxima*”

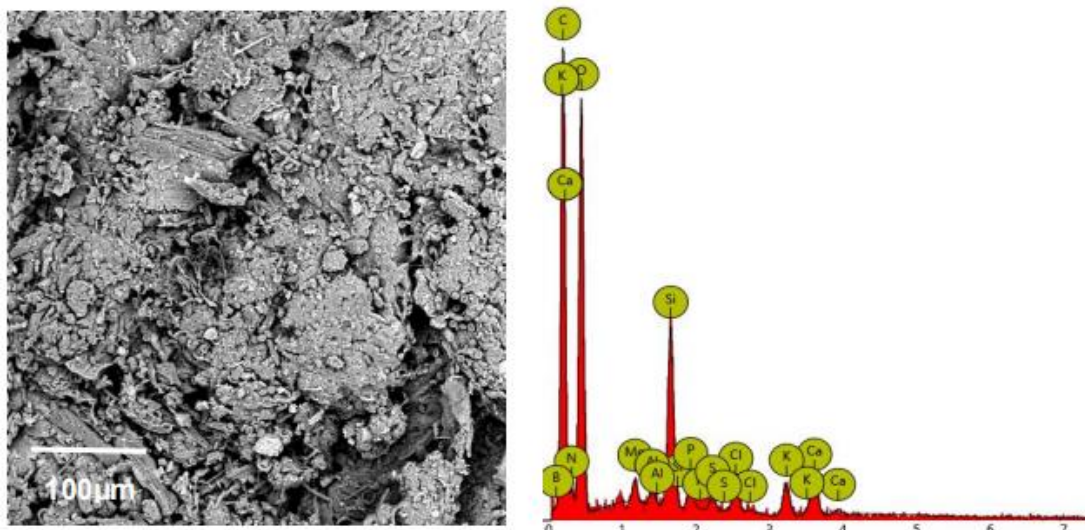


Fig.4.3 (b): SEM- EDX micrograph of the inoculum

Table 4.1(b): Elemental composition and stoichiometric concentration of inoculum

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
C	48.43	39.18	C	61.15
O	38.35	35.94	O	
K	2.26	5.95	K	9.28
B	8.09	5.89	B	9.19
Cl	1.44	3.44	Cl	5.37
Na	1.83	2.83	Na	4.42
N	2.70	2.54	N	3.97
Ca	0.75	2.29	Ca	3.58
Mg	0.63	1.03	Mg	1.61
S	0.22	0.48	S	0.75
P	0.21	0.43	P	0.67

4.2. Proximate Analysis

The proximate analysis of samples was carried out in order to confirm the anaerobic biodegradability of substrates and further predict the precise amount of substrate per parts inoculum to use for the experimental runs. The pH, total and volatile solids before and after anaerobic digestion (AD) were recorded for the two inoculum sets. The proximate analysis for seaweed on the other hand, was carried out only before anaerobic digestion (AD). This was because the seaweed was mixed as substrate with the inoculum and unlike the inoculum sets, there were no blank seaweed runs for reference.

The %TS and %VS of the samples showed no significant difference after twenty days. The volatile solids of the acclimatised inoculum for instance as recorded in table 4.1 recorded 2.855 ± 0.03 before digestion and 2.021 ± 0.7 after digestion. This implies that about 96% of its content was organic matter and half of this value was digestible and likely to have to been converted to gas. The post anaerobic digestion results then confirms the fact than more than 90% of the organic matter in both inoculum were converted to biogas. The results of the proximate analysis carried out on the individual feedstock are detailed in the table 4.1.

Table 4.2(a): Results from proximate characterisation of inoculum and seaweed samples

Analysis	Pre-Anaerobic digestion		
	<i>Blank Acclimatised</i>	<i>Blank Unacclimatised</i>	<i>Seaweed</i>
Total Solids (% TS)	3.703 ± 0.04	3.872 ± 0.15	9.683 ± 0.09
Volatile Solids (% VS)	2.855 ± 0.03	3.063 ± 0.12	7.528 ± 0.04

Table 4.2(b): Results from proximate characterisation of inoculum and seaweed samples after anaerobic digestion

Analysis	Post-Anaerobic digestion	
	<i>Blank Acclimatised</i>	<i>Blank Unacclimatised</i>
Total Solids (% TS)	3.143 ± 0.9	3.38 ± 0.8
Volatile Solids (% VS)	2.021 ± 0.7	2.857 ± 0.6

4.2.1. pH measurements

The pH like other parameters subtly influences the performance of samples and biogas yield. Prior works have confirmed significant biogas yield at pH ranging from 6.8 to 7.2. The pH of all samples was thus adjusted to 7.0 ± 0.032 and the values recorded for all ten reactors before and at the end of the experimental run. The values are given in Table 4.2. The average

pH before and after neutralisation for all runs were recorded as 7.595 ± 0.22 and 7.032 ± 0.005 respectively. The final pH measured after the end of the experiment looked similar to their respective initial pH values. The initial pH for the “Blank Accl.” and “Sample Accl.” for instance, was 7.56 ± 0.005 and 7.51 ± 0.00 respectively, not much of a difference recorded as compared to their final values of 7.51 ± 0.00 for “Blank Accl.” and 7.50 ± 0.02 for “Sample Accl.” Methanogenesis is noted to occur under alkaline conditions thus these recorded pH values confirm the presence and activeness of methanogens. However, “Control Unaccl.” recorded a contrasting final pH and it was however not surprising the abrupt cease in its biogas yield. The highly acidic value ~of 5.16 recorded for the “Control Unaccl.” indicated the end of methanogenesis process due to the inability of methanogens to survive in an acidic medium.

Table 4.3. pH values recorded for all test runs before and after neutralisation and at end of experiment.

Experimental Runs	Pre- Anaerobic Digestion		Post-Anaerobic Digestion
	<i>pH before</i>	<i>pH after</i>	<i>Final pH</i>
Blank Accl.	7.56	7.06	7.51
Blank Accl.	7.57	7.05	7.51
Control Accl.	7.65	7.03	7.38
Blank Unaccl.	7.59	7.02	7.35
Blank Unaccl.	7.56	7.05	7.29
Control Unaccl.	7.74	7.02	5.16
Sample Accl.	7.63	7.02	7.48
Sample Accl.	7.63	7.01	7.52
Sample Unaccl.	7.51	7.02	7.36
Sample Unaccl.	7.51	7.04	7.32

4.3. Biogas yield

Results of biogas produced from the anaerobic digestion of *Ecklonia maxima* residues at inoculum to substrate ratio (ISR) of 2:1 are shown in Fig 4.1. These curves show averages of the replicated samples of Controls Acclimated and Unacclimated, Sample Acclimated and Sample Unacclimated. The biogas yield plotted for all the runs excludes yield contribution from the blank inoculum.

The meaning and interpretation to these curves according to J. Filer et al [62], gives insights into the rate limiting step of the anaerobic digestion. The graph for sample acclimated depicts a readily biodegradable substrate as the production of biogas was observed right at the onset and levelled off quickly. The curve for sample unacclimated, however proved biodegradable but after a period of lagging. The lag phase indicated that hydrolysis could have been the rate limiting step in this sample.

Furthermore, sample acclimated observed a rapid biogas production of 219 ml VS/ g and 185.5 ml VS/g for sample unacclimated within the first 24 hours. The cumulative biogas produced at the end of the experiment by the sample prepared with acclimated inoculum was 6.8% more than that prepared from the unacclimated inoculum. The sample acclimated yielded a cumulative biogas volume of 1570.6 ml VS/ g of volatile solids added which differs significantly with documented data on other brown seaweeds such as works by Bird et al [63] and Hansen et al [64]. *E. maxima* in this work yielded about ~55% more than that obtained from *Laminaria sp.* recorded by Tedesco et al [65].

This significant differences in yield further confirms that the microbes affinity for the substrate as a result of the acclimatisation process and an induction of a suitable metabolic pathway as shown by Raposo et al [28].

However, “control acclimatised” recorded the highest biogas yield whereas control unacclimatised yielded least and also unexpectedly ceased production before the tenth day.

Raposo et al [25] has stated that higher inoculum concentration leads to a quick anaerobic conversion of substrates implying dully completion of test. The increased biogas production recorded for the control for acclimatised inoculum could be attributed to the upsurge in the level of concentration as it is known to affect the rate of biodegradation[25].

The abrupt cease in yield observed in sample unacclimatised indicates methanogenesis inhibition. The suspected inhibition could have resulted from improper mixing of Avicel® cellulose and inoculum because portions of the former were seen on the neck of the reactor bottle at the end of experiment. This defect may have resulted in several factors that hindered the completion of the methanogenesis. The improper mixing of the unacclimatised inoculum could have affected the intended ISR and the effects of such has been identified by Chynoweth *et al* [66]. Moreover, since the sample was improperly mixed, it implied an erroneous initial pH and thus adjustment were done on wrong conditions. In addition, there could also have been an incidence of gas escape thus the inability of the system to record the produced gas from that very test run.

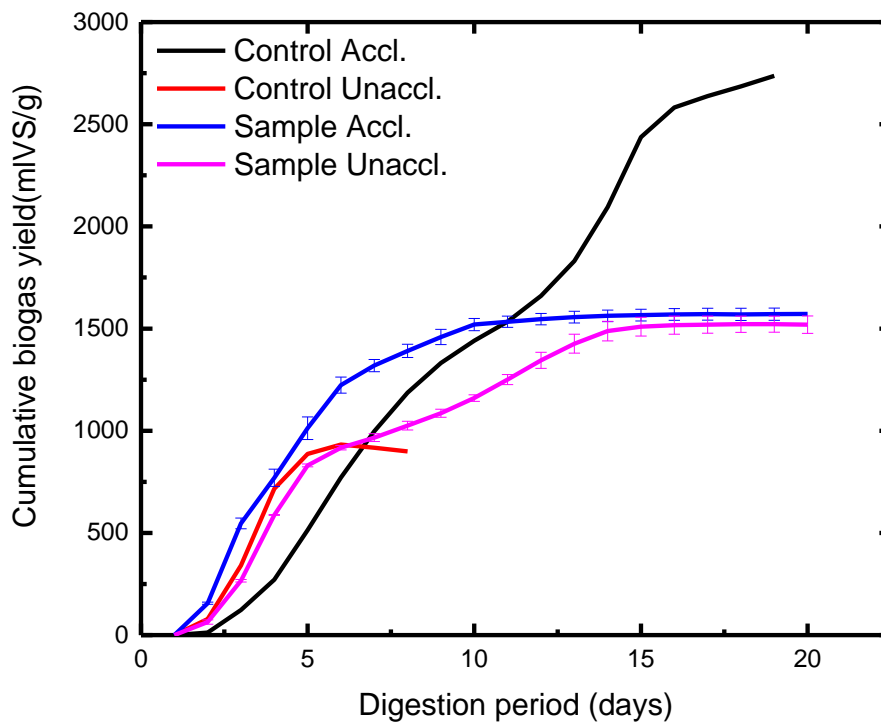


Fig.4.4. Biogas production curves for samples and positive controls

4.4. Effect of Inoculum acclimatisation on methane production

The cumulative methane yields for both samples with either acclimatised or unacclimatised inoculum are illustrated in Fig.4.2. The appearance of the graphs conforms with the typical curves as per Hansen et al [64] and Angelidaki et al [47].

Real-world yields from anaerobic digestion of fresh seaweed according to Roesijadi [19] have been testified between 140 and 400 ml CH₄ g/V_S and these values are in step with the methane yield from the residues used in this work. Cumulative methane (CH₄) from the acclimatised inoculum is highest when compared to the other test runs. This sample produced an average of 90 Nml of methane more than sample unacclimated on a daily basis. Consistent with this yield, another difference between the inocula was observed during the last week of digestion. The sample prepared from the acclimatised inoculum suddenly recorded a boost in its yield whereas the unacclimatised inoculum remained levelled. The cumulative methane

yield for the acclimatised sample was 862 ml CH₄ g /VS which is about 73% greater than the values recorded for Membere et al [67]. Substrates such as *Saccharina latissima*, *Laminaria digitata*, *Palmaria palmata* and hydrolysis leachate from brown seaweed have been used by Vanegas and Bartlett [51], Jard et al [16] and Nkemko and Murto [13] respectively . These samples recorded 340 ± 48, 184, 257 ± 22 and 110 of litres kilogram of methane per volatile solids added (LKg/Vs) respectively. Comparison of these values to that obtained on *E. maxima* proves the superiority over their counterparts.

In G. Hansson [68] work, fresh seaweed was used also, however, the highest methane achieved was 480 (L CH₄ Kg/Vs). Tedesco and Daniels [11] evaluated the geographical and seasonal influence on *Laminaria species* yield and this study produced some of the highest levels of methane recorded (523 ml CH₄ g/Vs).

Figure 4.2 presents the amount of methane produced using inoculum acclimatised with *E. maxima* which differs significantly from the sample with no prior acclimatisation and this confirms the effect acclimation have on biogas generation. The average methane yield is recorded as 671.02 ± 60.0 ml VS/g for acclimatised sample and 576. ± 62.4 ml VS/ g for sample with unacclimatised inoculum.

In summary, this trend observed indicates a critical influence of acclimation on the methanogenesis of *E. maxima*.

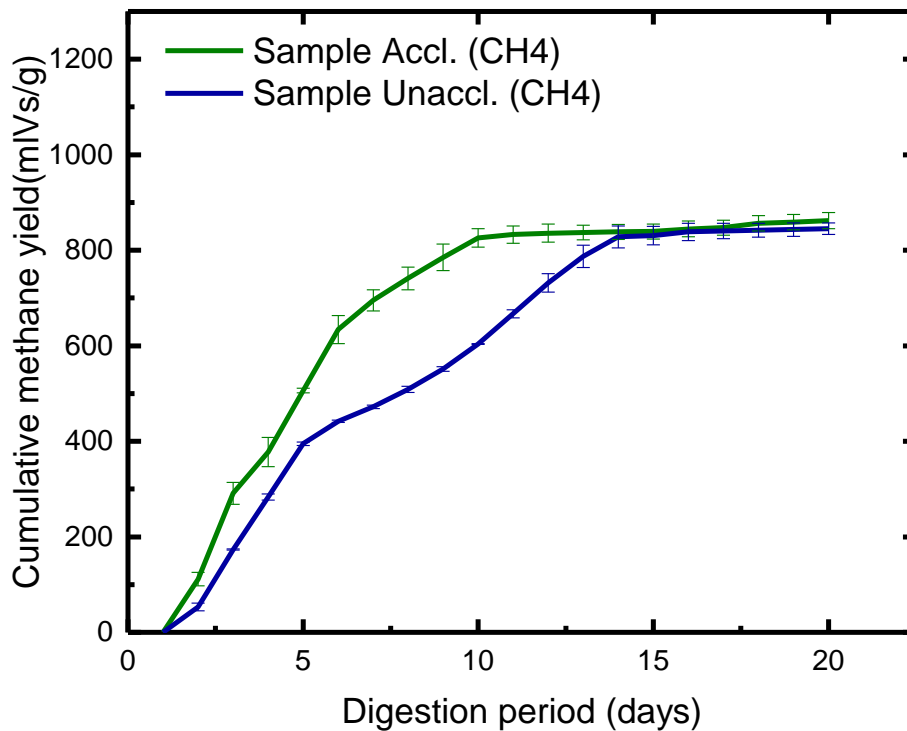


Fig.4.5. Cumulative biogas and methane yield

4.5. Kinetic modeling biogas yield using modified Gompertz model

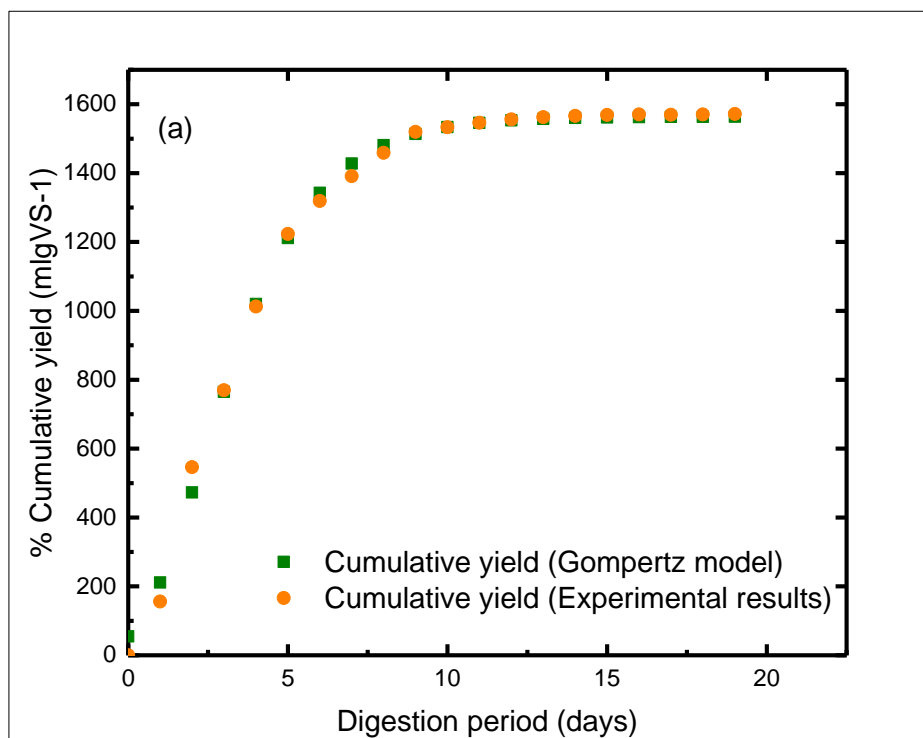
The modified Gompertz equation was used to fit the cumulative biogas produced from the acclimatised samples. Kinetic constants (A , μ , λ) were determined using non-linear regression. The results of the estimated kinetic parameters based on the modified Gompertz equation are indicated in Table 4 below. Membere & Sallis, 2018 have shown that the constants calculated predict the biogas potential (A), maximum biogas production rate (μ) and the duration of lag phase or the minimum time to produce (λ). The results obtained from the model and experimental results are plotted as shown in Fig. 4.6.

The biogas potential (A) and production rate (μ) of 1564.0 ml/gVS and 296.2 ml/day respectively recorded for the acclimatised sample were higher compared to that of the unacclimatised sample. The value of the lag time λ , however, was lower than that of the

unacclimatised sample. This meant that, the microorganisms thrived better under the acclimatised condition which led to the production of biogas at a faster rate. The unacclimatised sample produced smaller biogas yield and also at the slower rate.

As can be seen from Table 4.4 and Fig.4.6, the acclimatised sample qualified as the best substrate due to the higher values of A , μ and the lowest value of λ .

The kinetic constant, A , in this study compared to other studies was higher. This means that the biogas yield potential of the brown seaweed adopted in this study was higher compared with other sources of marine biomass. The substrates used, unlike in other studies did not contain toxic materials that could have hindered bacterial activities. The lag phase however recorded was between 0.4 - 3.4 days which is relatively higher than prior studies reported in literature. The lag was due to the presence of relatively high amounts of the lignocellulose content in the inoculum.



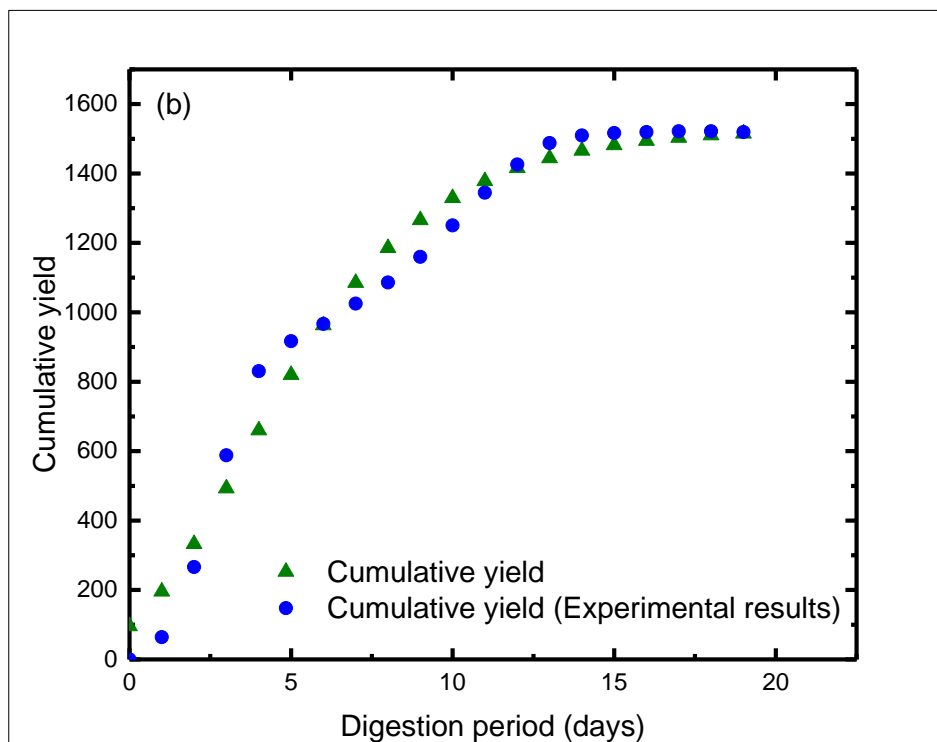


Fig.4.6 (a-b). Comparison of experimental data the modified Gompertz model

The R-squared (R^2) value which denotes the determination coefficient was recorded as 0.99 and 0.98 respectively for acclimatised and unacclimatised samples. This shows a good fit of the predicted values to the experimental data

Table 4.4. Results of kinetic modeling using the Gompertz model

Samples	A (mL/gVS)	μ (mL/day)	λ (day)	R^2
Acclimatised inoculum	1564.0	296.2	0.4	0.99
Unacclimatised inoculum	562.7	60.3	3.4	0.98

4.6. Gas Chromatography Analysis of Biogas Yields

The end products of anaerobic digestion are in greater percentages of methane (CH_4) and Carbon dioxide (CO_2). The carbon dioxide content by reason of its negative influence on the

environment is desired to be as low as possible. The AMPTS II by virtue of its design fixes or removes the carbon dioxide content produced. Regardless, the original percentage is intrinsic of a sample, unfixed biogas samples were then taken to characterize the level of CH₄ and carbon dioxide(CO₂)in the substrates used. A chart representing the respective CH₄ and CO₂ of the runs is given by Fig. 4.3. The sample acclimatised recorded the highest CH₄ content of $\sim 55 \pm 0.8$ and a CO₂ percentage of $\sim 25 \pm 2.6$. The least CO₂ percentage was however recorded by Blank acclimatised with a value of 18 ± 1.9 .

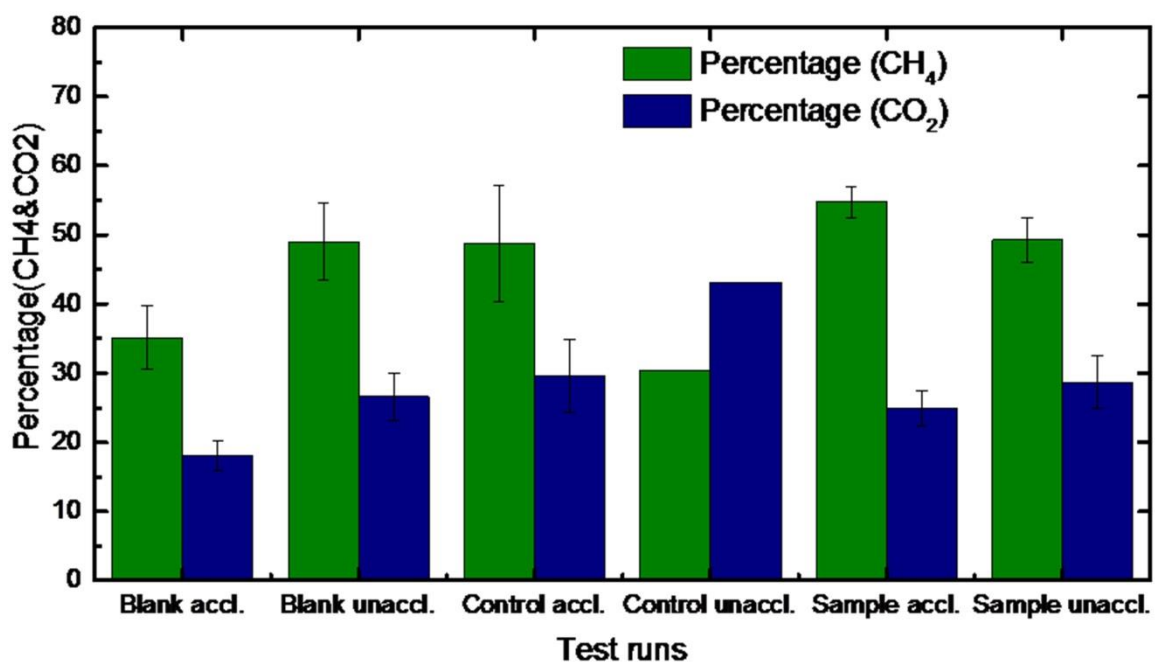


Fig.4.7. Gas chromatography results for methane and Carbon dioxide percentage

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

The production of biogas from *Ecklonia maxima* has been confirmed to be superior over other brown seaweed species. The yield further improved significantly upon acclimatising inoculum to residues of the *Ecklonia maxima*. The biogas produced from the acclimatised sample had ~55% of its constituents to be methane (CH₄) and ~25% carbon dioxide (CO₂) which corresponds to the biogas composition standards have established. Acclimatising inoculum prior to anaerobic digestion has proven to be very useful and effective regarding the rate and cumulative quantities produced. The modified Gompertz equation predicted a biogas potential (A) of 1,564.0 ml/gVS at a production rate (μ) of 296.2 ml/day for the acclimatised sample which is three times greater than that of the unacclimatised sample. The Gompertz equation further confirmed that acclimatising the inoculum enables easy degradation of the feedstock and rapid biogas yield since the model predicted a minimum production time (λ) of 0.4 days and 3.4 days for the unacclimatised sample. The characterization carried out on the feedstock and the end product have shown them promising for use as sustainable energy option.

5.2. Recommendations

Anaerobically digesting *Ecklonia maxima* under thermophilic conditions should be investigated to compare most optimum conditions for very improved yields. The microbiology of the inoculum and seaweed should be studied to understand the exact methanogens present during acclimatisation in order to devise means of introducing nutrients when necessary for higher yields. Finally, advanced purification methods could be studied, modified and employed to further purify biogas produced from *Ecklonia maxima*.

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