MICROALGAE BIOMASS AS FERMENTATION FEEDSTOCK

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By

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Abstract

The search for alternative energy is as a result of pollution generated by the utilization of fossil fuel. Bearing in mind the increase in demand which exceeds supply, alternative energy must reduce the carbon foot print in order to relieve use of fossil fuels. Biogas generation from wastes is an old technology that has been in existence for decades. This same concept was behind the development of the integrated algae ponding system (IAPS), where the use of microalgae biomass is adopted for waste water treatment and, anaerobic digestion which is a component of the IAPS, simultaneously generates biogas. The biogas from the IAPS was quantified in order to evaluate efficiency of the system and the anaerobic fermentation pit was also simulated in the laboratory to optimize biogas production using microalgae as co-fermentation feedstock. Microalgae biomass was evaluated as potential feedstock for ethanol fermentation and the use of biogas was investigated as an alternative transportation fuel. In an IAPS substantial biomass is produced on an annual basis. For effective treatment of waste water and efficient nutrient removal continuous harvest of the biomass is required.

In the present study, water treatment efficiency of the EBRU IAPS was determined by carrying out a series of tests to investigate the decline in nutrient content from port of influent entry to effluent discharge. There was more than a 60% reduction in nutrient content with a concomitant increase in biomass and growth rate of 0.25 g/L. Biogas generated from the IAPS was quantified using a flow meter and the composition determined by gas chromatography. Methane which is the principal constituent of biogas was 75% (±SD, n=10) and 2.34 $m^3.d^{-1}$ was measured as biogas yield from the EBRU IAPS. The study also investigated the use of the excess microalgae biomass as a fermentation feedstock for ethanol production and as a co-substrate in order to increase biogas yield from the system. Positive results were achieved for ethanol production from microalgae although yield was generally low. About 385 $mg.g^{-1}$ of ethanol was recovered when glucose was used as substrate, where as only 115 $mg.g^{-1}$ of ethanol was recovered with microalgae as substrate. Suitability of microalgae as feedstock for ethanol generation and biogas generation was determined by characterisation which involved estimation of the carbohydrate,

protein and lipid content, and analysis of the C, H, O, N and S content. Laboratory fed batch reactors simulated the anaerobic digestion process in order to study the effect of microalgae biomass as co-substrate for biogas generation. The fermenters were inoculated with an active consortium obtained from the Makana municipal waste water works and microbial studies were carried to confirm the presence of the anaerobic consortium. Different pre-treatments (concentrated, rupturing and freeze-drying) were used to disrupt the microalgae prior to introduction into fermenters in a ratio of 3:1. COD, TC, TOC, SO_4^{-2}, and TN analyses were carried out to monitor nutrient depletion in the system, and biogas generated by the system was quantified by volumetric analysis and the gas composition determined. Statistical analysis (ANOVA) was used to test for significant difference pre and post addition of microalgae. In the most effective fermenter, biogas production was at an average of 394 $mL.d^{-1}$ and CH_4 ratio in the biogas increased by over a 100%. Theoretical methane potential of the IAPS and the Makana municipal waste water works treating 5 $ML.d^{-1}$ of domestic waste was determined using the empirical formula of waste water and shown to yield 1,037,342.40 m^3/yr. The projected biogas yield from this system was used to evaluate its potential use as transportation fuel. In total, 198,673.55 m^3 of biogas was estimated to be required to fuel the Rhodes University's fleet of vehicles, with a residual biogas stream of 838,668.85 m^3.

It was also demonstrated in the present study that renewable energy sourced from biomass has the potential of supplanting the use of fossil fuel resulting in less pollution leading to a cleaner and healthier environment.

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I dedicate this work to my sweet mom who is always there to catch me when am falling. You always seek and find that which is good in every human and every situation. Mom, I love you so much and it is my prayer that Allah grant me the opportunity to show you how much I appreciate you. To my husband and best friend who always keeps me from falling apart during the difficult hours of my research lab work as well as during the endless hours of writing my thesis. Temi, thank you so much for being so caring, and for always being there during my most trying moments. To Fawaz, my son, who had to bear with my absence during the writing of my research work thesis. I will make it up to you my sweetie. I love you and you are my divine treasure. I dedicate this thesis to you with all my heart. My profound gratitude also goes to my mentor, Mr. Adewale Koiki, thank you so much for your support.

My family, I owe every one of you much and the dedication I write to you is merely a small reflection of how much I cherish everyone. The Abdulrazaks, Nike, Shola, Salma, Mustapha and Maryam.

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Equations

List of Acronyms

AIWPS – Advanced integrated wastewater pond systems

BOD – Biochemical Oxygen Demand

BSA – Bovine Serum Albumen

CH_4– Methane

CO – Carbon monoxide

CO_2 –Carbon dioxide

COD – Chemical Oxygen Demand

C/N – Carbon/Nitrogen

dH_2O – Distilled water

EBRU– Institute for Environmental Biotechnology Rhodes University

FID – Flame Ionization Detector

GC – Gas Chromatography

GHG – Green house gases

GJ/t – Gigajoule/tonne

HRAP – High Rate Algal Pond

HRAOP – High Rate Algal Oxidation Pond

HRT – Hydraulic Retention time

H_2S – Hydrogen Sulphide

ICP-AES – Inductively Coupled plasma-Atomic Emission Spectrometry

IC – Inorganic Carbon

IAPS – Integrated Algal Ponding Systems

MJ – Mega joules

m^3 – Cubic meter

mL – Milliliter

mm – Millimeter

MWh - Megawatts

NDIR- Non dispersive infrared sensor

nm – Nanometer

N_2 – Nitrogen

PE – Person equivalent

PFP – Primary Facultative Pond

SEM – Scanning Electron Microscopy

SO_4^{2-} - Sulphate

TC – Total Carbon

TCD –Thermal Conductivity Detector

TN – Total Nitrogen

TOC – Total Organic Carbon

VFA – Volatile Fatty Acids

v/v – Volume/Volume

WHO – World Health Organisation

WRC – Water Research Commission

Chapter 1: Literature Review

When one considers fossil fuel, the first thing that comes to mind is: 'How long is it going to last?' Fossil fuel formation takes millions of years and as the rate of new discovery is much slower than that of production, demand exceeds supply as depicted in Figure 1-1.

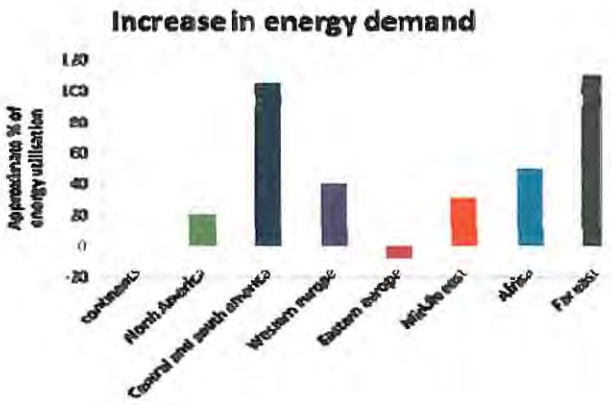


Figure 1-1: The estimated rise in energy demand from 1980 to 1998 (Rocky, 2008).

The use of fossil fuel cannot be completely eliminated; however, it can be supplemented by seeking alternative and more sustainable sources of energy. These alternatives may lessen the current global pressure on fossil fuel. The urgent need to seek a cleaner form of energy is due to the imminent fear of fossil fuel exhaustion and also the contribution of fossil fuel combustion to climate change.

Ozone serves as a protective stratum around the earth. It absorbs harmful ultraviolet rays from the sun, and its current depletion is mainly due to accumulation of greenhouse gases and chlorofluorocarbons. Human activities such as burning of fossil fuels for the generation of electricity and transportation coupled with increased deforestation have resulted in higher greenhouse gas emissions, exceeding the accepted threshold leading to dangerously high levels of 385-390 ppm carbon dioxide (CO_2). Carbon dioxide is one of the most important and powerful greenhouse gases and levels have steadily risen since 1850, purportedly due to the burning of coal, petroleum and natural gases as presented in Figure 1-2.

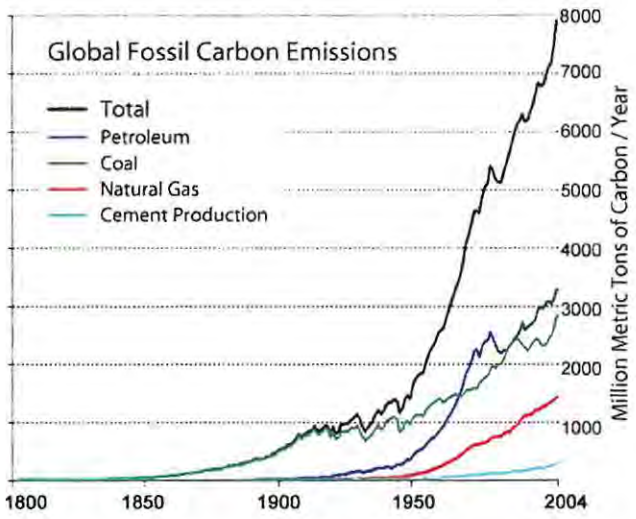


Figure 1-2: The steady increase in carbon emissions through fossil fuel usage from 1800 to 2004 (Thorpe Mak, 2010).

In order to 'save our planet' we need an environmentally friendly, sustainable, renewable, economical and carbon-neutral alternative to fossil fuel. Furthermore, the growing interest in alternative forms of energy is so that some communities and countries as a whole can depend less on fossil fuels and reduce resulting pollution caused by fossil fuel usage to our immediate environment.

One renewable form of energy that can be easily obtained is methane (CH_4) by digesting wastes anaerobically. Anaerobic digestion is the conversion of organic matter to its most oxidised and reduced states which include CH_4 and CO_2 (biogas). This form of energy can be generated by various types of waste water treatment facilities, some instances are the trickle bed filters, package plants and batch fermenters, and integrated algal ponding systems (IAPS). In some parts of the world like Scandinavia, South East Asia, India and even parts of Canada, the biogas production process has been optimised to produce maximum CH_4 for cooking, heating and transportation. Ethanol is another form of energy that can be generated by fermentation of biomass. Examples include corn to ethanol and sugar cane to ethanol (Harun *et al.*, 2010). Unfortunately most feedstock such as corn and cane sugar compete with food crops for arable land rendering this an unsuitable source of ethanol (Harun *et al.*, 2010). A potential alternative is microalgae biomass obtained from different cultivation facilities including the high rate algal

ponds (HRAP) associated with wastewater treatment. A good example here is the HRAPs of the IAPS (Green *et al.*, 1995). Microalgae do not compete with crops for arable land, can be cultivated in any habitat and are known to contain high amounts of carbohydrate for successful fermentation to ethanol as well as other alcohols which can serve as liquid fuels. Integrated algae ponding systems are a sustainable process that would not only resolve the problems associated with domestic waste effluent treatment and disposal but could potentially reduce fossil fuel dependency. Residual sludge from the anaerobic digester can also serve as a fertiliser and the biogas produced is carbon-neutral. The biologically generated CH_4 has a high calorific value (50 MJ/kg), is non-toxic, can be used to generate power as well as facilitate transportation and heating. Similarly, bioethanol which is an important solvent and precursor for other products can be used as a fuel for heating, lighting and transportation (e.g. Brazil).

In the present thesis, experiments were carried out to confirm the production of CH_4 by an IAPS system, and to optimise CH_4 yield by investigating the potential of microalgae to serve as a co-feedstock. Additionally, fermentation of microalgae biomass to ethanol was studied. It is hoped that the outcome of the work described in this thesis will pave the way to a better understanding and utilisation of waste water treatment facilities, to provide a sustainable supply of renewable energy in addition to clean waste water treatment.

1.1 Introduction

The commotions in the international oil market during the 1970s signalled the inevitable depletion of fossil fuel energy resources and reinforced a worldwide interest in energy conservation, increased efficiency and the development of renewable energy resources. These factors have been influential in the development of innovative and energy efficient waste water treatment systems (Holdren, 1987). Oswald *et al.*, (1963) developed the advanced integrated wastewater pond systems (AIWPS) to address the issue of wastewater treatment in conjunction with energy generation (Green *et al.*, 1996). This intensively engineered application is found to be attractive in low income communities, small organisations, developing and underdeveloped countries. The water research commission (WRC) in 1990 funded a series of studies for the application of Integrated Algae Ponding System (IAPS), which is similar to the AIWPS, to a range of waste water treatment problems in South Africa (Rose, 2002). The project officially started in 1994, where the AIWPS technology transfer exercise was executed under the supervision of Professor Oswald and Dr Bailey Greene both from the University of California. The AIWPS design was implemented in the form of IAPS at the Institute for Environmental Biotechnology Rhodes University (EBRU) Experimental Field Station in Grahamstown. This series of studies demonstrated the principles and feasibility of IAPS technology as a low cost sewage treatment system for South African communities (Rose, 2002).

1.2 Integrated algae ponding system (IAPS) as a waste water plant

The IAPS is a bioprocess that relates generally to wastewater treatment, by initially applying simple liquid waste treatment which is safer and less expensive than current methods used for domestic waste water treatment (Oswald, 1989; Green *et al.*, 1995). The IAPS converts domestic organic wastes via normal algae photosynthetic activity into a protein rich biomass. This biomass indirectly generates a high quality effluent when compared to that of conventional waste stabilisation pond water treatment plants. Integrated algae ponding and the traditional pond systems comprise of primary sedimentation, flotation, fermentation, aeration, secondary sedimentation, nutrient removal, effluent storage and final liquid disposal. However, IAPS do not require daily sludge management and sludge retention time is of the order of decades (Oswald, 1990; Oswald, 1991). The IAPS consists of a minimum of four ponds in series as shown in Figure 1-3. The reasons for this are:

- Several unit processes involved require distinct environments which cannot be overlapped;
- Use of four ponds in series with properly designed transfer structures prevents all conceivable short circuiting of influent to effluent;
- In areas where annual evaporation generally exceeds annual precipitation, it is necessary to divide the ponding area into sectors to maintain sufficient depth in order to avoid drying (Oswald *et al.*, 1994).

Each pond is designed to best perform one or more of the basic treatment processes. The series of ponds and a description of the function of each are as follows;

1. Primary facultative pond

The facultative pond has an aerobic surface and an anoxic internal fermentation pit for sedimentation and digestion (Figure 1-3), for optimal methane generation and as protection to oxygen intrusion (Oswald, 1990). Facultative ponds are designed to foster three distinct microbial consortia; the obligate microbes found in the deep anaerobic strata overlain by those in the deep facultative strata and lastly those overlain by aerobic surface strata (Oswald *et al.*, 1994). Methane generation was first recognized as one of the most efficient ways to manage and recover waste organic matter by Oswald *et al.*, (1994).

2. Fermentation pit

Due to the large pit volume, settled solids ferment to a point where only inorganic ash remains, hence sludge removal is seldom required (Bouwer and McCarty, 1983). Methane generation is the principal mechanism for biochemical oxygen demand (BOD) removal in the fermentation pit. Raw waste is introduced near the bottom of the pit and since these are deep and are loaded with low up-flow velocity, most of the solids remain within the pit (Oswald, 1991; Oswald *et al.*, 1994). The fermentation pit is located under the PFP in order to avoid premature discharge of effluent into the High Rate Algae Ponds (HRAP).

The gas produced in the fermentation pit can be collected using a surface gas collector or submerged gas collector (Oswald *et al.*, 1963; Green *et al.*, 1995). The advantage of a surface gas collector is that the biogas gets scrubbed of CO_2 while rising to the surface, thereby

increasing CH_4 concentration by more than 50%. By the time the gas gets to the pond surface, the ratio of the CH_4 in the biogas will be about 86% (Oswald *et al.*, 1963; Green *et al.*, 1995).

3. High rate algal pond (HRAP)

Effluent from the facultative pond flows under gravity directly into one or more HRAPs linked in series, passing through a connecting pipe. The HRAP is shallower than the facultative pond with a shorter retention time of 2 – 4 days and it produces much more dissolved oxygen since it is aerated (Green *et al.*, 1995). High rate algae ponds are designed to enhance the symbiotic relationship between microalgae and aerobic bacteria, each utilizing the major metabolic products of the other (Green *et al.*, 1995). An example of this relationship is utilization of the O_2 produced by microalgae through photosynthesis by aerobic bacteria to oxidize biochemical oxygen demand (BOD), and the microalgae subsequently uses the CO_2 and nutrients released through bacterial oxidation (Green *et al.*, 1995). HRAPs are powered using a paddle wheel as shown in Figure 1-3 which enables proper circulation of microalgae and aerobic bacteria. Algae elevates the pH to approximately 9.2 which is believed to eliminate *Escherichia coli* and most other disease causing bacteria (Green *et al.*, 1996).

4. Algal settling pond

The effluent from the HRAP decants into the algal settling pond; concentrated algal slurry can be harvested from the settling ponds to avoid accumulation in the HRAPs (Nurdogan, 1988). Metting and Pyne (1986) showed that dried algal sludge harvested from the HRAPs of waste water facilities was rich in N, K and P, therefore making it an excellent fertilizer for fast growing crops (Metting and Pyne, 1986). Water from the settling pond is considered to be sufficiently low in BOD and suspended solids such that it can be used for irrigation or released directly into naturally occurring water bodies (Sheikh *et al.*, 1986; Oswald, 1989).

5. Maturation pond

The final effluent from the algal settling pond passes into the maturation pond. Bacteriological guidelines recommended by the World Health Organization (WHO) for agricultural re-use is met by maturation pond effluent. This pond ensures further disinfection of water regarding human health risk by providing a longer incubation period (Shuval, 1988).

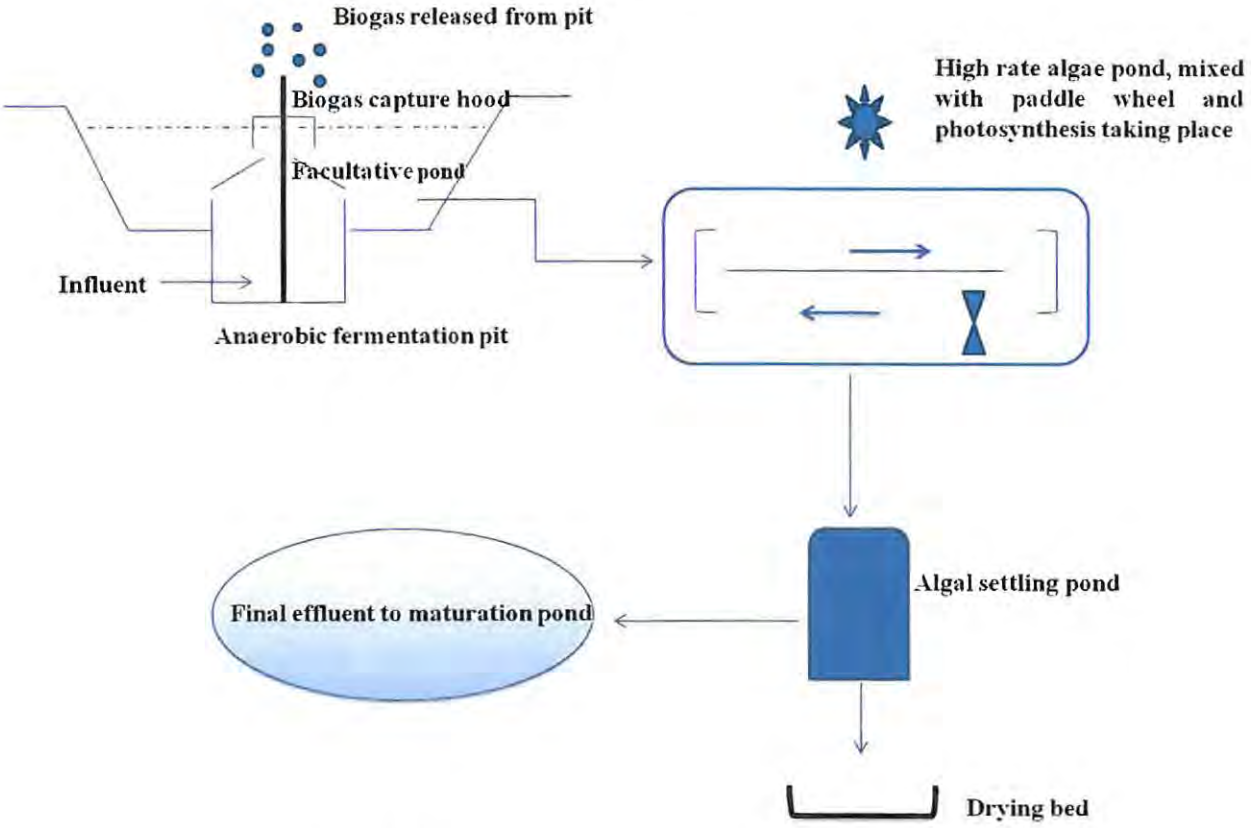


Figure 1-3: Schematic of the principal unit operations associated with the IAPS Plant design constructed at the Institute for Environmental Biotechnology Rhodes University experimental field station, Grahamstown Disposal Works (Rose, 2002).

1.3 Anaerobic digestion of domestic wastes to methane

A wide range of feedstocks including industrial and municipal wastewater, agricultural wastes, food industry wastes, domestic wastes and plant residue can be subjected to anaerobic digestion to generate biogas (Mata-Alvarez, 2002) as presented in Appendix 1-A.

The production of biogas through anaerobic digestion offers significant advantages over other forms of waste treatment.

- Less sludge is produced in comparison to aerobic treatment technologies;
- Effective pathogen removal (Bendixen, 1994);
- High degree of compliance with many national waste strategies implemented to reduce the amount of biodegradable waste entering landfill;
- The slurry produced (digestate) is an improved and clean form of fertilizer in terms of its availability to plants (Tafdrup, 1995);

- A source of carbon neutral energy is produced in the form of biogas

Biogas produced from sewage digesters usually contains 48 - 65% CH_4, 36 - 41% CO_2, up to 17% N_2, < 1% O_2, 32 -169 mg/L H_2S and traces of other gases (Rasi *et al.*, 2007).

Both CO_2 and CH_4 are potent greenhouse gases and possibly 18% of global warming is thought to be caused by anthropogenically derived CH_4 emissions (Ghosh, 1997). CO_2 released through natural mineralization is considered neutral in greenhouse gas terms as the carbon has been recently removed from the atmosphere by plant uptake to be released again as part of the carbon cycle.

Incorporation of the HRAP into wastewater treatment facilities results in production of considerable amount of biomass. Microalgae cultivation is mainly dependent on the species and the product needed from the culture (Chisti, 2007). Microalgae can be cultivated intensively (photobioreactors) or extensively (raceways). The raceways are artificial, minimally controlled and are used to simulate natural water bodies like ponds, lakes and lagoons. These open ponds are mainly used for large scale algae biomass production coupled with improvement of water quality (Chinnasamy *et al.*, 2010). The ponds or raceways are powered by paddle wheels to ensure adequate mixing and growth of the microalgae biomass confined to a closed loop recirculation channel (Chisti, 2007). The raceways are designed to promote the symbiotic relationship between the microalgae and aerobic bacteria as described by Equations 1-1 and 1-2.The microalgae take up CO_2 released by aerobic bacteria as a result of oxidation of soluble and biodegradable BOD to release O_2 through photosynthesis as shown in Figure 1-3 (Oswald, 1990; Green *et al.*, 1995).

$CO_2 + H_2O \longrightarrow CH_2O + O_2$ -------- **Equation 1-1**

$CH_2O + O_2 \longrightarrow CO_2 + H_2O$ -------- **Equation 1-2**

1.3.1 Anaerobic Digestion Pathway and Methanogenesis

Methanogens belong to the *Euryarchaeota* family that produces CH_4 as an integral part of their energy metabolism. The key genera of this class are *Methanobacterium, Methanocaldococcus and Methanosarcina* and some of the defining characters are listed in Appendix 1-B (Madigan and Martinko, 2006). Although methanogens are mostly coccoid and rod shaped they show a variety of morphologies. Appendix 1-B displays the taxonomy and is based on both phenotypic

as well as phylogenic analyses, with several taxonomic orders now recognized. Methanogens also show diversity in their cell wall structures:

- Pseudopeptidoglycan walls of methanobacterium species and relatives.
- The methanochondroitin (because of its structural resemblance to chondroitin; the connective tissue polymer of vertebrate animals) walls of *methanosarcina* and relatives.
- The protein walls of *methanocaldococcus* species.
- The glycoprotein walls of *methanoplanus* species.
- S- Layer walls of *methanospirillum* (Madigan and Martinko, 2006).

Methanogens are obligate anaerobes, so strict anoxic conditions are necessary for culturing. As a result of the removal of carbon, organic bound non-carbon compounds are released in their soluble inorganic form during the anaerobic process (Pain and Hepherd, 1985). There are four steps defined in anaerobic digestion, each attributed to different metabolic groups. The first three steps are hydrolysis, acidogenesis, and acetogenesis as presented in Figure 1-4. Different bacterial populations perform each step. The final step is methanogenesis shown in Figure 1-4 is performed by a class of *Archaea* termed the methanogens (Griffin *et al.*, 1998).

The exothermic reaction for this process is shown in Equation 1-3(Horan, 1991; Maier, *et al.*, 2000)

$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O\ \Delta G = -130$ kJ/mol **---Equation 1-3**

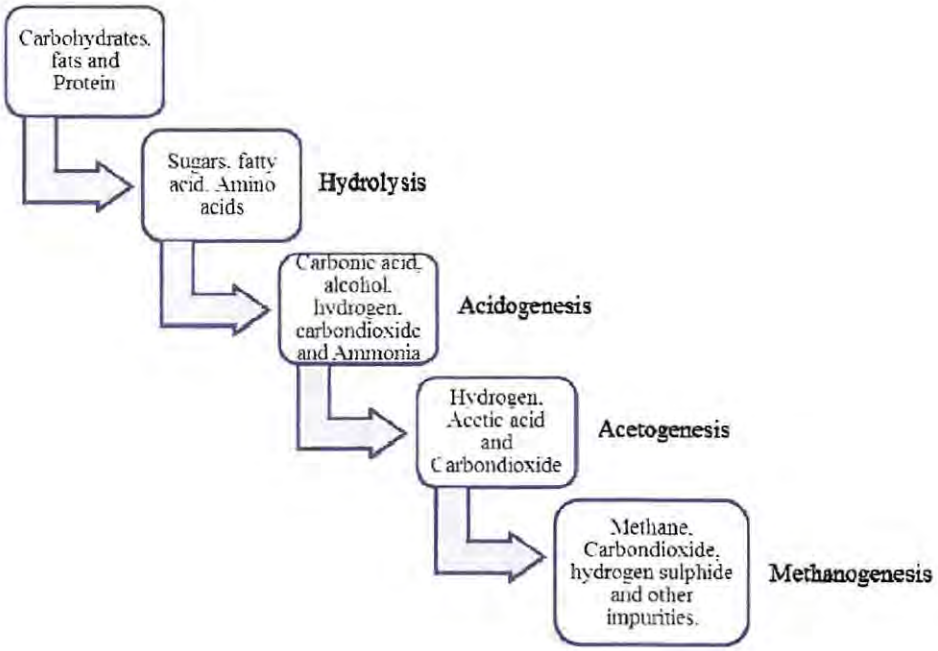


Figure 1-4: Anaerobic digestion pathways, showing the breakdown of complex organic compounds to intermediate products and to final end products (Gujer and Zehnder, 1983; Weiland, 2010).

1.4 Ethanol Fermentation

Yeast microbes were one of the earliest domesticated organisms and have been used for fermentation and baking throughout history (Gray *et al.*, 2006). Yeasts are chemoorganotrophs as they use organic compounds as a source of energy and do not require sunlight to grow. Yeasts and in particular *Saccharomyces cerevisiae* are the recommended catalysts in ethanol fermentation processes, requiring O_2 for aerobic cellular respiration as either an obligate aerobe or facultative anaerobe (Barnett, 1975).

The use of algae as a substrate for the bio-generation of alcohols for energy has not been fully investigated. Microalgae could potentially substitute the food sources originally used for fermentation to produce liquid fuel, these microalgae are very easy and inexpensive to cultivate, and do not contain lignin (Cowling, 1975; Chynoweth *et al.*, 1985; Tong *et al.*, 1990). The cellulose component of algae is easier to convert to alcohol since it is comprised of hexose sugars unlike the hemicellulose component which comprises pentose sugars (Doi and Kosugi, 2004; Lynd *et al.*, 2005). These particular components of microalgae can be converted to bioethanol with high yield and productivity using *Saccharomyces cerevisiae* as the biocatalyst

(Gray *et al.*, 2006). A very good example of hexose sugar is glucose which when fermented by yeast yields 2 moles of ethanol and 2 moles of CO_2 (Equation 1-4)

$C_6H_{12}O_6 \longrightarrow 2(CH_2CH_3OH) + 2(CO_2)$ --- **Equation 1-4**

1.4.1 Enzymatic depolymerisation of cellulose and hemicellulose components of algae

Cellulase comprises three types of enzymes;

a) Endoglucanases

b) Exoglucanases

c) β- glucosidases

Endoglucanase cleaves internal β–1, 4 glycosidic bonds, exoglucanases act on the reducing and non-reducing ends of cellulose chains to release short chain cello-oligosaccharides (cellobiose). Lastly β–glucosidases hydrolyse soluble cello-oligosaccharides to glucose as shown in Figure 1-5 below (Doi and Kosugi, 2004; Lynd *et al.*, 2005).

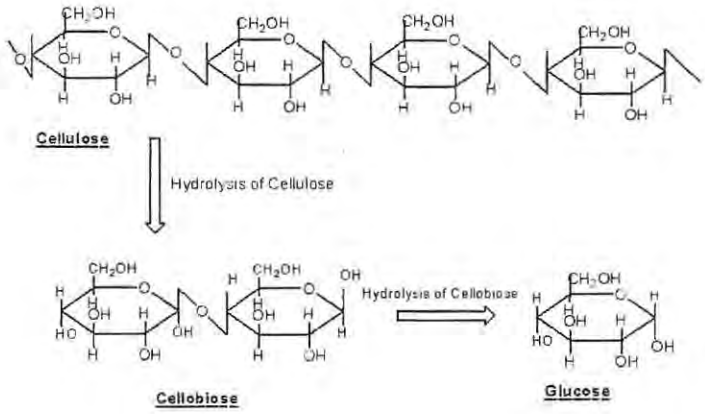


Figure 1-5: Hydrolysis of cellulose by cellulase to cellobiose and eventually glucose (Doi and Kosugi, 2004; Lynd *et al.*, 2005).

1.5 Microalgae biomass as feedstock for biofuel

1.5.1 Brief introduction to microalgae

Microalgae are either unicellular or multicellular; lack roots, stems and leaves; and are photosynthetic organisms with chlorophyll as the principal primary pigment (Lee, 1980; Falkowski and Raven, 1997). The mode of nutrition in algae can be autotrophic, heterotrophic or mixotrophic. Autotrophic algae utilise inorganic compounds as their carbon source and are either chemoautotrophic (oxidising inorganic compounds for energy) or photoautotrophic (using sunlight as the source of energy). The latter algae convert solar radiation and CO_2 into ATP and O_2 which are used to produce energy for growth (Zilinskas Braun and Zilinskas Braun, 1974; Lee, 1980). Heterotrophic algae require an external source of organics and nutrients, like glucose. Mixotrophic algae have the ability to grow by photosynthesis and by acquisition of organic nutrients (Lee, 1980).

Microalgae have the ability to fix CO_2 and accumulate high contents of stored polysaccharides in the form of starch coupled with an ability to reproduce rapidly, grow under harsh conditions (Chisti, 2007) as well as adapt to various environments. These sunlight-driven microorganisms are useful in different bioremediation applications (Brennan and Owende, 2010). These advantages make microalgae with little or no genetic manipulation a safe and preferential potential feedstock for bio-ethanol production (Choi *et al.*, 2010) while the absence of lignin makes this renewable energy source readily convertible to biogas by anaerobic digestion (Vergara-Fernández *et al.*, 2008).

1.5.2 Biochemical composition of microalgae

The major components in microalgae are C, O, H, N, P and a well-accepted generic molecular formula for microalgae biomass is $C_{106}O_{44}H_{181}N_{16}P_{01}$ (Grobbelaar, 2004). Microalgae apart from micro nutrients like Fe, Co, Zn, contain lipids, carbohydrates, proteins and nucleic acids in different proportion depending on species and cultivation conditions (Grobbelaar, 2004) (Appendix 1-C). Microalgae protein content ranges from 6 - 52%, carbohydrate from 5 - 23%, and lipid from 7 - 23% (Brown *et al.*, 1997). High proportion of protein is characterized by a low C/N ratio.

1.5.3 Microalgae as feedstock for fermentation

The anaerobic digestion of microalgae was first mentioned by Golueke *et al.* (1957) when digestion of algae biomass for the production of biogas was studied (Sialve *et al.*, 2009). Golueke and Oswald (1957) proposed an integrated process associating the production of microalgae biomass in raceways for the treatment of wastewater with anaerobic digestion of the resultant microalgae biomass for energy recovery (Sialve *et al.*, 2009). Table 1-1 shows experiments that were carried out to demonstrate anaerobic digestion of microalgae biomass by different researchers (Sialve *et al.*, 2009).

Table 1-1: Experiments which involve the anaerobic digestion of microalgae to generate CH_4 carried out by some renowned researchers.

Fermenter	Substrates	CH_4 yield (%volume)	Hydraulic retention time (HRT)	T (^{0}C)	Researchers
11 L batch fermenter	Algae sludge (*Chlorella* and *Scenedesmus*); Algal biomass; *Spirulina* and *Dunaliella.*	62 – 72	3 – 30 d.	35 - 50	Golueke *et al.*, 1957; Chen, 1987
2-5 L Continuous stirred-tank reactor.	Dried *Tretraselmis*; fresh *Tretraselmis*; Dried *Tretraselmis*+ NaCl 35g/L;	72 -74	14 d.	35	Asinari Di San Marzano *et al.*, 1982
5 L batch fermenter	*Chlorella vulgaris*	68 -75	64 d.	28 - 31	Sanchez and Travieso, 1993
10 L Semi-continuous (daily fed)	*Spirullina maxima*	68 -72	33 d.	35	Samson and Leduy, 1982
2 L fed batch reactor	*Spirullina maxima*	46 -76	5 – 40 d.	15 - 52	Samson and Leduy, 1986
4 L Continuous stirred-tank reactor	*Chlorella* and *Scenedesmus*	69	10 d.	35	Yen and Brune, 2007

The cell wall of microalgae represents a principal barrier which an "attacker" must disrupt and depending on biochemical composition, biodegradability of microalgae can be low. The cell wall is the major determinant of resistance to microbial decomposition even in natural environments (Gunnison and Alexander, 1975). The major concern stated in the literature about anaerobic

digestion of microalgae is high protein content which can lead to high NH_4 release (Tong *et al.*, 1990) and high Na concentrations in the case of marine algae.

1.6 Factors affecting biogas yield in an anaerobic digester

- **Effect of Chemical Oxygen Demand (COD) level**

The Chemical oxygen demand (COD) content describes the amount of O_2 needed to completely oxidize waste under aerobic conditions (Angelidaki and Sanders, 2004). COD is used as a measure of the O_2 equivalent of the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidant (APHA, 2002; Angelidaki and Sanders, 2004). COD is usually used in the description of waste water and it gives a good first impression of the strength of the waste, high COD results in high CH_4 (Angelidaki and Sanders, 2004). Table 1-2 shows how an increase in COD results in increased CH_4 yield (Angelidaki and Sanders, 2004).

Table 1-2: COD and CH_4 yield in some organic materials

Substrates	COD-g	CH_4 yield (%)
Lipids	2.90	70
Carbohydrates	1.19	50
Ethanol	2.09	75
Propionate	1.51	58
Acetate	1.07	50
Protein	1.42	50

- **C/N ratio**

The relationship between the amount of carbon and nitrogen present in organic materials is expressed as the C/N ratio. The optimum range for C/N ratio in feedstock for anaerobic digestion is 20:1-30:1 (Parkin and Owen, 1986). Low nitrogen content coupled with high carbon content results in low gas yield. However, high concentrations of nitrogen leading to ammonia decreases methanogenic activity and further accumulation could completely halt the anaerobic digestion process (Parkin and Owen, 1986). C/N ratio varies according to the organic material, and a desired C/N ratio can be achieved by adjusting a low C/N ratio feedstock with a feedstock that has a higher C/N ratio. Karki and Dixit (1984) demonstrated the use of feedstock with a high C/N ratio to complement feedstock with a low C/N ratio thereby improving the digestion performance. This practice was used by Sosnowki *et al.*, (2003) for co-digestion of sewage sludge and municipal waste water (Sosnowski *et al.*, 2003). Yen and Brune (2007) also demonstrated the co-digestion process by adding high carbon content waste paper in algal sludge

feedstock to give a balanced C/N ratio. Their study showed addition of waste paper to algal sludge resulted in significant increase in CH_4 production as compared to algal sludge digestion alone (Yen and Brune, 2007). Co-digestion is also beneficial because potential toxic NH_4 is diluted which allows increased loading rate and improved biogas yield (Sosnowski *et al.*, 2003).

- **Effect of Temperature on anaerobic digestion**

Anaerobic digestion can take place at temperatures below 20 °C but methanogens and sulphate reducing bacteria (SRBs) which are part of the anaerobic food chain are inactive at extremely high and extremely low temperatures (Bouallagui *et al.*, 2003). This was confirmed by an experiment carried out by Bouallagui *et al.*, (2003) where no methanogenic activity was recorded at temperature below 10 °C. Most reactors operate optimally between 30 °C and 55 °C. When ambient temperature is below 10 °C, gas production virtually stops (Lund *et al.*, 1996). Satisfactory gas production takes place in the range of 25 °C to 30 °C, and proper insulation of the digester helps to maintain the temperature range and increase biogas production (Lund *et al.*, 1996).

- **Effect of pH on anaerobic digestion**

The ideal pH for anaerobic digestion is 6.8-7.8 as is the case for most biological processes. Optimum biogas production is achieved when pH of influent to the digester is within this range. The growth rate of methanogens is greatly reduced at low pH (Mosey and Fernandes, 1989) which can be caused by large amounts of organic acids produced by acid forming bacteria during the initial stage of fermentation.

- **Toxicity**

Mineral ions, heavy metals and the presence of detergents are some of the toxic materials that inhibit the normal growth of anaerobic bacteria. Addition of certain metals to the feed material has been found to increase biogas production (Wang *et al.*, 2006). Heavy metals such as Cu, Ni, and Zn are essential in very small quantities for the growth of the bacteria but at higher concentrations these have a toxic effect (BRTC, 1989). Addition of detergents such as soaps, antibiotics and organic solvents are known to inhibit the growth of CH_4 producing bacteria (Funk *et al.*, 2004).

- **Loading Rate**

Loading rate is the amount of raw material fed into the digester per unit volume of its capacity per day (BRTC, 1989). If the fermenter is underfed, gas production will be low, and if it is overfed, acid is likely to accumulate and CH_4 production will be inhibited (Karki and Dixit, 1984).

- **Hydraulic retention time (HRT)**

This is the average time that a given quantity of digester input remains in the fermenter for it to undergo due processes. Hydraulic retention time is a key parameter in the anaerobic process; Speece (1996) recommended that the HRT should be long enough to allow active populations to remain in a reactor (Speece, 1996; Sialve *et al.*, 2009). HRT is an important determining factor in the case of complex organic pollutants that degrade slowly (Speece, 1996). When an anaerobic digestion process is operated at low loading rate and long retention time, CH_4 yield tends to be higher than when the operation is at a high loading rate and short retention time (Chen, 1987). For an efficient and effective conversion of organic matter to gas, optimum loading rate and retention time is required depending mainly on composition of substrate. The optimum retention time is also dependent on temperature and how accessible the substrate is to the anaerobes (Asinari Di San Marzano *et al.*, 1983). Chen (1987) and Asinari Di San Marzano *et al.*, (1982) showed that loading rate and HRT is mostly dependent on the characteristics of the substrate (Asinari Di San Marzano *et al.*, 1983; Chen, 1987).

1.7 Problem Statement

IAPS is a bioprocess with the primary purpose of domestic wastewater treatment. The IAPS technology researched at EBRU applies to small South African communities in cognisance with effective faecal pathogen removal and public health consideration for the purpose of water reuse (Rose, 2002). The system treats domestic effluent with an efficiency of approximately 99.99 % reduction of *E. coli* (Rose, 2002). As part of the IAPS, a fermentation pit located at the bottom of the PFP, ensures complete fermentation of volatile solids and simultaneously generates biogas. The HRAP produce microalgae biomass that serves as a polishing step for waste water treatment and subsequent pathogen removal. Biomass is generated in the HRAP as a result of nutrient removal procedures and water remediation. This present research describes experiments to demonstrate biogas generation from the IAPS and encompasses use of the microalgae biomass generated to supplement anaerobic digestion feedstock in order to investigate its effect on biogas

yield. Furthermore, studies are described to demonstrate that the microalgae biomass can be used alternatively for fermentation to ethanol.

The aim of the current research was to seek a way of integrating microalgae biomass obtained from HRAPs in the IAPS treatment of domestic wastewater into an alternative renewable energy generation bioprocess.

Hypothesis

- Microalgae biomass from the high rate ponds can serve as co-feedstock in the anaerobic digestion process to improve methane yield and the use of HRAP microalgae biomass to produce bioethanol.

Objectives

1. Quantify biogas yield from an IAPS treating domestic waste water;
2. Simulate fermentation pit conditions in a lab scale fermenter to optimize and test the effect of microalgae biomass as a co-feedstock on CH_4 output;
3. Assess the potential to use microalgae biomass as feedstock in the fermentation to ethanol;
4. Evaluate the use of biogas and bioethanol as alternative fuels for transportation.

Chapter 2: Methane Production Potential of an Integrated Algae Ponding System (IAPS)

2.1 Introduction

The existence of anaerobic digestion dates back to the time of the first engineered wastewater plants (Foresti *et al.*, 2006). Although the first anaerobic digester configuration was designed for the treatment of industrial wastewater, it has also been recently applied to the treatment of low strength domestic sewage (Foresti *et al.*, 2006). In addition to domestic water treatment, organic matter is reduced with no energy consumption and methane gas is also generated. Methane was first described as highly flammable and non-toxic by Sir Alessandro Volta in the 1770s (Mardigan and Martinko, 2006) and it has a strong potential as an alternative to fossil fuel. It is given much consideration as a substitute for fossil fuel because of its clean burning process and relative abundance, largely obtained anaerobically. Methane occurs mostly in natural environments where light and inorganic electron acceptors are absent such as landfills, rice fields, and intestinal tracts of livestock (Angelidaki *et al.*, 2011).

In order to avoid generation of effluent that cannot be directly discharged into water bodies, anaerobic digestion must be combined with other technologies (Foresti *et al.*, 2006). Hence, the incorporation of an anaerobic digester in integrated algae ponding systems, as a form of post treatment of the effluent. The integrated algae ponding system (IAPS) sparked a lot of interest right from the inception stage. Professor Oswald (1988) was the initiator of this unique invention, where he started the formulation of a ponding system that would remove suspended solids, support the growth of methanogens, oxygenate primary effluent via photosynthesis as well as disinfect domestic waste water (Oswald, 1991). The integrated algae ponding system combines both anaerobic and aerobic biological processes for efficient and effective wastewater treatment. The system is meant to simulate natural waters such as lagoons, ponds and lakes; it is an artificial, minimally controlled process with raceways usually built at a depth of between 0.2 and 0.5 m (Brennan and Owende, 2010). The open pond is mainly used for large scale algae biomass production and requires little or no man power. They are usually designed in a closed loop re-circulation channel and adequate mixing is achieved using a paddlewheel to ensure proper algae growth, optimal activity and productivity (Chisti, 2007). A fermentation pit can be

located deep within the primary facultative pond (PFP) with a volumetric capacity of 0.45 m^3 per capita (Rose, 2002). In an attempt to quantify and analyse the biogas generated from the fermentation pit of an IAPS pilot plant, gas capture apparatus in the form of a hood with a flow meter for evaluation of the biogas production were installed. The volume of biogas was quantified using a flow meter and biogas composition determined by gas chromatography. In addition, the efficiency of the IAPS for waste water treatment was determined and microalgae biomass yield quantified.

2.2 Materials and Methods

2.2.1 Integrated Algae Ponding System Configuration

The IAPS pilot plant utilised in this research project is situated at the Institute for Environmental Biotechnology Rhodes University (EBRU), adjacent to the Grahamstown Disposal Works (33° 19' 07" South, 26° 33' 25" East) (Figure 2-1).

Figure 2-1: The PFP component of the IAPS pilot plant, with a fermentation pit underneath

The system operates uninterrupted and consists of a primary facultative pond (PFP) which contains a single fermentation pit, two similar high rate algal ponds (HRAP), two algal settling ponds and four bottom drained algal drying beds. A schematic illustrating this configuration is shown in figure 2-2. The hydraulic retention time (HRT) in the PFP was 20 d while in the fermentation pit the HRT was 3 d. The IAPS has a capacity of treating the domestic waste of 500 person equivalent (PE) which is based on an assumption that each person generates 160 $L.d^{-1}$ of liquid waste resulting in a total capacity of 80 $m^3.d^{-1}$. The wall of the fermentation pit extends 3.0

m below the base of the PFP and 1.5 m above the bottom of the PFP. At an overall depth of 4.5 m and a volumetric capacity of 225 m^3 the area of the fermentation pit is 50 m^2 for effective successful separation of gas bubbles from solids (Rose, 2002).

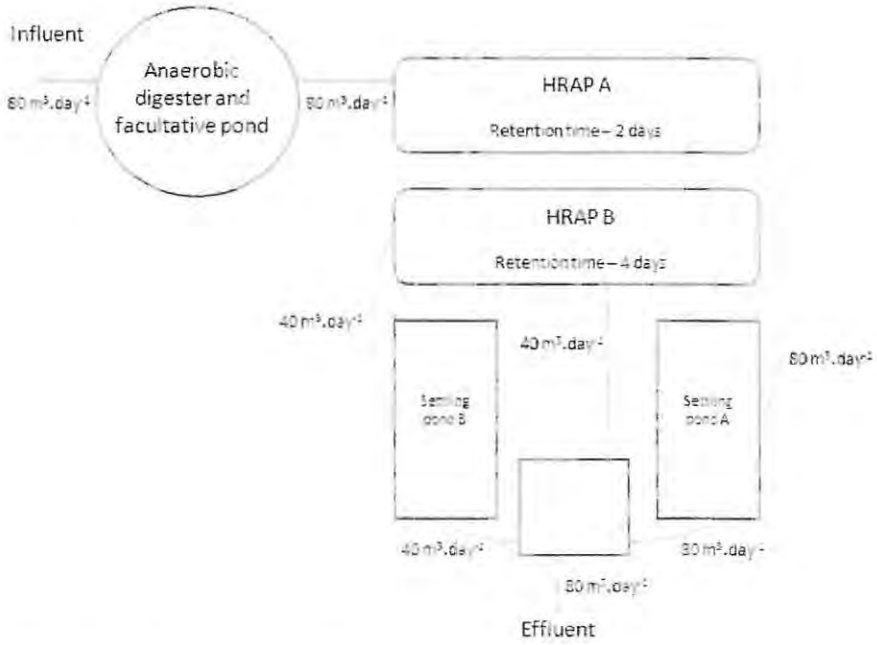


Figure 2-2: IAPS configuration, illustrating the effluent pathway from influent to discharge

From the PFP, effluent decants under gravity into the first HRAP through a transfer point located 0.5 m below the water surface of the PFP. The two HRAP (60 × 8 m) are linked by settling pond A and the water depth is maintained at 0.3 m to give a total volume of 150 m^3 with surface area of ±500m^2 per raceway. The HRT was 2 d in the first raceway (HRAP A) with an influent rate of 80 $m^3.d^{-1}$. A HRT of 4 d with influent rate of 40 $m^3.d^{-1}$ into the second raceway (HRAP B) was achieved by diverting 40 $m^3.d^{-1}$ back into HRAP A. Efficient mixing in the raceways was powered by a small electric motor (0.35kWh) attached to the paddle wheel which provides a linear velocity of 0.3 $m.s^{-1}$ in each raceway. The biomass generated in HRAP B is removed to thickener tanks or settling cones where the slurry yield was about 3-5% solids.

2.2.2 Gas Capture Hood Installation

The hood employed for biogas capture was a partitioned scrim-reinforced laminated PVC fabric manufactured by Basil Humin Inc. (Grahamstown, Eastern Cape, South Africa) manually

installed as shown in figure 2-3. The gas capture hood functions as a form of submerged gas collector, laminated between two 5-7 mm thick PVC sheets using a PVC adhesive plastinol. A nylon mesh matrix was constructed across the surface of fermentation pit and used to break sludge rafts in order to prevent hood rupture as shown at the extreme left in Figure 2-3. A flow meter (Schlumberger, England) obtained from Sowerby Suppliers' (South Dale, Johannesburg, Gauteng, South Africa) with a maximum capacity for gas measurement of 6 $m^3.h^{-1}$ and a minimum capacity of 0.06 $m^3.h^{-1}$ was utilized for the quantification of total biogas produced as shown in extreme right Figure 2-3.

Figure 2-3: Installation of laminated reinforced PVC hood for gas capture with the nylon mesh matrices evident (left) above the fermentation pit and Flow meter (right)

2.2.3 Inoculation of Anaerobic Digester

In order to generate an active and robust culture for the anaerobic digester, active inoculums were obtained from the adjacent waste water treatment plant. Approximately 100-150 m^3 of vibrant established culture was pumped into the anaerobic digester of the IAPS PFP and allowed to establish for a period of 3 weeks. Once gas production was stable, quantification of total biogas and gas composition measurement commenced.

2.2.4 Quantification and Measurement of Biogas Composition

a. Quantification of biogas

Volume of biogas produced by the anaerobic digester in the PFP of the IAPS pilot was measured using a flow meter (Schlumberger, England obtained from Sowerby, Gauteng South Africa). The daily cumulative readings of the biogas were recorded.

b. Determination of biogas composition

The composition of biogas was determined by gas chromatography (GC) using a fused silica capillary column (30 m × 0.25 mm I.D.) fitted to an Agilent 6820 GC instrument operated in splitless injection mode. The initial oven temperature was 50 °C, isothermal for 4 min, then ramped to 160 °C at 20 °C per min. Total run time was 11 min 50 s per sample with 150 µL as quantity of sample injected into the GC. A thermal conductivity detector (TCD) set at 300 °C was used with helium as the carrier gas at a flow rate of 0.9 mL.min $^{-1}$. The identity of the separated gases was established by comparison of retention times with known standards of specified gas mixtures obtained from Afrox South Africa and levels quantified by peak integration as presented in Appendix 2-A.

2.2.5 Analytical Procedures

2.2.5.1. Characterisation of Anaerobic Digester Feedstock

The anaerobic digester feedstock was characterised by analysis of chemical oxygen demand (COD), sulphate (SO_4^{2-}), total nitrogen (TN), total carbon (TC), and total organic carbon (TOC).

a) Chemical oxygen demand (COD)

COD expresses the amount of oxygen originating from potassium dichromate which reacts with the oxidizable substances contained in 1 L of H_2O (Angelidaki and Sanders, 2004). Determination of COD was carried out using a COD test kit obtained from Merck®, South Africa. The procedure involved pre-treating 3 mL of filtered sample by adding 0.3 mL of solution A followed by 2.3 mL of B according to manufacturers' instructions. The sample was then heated in a thermo reactor for 2 h at 148 °C. Measurement was carried out using a Nova 60 spectroquant (Merck Chem. Co., Darmstadt, Germany) against a reagent control. Potassium dichromate (1 mol) is equivalent to 1.5 mol oxygen released and results were expressed in mg.L^{-1}.

b) Sulphate (SO_4^{2-})

Sulphate ions react with barium iodate releasing iodate ions in the process; these oxidize tannins to form a brown-red compound which is an indication of the presence of sulphate in solution. Determination of SO_4^{2-} was carried out using a SO_4^{2-} test kit obtained from Merck®, South Africa. The analysis included filtering 2.5 mL of sample, followed by the addition of 2 drops of reagent 1 and one level green micro spoon of reagent 2. This was heated in a water bath for 5

min at 40 °C, and then 2.5 mL of reagent 3 was added followed by filtration through Watman (110mm) glass microfiber filter paper. Four drops of reagent 4 were then added to the filtrate and the mixture finally transferred to a water bath for 7 min at 40 °C. Measurement was carried out using a Nova 60 spectroquant (Merck Chem Co., Darmstadt, Germany) and results expressed in $mg.L^{-1}$.

c) Total carbon (TC) and Total organic carbon (TOC)

Total carbon was analysed using an Apollo 9000 TOC analyser (Teledyne Tekmar, Mason, OH USA). The instrument executed the analysis by the combustion method. The combustion temperature set for the method was 680 °C and the carrier gas used was medical air (Afrox, Port Elizabeth, South Africa) at a flow rate of 0.9 $mL.min^{-1}$. Total carbon was analysed by automated injection of an aliquot of liquid sample into the preheated furnace. Through catalytic oxidation the sample was completely oxidized to CO_2 and H_2O. The carrier gas transports the CO_2 through the nafion drier for scrubbing in order to eliminate water vapour and then through a halogen scrubber for further scrubbing and finally to the non-dispersive infrared sensor (NDIR) for CO_2 quantification (Apollo 9000 TOC analyser manual) and results presented as $mg.L^{-1}$.

Total organic carbon (TOC) was also analysed using an Apollo 9000 TOC analyser (Teledyne Tekmar, Mason, OH USA). The instrument executed the analysis by the combustion method and the combustion temperature was set at 680 °C with medical air (Afrox, Port Elizabeth, South Africa) as the carrier gas at a flow rate of 0.9 $mL.min^{-1}$. For total organic carbon analysis, liquid samples were first acidified (5 M) with phosphoric acid and sparged with carrier gas in order to vent any inorganic carbon (IC). Thereafter, samples were injected into the furnace. Results were presented as $mg.L^{-1}$.

d) Total nitrogen (TN)

According to Koroleff's method, treatment of organic and inorganic nitrogen compounds with an oxidizing agent in a thermo reactor transforms these compounds to nitrate (Ambe, 1978). In concentrated sulphuric acid, the transformed nitrate reacts with a benzoic acid derivative to form a red nitro compound which can be used as an indication of total nitrogen (TN) present in the sample. Determination of TN was carried out using a Total Nitrogen test kit obtained from

Merck®, South Africa. The procedure included addition of 1 level blue micro spoon of reagent 1 and six drops of reagent 2 to 10 mL of filtered sample, which was then heated in a thermo reactor for 1 h at 120 °C (digestion step). One level blue micro spoon of reagent 3 was added to another cell containing sulphuric acid, after vigorous mixing by vortexing 1.5 mL of the digested sample was then added and absorbance readings were determined using the **Nova 60 spectroquant** against reagent control supplied by the manufacturer. Results were recorded and expressed as $mg.L^{-1}$.

2.2.6 Gas Chromatography of Volatile Fatty Acids

For analysis of volatile fatty acids 1 mL of sludge sample obtained from the IAPS anaerobic fermenter was acidified with 34% phosphoric acid and centrifuged at 12,000 rpm for 10 min (Eppendorf 5810R centrifuge) to homogenise the sample. An aliquot of supernatant was injected onto a HP-Innowax 19091N-213; 30m x 0.32 mm; 0.5 micron column fitted to an Agilent 6820 GC instrument equipped with flame ionisation detector (FID). The oven temperature was set at 150 °C and the detector temperature at 180 °C (Jackson-Moss C.A., 1990). Helium was the carrier gas with a flow rate of 1.0 $mL.min^{-1}$. A standard mix containing C1-C7 acids obtained from Supelco, South Africa was used for calibration and to identify VFAs present in the sludge as presented in appendix 2-B and results were presented as percentage area of identified volatile acid.

2.2.7 Inductive Couple Plasma (ICP) of Metals

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used to determine the concentration of metals in solution. Prior to analysis, sludge samples obtained from the IAPS anaerobic fermenter were ashed in a muffle furnace (Carbolite England) for 6 h at 575 °C. The leftover ash was re-suspended in milliQ H_2O and filtered through Watman glass fibre filter paper (0.25 μm) and the process repeated in order to remove all particulates. The instrument measures characteristic atomic-line emission spectra by optical spectrometry after nebulisation and transport of the sample to the plasma torch. The spectra were dispersed by a grating spectrometer, and the intensities of the line spectra were monitored at specific wavelengths by a photosensitive device. Photocurrents from the photosensitive device were processed and controlled by a computer system. A background correction technique was required to

compensate for variable background contribution to the determination of the analytes (Method 200.7, 2001). The background was measured adjacent to an analyte wavelength during analysis. Standards were prepared from stock solutions of Mn, Mg, Cu, Ni, Co, Cd for calibration and quantification of metals present in sludge samples. Table 3 presents the metals analysed and the respective wavelengths used. Results were presented as $mg.L^{-1}$.

Table 2-1: Metals analysed and their wavelengths

Metals analyzed	Wavelengths (nm)
Manganese	257.610
Magnesium	279.079
Copper	324.754
Nickel	231.604
Cobalt	228.616
Cadmium	226.502

2.2.8 Yield of Microalgae Biomass and Measurement of Water Treatment Capacity

a. Microalgae biomass yield:

Liquid culture containing various microalgae species was harvested from HRAP pond A using glass cylinders. The microalgae containing media was allowed to settle for 2 d using settling cones followed by harvest of the microalgae slurry by centrifugation at 3500 × g for 20 min and a dewatering step by freeze drying using a bench top freeze drier (VIS-TIR Bench top SLC). The dry weight of the biomass was recorded in $g.L^{-1}$.

b. Water treatment capacity

Water treatment capacity was determined by analysing the final discharge from the IAPS for COD, SO_4^{2-}, TN, TC and TOC. The analyses were carried out as described in section 2.2.5.1 and the results presented in $mg.L^{-1}$.

2.2.9 Biochemical Characterization of Microalgae Biomass

Carbohydrate content of microalgae biomass was determined using a phenol - sulphuric acid assay (Dubois *et al.*, 1956). Microalgae biomass was treated with 0.5 M NaOH overnight at 15

°C and carbohydrate was extracted with a Pasteur pipette from the bottom of the solution. 0.5 mL of the clear aqueous solution extracted was placed in test-tubes, followed by addition of 0.5 mL of 5 % phenol and addition of 2.5 mL 98% sulphuric acid (Dubois *et al.*, 1956). The solution was mixed vigorously by vortexing and left to stand for 15 to 20 min. A yellow-orange colour developed indicative of the interaction between carbohydrate and the phenol. The absorbance was determined at 490 nm using a Thermo Spectronic Aquamate spectrophotometer (Fisher, England). Sulphuric acid oxidises non-reducing sugars to reducing sugars so that total sugars present can be quantified relative to D-glucose. Since the method is non-stoichiometric, a calibration curve (Appendix 2-3) was prepared using known concentrations of D-glucose and the carbohydrate content determined by interpolation (Dubois *et al.*, 1956). The results were expressed as $mg.L^{-1}$.

Protein content of algae biomass was determined using the Bradford dye binding assay which is a spectroscopic analytical method used to determine the concentration of protein in a solution (Bradford, 1976). This assay is dependent on the interaction between amino acids present in the protein and Coomassie brilliant blue G-250 dye. To protein containing extracts (60 µL) 3 mL of Bradford reagent (Sigma, South Africa) was added. After vigorous mixing colour change from brown to blue was noticeable in proportion to the amount of protein present in the sample. The absorbance was measured after 5 min and within 1 h at 595nm using a Thermo Spectronic Aquamate spectrophotometer and the quantity of protein was determined by interpolation from a standard curve (See appendix 2-4) prepared using a series of known dilutions of Bovine Serum Albumin (BSA, Chem Co. St Louis MO).

Lipid content of microalgae biomass was determined using a potassium dichromate assay, after extraction of total lipid as described by Folch *et al.*, (1957). To 0.1 g of dry microalgae in a clean test tube was added 500 µL of dH_2O followed by 10 mL of chloroform/methanol (2:1, v/v) and the extract vortexed and then centrifuged at 4000 × g for 5 min. The supernatant was discarded and the extract was washed with 0.5% $CaCl_2$ followed by centrifugation at 4000 × g for 5 min and the lower phase was extracted. The extraction procedure was repeated 3 times and the lower phases combined. Solvent was evaporated in an incubator at 100 °C followed by the addition of 3 mL of 2% potassium dichromate in 98% sulphuric acid. The samples were then heated in

boiling water bath for 15 min and cooled. 4.5 mL dH_2O was added to test tubes containing evaporated samples and thoroughly mixed. Absorbance was measured at 590 nm using the Thermo Spectronic Aquamate spectrophotometer (Fisher, England) and results were determined by interpolation from a standard curve which was prepared using known concentration of palmitic acid (Folch *et al.*, 1957) (Appendix 2-5).

2.3 Results

2.3.1 Quantification of Microalgae Biomass and Measurement of Water Treatment Capacity

Experiments that focused on biomass production potential and water treatment capacity of the IAPS system were carried out. The experiment for microalgae biomass quantification was conducted using 100 L capacity settling cones with 80 L experimental volume and was carried out in triplicate. Algal slurry was harvested by settling, and after 2 d the material was frozen and freeze dried (VIS-TIS SLC bench top) to a final dry weight of 20 g. Thus, based on the volume harvested the microalgae culture yielded 0.25 $g.L^{-1}$.

Analysis of COD, SO_4^{2-}, TN, and TOC was carried out as described in section 2.2.5.1 to determine the nutrient removal trend and water treatment capacity of the EBRU IAPS pilot plant (Figure 2-4).

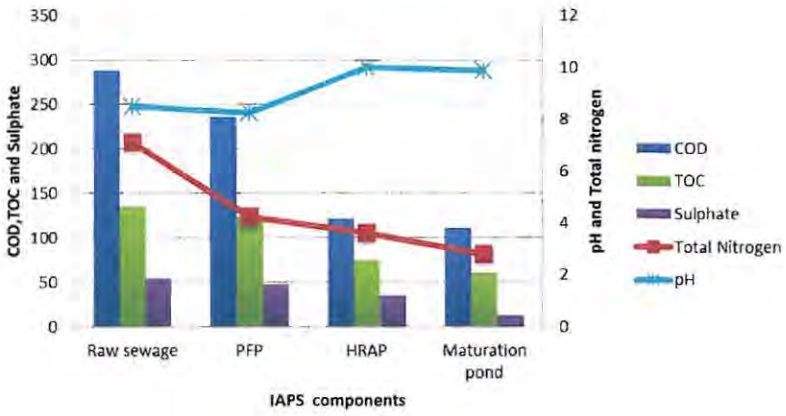


Figure 2-4: Analysis of effluent at the start of each stage in water treatment by an Integrated Algae Ponding System.

At port of entry, high COD of about 280 mg/L, TOC of 140 mg/L, SO_4^{2-} of 50 mg/L and TN levels of 8 mg/L were recorded. Post anaerobic digestion, a stable pH was noted across the IAPS and COD, TOC, SO_4^{2-} and TN were reduced remarkably in the discharge by over 40%. As expected, this level of decline is acceptable for treatment of water under South African standards. The result confirm that IAPS effectively treats domestic wastes to provide clean water and substantial biomass.

2.3.1 Biochemical Characterisation of Microalgae Biomass Harvested from HRAP

Microalgae species and composition mostly depends on its environment. Typical species of microalgae found in the HRAPs include, *Pediastrum, Scenedesmus, Micractinium, Chlorella* and *Euglena* (Oswald, 1989; Green *et al.*, 1995; Canovas *et al.*, 1996; Craggs *et al.*, 2003). Biochemical analyses were carried out as described in section 2.2.9. The highest component of the biomass was protein at 30% followed by lipids at 19.7% and carbohydrate at 18.63% (Table 2-2).

Table 2-2: HRAP Microalgae biomass, chemical analysis and biochemical composition

Parameter	Microalgae
Carbohydrate (%)	18.63 ± 1
Protein (%)	30.00 ± 0.5
Lipids (%)	19.70 ± 1
Total Carbon mg/L	958.00 ± 20
Total organic carbon mg/L	598.00 ± 20
Sulphate mg/L	40.00 ± 10
COD mg/L	645.00 ± 20
Total nitrogen mg/L	75.00 ± 10
C:N ratio	12:1

Table 2-2 indicates the potential digestibility of microalgae biomass as a substrate for fermentation. Going by the fact that COD gives a good first impression of the strength of wastes, 0.645 g/L (Table 2-2) should yield a considerable amount of CH_4. Also, the C/N ratio of this biomass was 12:1 which although not the ideal C/N ratio for high CH_4 yield but this can be acceptable since the biomass was being used as a co-substrate in the present situation to

supplement sludge with a C/N ratio of 24:1. In the biomass, protein content was highest followed by carbohydrate and lipids which were within acceptable ranges for substrate digestibility.

2.3.1 Analysis of Digester Sludge: Volatile Fatty Acids and Metal Analysis, Pre and Post Inoculation

The digestate supplies nutrient for growth of microalgae in the HRAOPs. In preparing an inoculum for anaerobic digestion various analyses were carried out in order to determine the functionality of the anaerobic consortium present in the sludge used to seed the fermentation pit. Thus, prior to inoculation of the fermentation pit, pH, COD, sulphate, total nitrogen, total carbon and total organic carbon were measured (Table 2-3). These analyses were repeated post inoculation in order to establish viability of the inoculums.

Table 2-3: COD, pH, Sulphate, Ammonium nitrogen, Total carbon and Total organic carbon analysis of sludge pre and post inoculation of fermentation pit

Parameters	IAPS sludge pre inoculation	IAPS sludge post inoculation
Analysis	Values	Values
pH	6.52	7.56
COD	964.67 ±20 mg/L	1554.33 ±20 mg/L
Sulphate	258.33 ±10 mg/L	97.67 ±10 mg/L
Ammonium Nitrogen	0.60 ±5 mg/L	50.33 ±5 mg/L
Total Carbon	490.47 ±20 mg/L	1207.67 ±20 mg/L
Total organic carbon	131.95 ±20 mg/L	819.67 ±20 mg/L

Pre inoculation, the pH of the digestate was lower in comparison to the sludge after inoculation, including an increase in the values for COD which was higher after inoculation. Pre inoculation, value of sulphate indicated an accumulation in sulphate from pre to post inoculation. TC and TOC were also increased post inoculation. This series of analyses indicated viability of the inoculum after establishment in the digester and it confirmed less activity in exposed inoculum pre inoculation.

Volatile fatty acids are important intermediary products in the biogas generation process and the concentration of these affects the efficiency of fermentation (Wang *et al.*, 2009). VFAs analysed pre-inoculation showed that the sludge contained little or no VFA activity whereas post inoculation substantial VFAs were present indicative of an active microbial consortium potentially capable of driving methanogenesis (Figure 2-5). The intermediate acids formed were

tentatively identified as acetate, propionic acid, isocaproic acid, butyric acid, n-caproic acid and isovaleric acid which are important precursors to eventual CH_4 generation (Wang *et al.*, 2009).

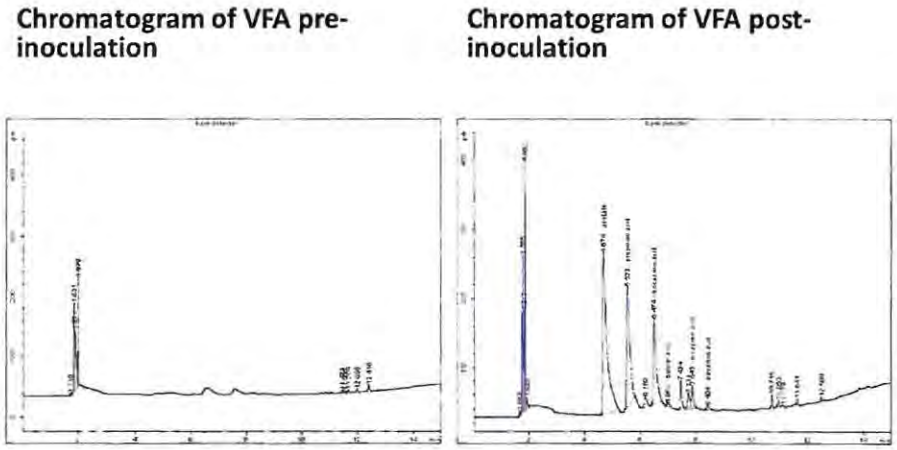


Figure 2-5: VFA chromatogram, pre and post inoculation of the fermentation pit.

Metal ions and in particular copper are known to play an important role in methanogenesis (Wang *et al.*, 2009). Analysis of metals in sludge samples was carried out. Mn, Mg, Cu, Ni, Co, Cd were quantified in the sludge both pre and post inoculation and the results are presented in Table 2-4. All the metals analysed were at acceptable levels in both samples except for magnesium which was very high in the pre inoculation sludge.

Table 2-4: Values for metal analysis in inoculum, prior to seeding and post inoculation

Metals analyzed	Values pre-inoculation (mg/L)	Values post inoculation (mg/L)
Manganese	0.0089	0.0000
Magnesium	9.0590	4.9930
Copper	0.0460	0.0150
Nickel	0.0100	0.0030
Cobalt	0.0020	0.0000
Cadmium	0.0020	0.0000

2.3.2 Quantification and Determination of Biogas Composition and Yield

Biogas obtained from the anaerobic digester of the integrated algae ponding system (IAPS) was quantified using a flow meter attached via a connecting pipe, from the gas capture cap of the PVC hood covering the fermentation pit in the primary facultative pond (Figure 2-6). The gas was measured in m^3.d as interpreted from the flow meter.

Figure 2-6: Biogas outlet from the fermentation pit in the primary facultative pond attached to the flow meter showing biogas inflated hand glove.

Biogas content and composition was determined by GC-TCD as described in section 2.2.4. After the installation of a gas capture hood followed by a 3 wk equilibration period for methanogens to establish, analysis of biogas composition revealed the presence of CO_2, N_2 and CH_4 which were tentatively identified based on retention time and Kovats indices (Figure 2-7). Biogas production was quite stable with the highest CH_4: CO_2 ratio of 85:7 % and the lowest ratio 60:7 %. The CH_4 ratio showed that this system was producing a significantly higher percentage of CH_4 in comparison to similar systems (Brune *et al.*, 2009).

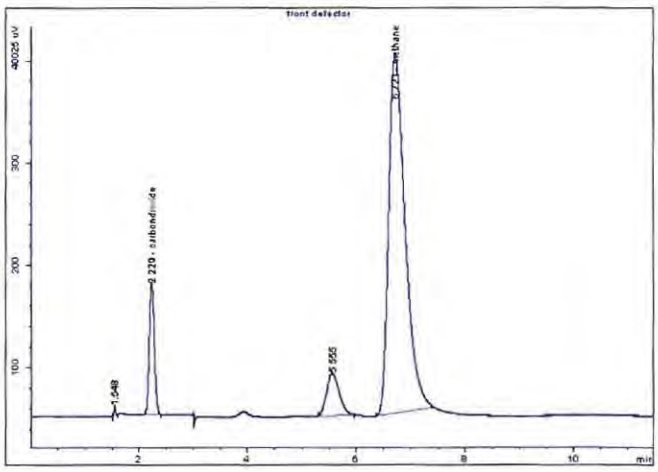


Figure 2-7: Chromatograms of biogas composition. A) 9.33% CO_2 at 2 min 22 s B) 7.51% N_2 at 5 min 55 s and C) 82.90% CH_4 at 6 min 72 s.

The cumulative biogas measured from the EBRU IAPS was 2.34 $m^3.d^{-1}$ with an average ratio of 75% CH_4 (± SD; n=10). From the methane ratio (75%) and density (0.67 mg/L), methane composition in the biogas from the IAPS fermentation pit was determined to be 0.6289 kg.

Note: 1mole of gas = 22.4 L at STP; molecular weight of CH_4 = 16 g/mol.

2.3.3 Potential Methane Yield from Algae Biomass

According to Angelidaki and Sanders (2004), calculation of theoretical CH_4 expected from anaerobic digestion is possible once the composition of organic matter is known (Angelidaki and Sanders, 2004; Sialve *et al.*, 2009). The formula adapted from Symons and Buswell (1933) by Angelidaki and Sanders (2004) can be used to calculate CH_4 yield (Symons and Buswell, 1933; Angelidaki and Sanders, 2004) (Equation 2-1).

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

--- Equation 2-1

The more reduced the carbon content of organic matter is, the more CH_4 will be generated. From the generic formula of microalgae, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar, 2004), potential CH_4 yield can be estimated. From Equation 2-1 above, the specific methane yield of microalgae as fermentation substrate can theoretically yield 0.180 L CH_4 gVS^{-1}

Biogas generated from the IAPS is made up of an average of ±75% CH_4 and by interpolation with data from the literature, this equates to a theoretical maximum of 39.57 m^3 biogas.d^{-1} for a 500 PE IAPS pilot plant.

2.4 Discussion

In order to determine water treatment capacity of the IAPS, samples were obtained from different points in the IAPS (Raw influent, PFP, HRAPs and Maturation pond) and analyses were carried out using standard methods (Figure 2-4). With regards to nutrient removal and water treatment capacity of the EBRU IAPS pilot plant, there was a decrease in COD, TOC, sulphate, TN and an increase in pH in the influent. Based on the series of analyses, the IAPS pilot plant treated the water resulting in a 40% reduction of nutrients across the system. The effluent was considered as an acceptable water quality for discharge according to the South African standard. The algae present in the system utilize the carbon which resulted in the high COD and TOC levels and also the nitrogen to form biomass. The resulting increase in biomass was therefore coupled to a decline in nutrients that was acceptable for treatment of water under South African standards. The pH also increased across the system due to optimum algae growth in the HRAP with its consequent oxygen production. Productivity of HRAP of the IAPS microalgae biomass is 0.25 $g/L.d^{-1}$, the biomass yield theoretically contains 0.6 t/kg (w/w) resulting in an estimated energy content of 29 GJ/t dry weight.

The algae biomass protein content was high which indicated a low C/N ratio and the protein, carbohydrate and lipids were still within acceptable ranges for substrate digestibility. Similarily, Yen and Brune (2007) demonstrated the co-digestion process by adding high carbon content waste paper in algal sludge feedstock to obtain a balanced C/N ratio. Their results showed that by adding 50% waste paper to algae sludge there was a significant increase in methane production rate as compared to algae sludge digestion alone (Yen and Brune, 2007).

From the VFA results for the digester pre innoculation, it was evident that very little activity was occuring in the sludge. This was confirmed by measuring the VFA post inoculation and finding good levels of microbial activity. Volatile fatty acids are important intermediary products in the biogas generation process and the concentration of these affects the efficiency of fermentation (Wang *et al.*, 2009). VFAs analysed pre-inoculation showed that the sludge contained little or no VFA activity whereas post inoculation substantial VFAs were present indicative of an active microbial consortium potentially capable of driving methanogenesis. It is therefore

recommended that the activity of a digester should be determined before further studies continue especially in long dormant or exposed reactors.

Metal ions and in particular copper are known to play an important role in methanogenesis (Wang *et al.*, 2006). Copper serves as one of the macro elements needed by methanogens for growth and from the results this growth factor is lacking in the pre-inoculation digestate. Manganese decreased post inoculation which is an indication that it was being used by the microorganisms for their growth during the digestion process (Wang *et al.*, 2006).

The cumulative biogas measured was 2340 $L.d^{-1}$ with CH_4 composition of 1.590 $kg.d^{-1}$ measured as yield from the IAPS fermentation pit. Biogas production was quite stable with the highest CH_4: CO_2 ratio of 85:7% and the lowest ratio was 60:7%. The methane ratio shows that this system was producing a significantly higher percentage of methane than similar systems (Rasi *et al.*, 2007).

Equation 2-1 above shows that actual yield is not as high as expected yield and there is the possibility of recovering about 51% of microalgae as feedstock in the form of CH_4. However, CH_4 recovery might vary from 46 to 76% (v/v) depending on species of microalgae and prevailing environmental conditions.

Chapter 3: Microalgae Biomass as Fermentation Feedstock

3.1 Introduction

Anaerobic fermentation is one of the most important biological processes. Its importance in the treatment of waste water cannot be overstated where it reduces the organic matter load by 45 to 60% (Rittmann and McCarty, 2001). Waste treatment is highly dependent on the activity of anaerobic microorganisms employed to decompose complex organic matter (Angelidaki *et al.*, 2011). As a consequence, anaerobic fermentation is utilised in the treatment of various wastes ranging from municipal solid wastes, agricultural wastes, and industrial wastes to domestic wastes. The waste water pond is a system which simulates natural water bodies, lagoons and is regarded in many parts of the world as the method of choice for waste water treatment (Oswald, 1991). The use of waste water ponds to cultivate algae has been documented for many years and Oswald and Gotaas (1957) made the earliest mention of it in relation to the utilisation of the resultant algae biomass to generate biofuel (Singh and Gu, 2010). In addition, they compared digestion of domestic wastewater sludge and green microalgae biomass (*Scenedesmus* and *Chlorella*) harvested from waste water ponds and found that the algae yielded 0.25-0.50 L CH_4/g VS at an 11 d retention time. (Golueke *et al.*, 1957; Salerno *et al.*, 2009). Microalgae biomass can be used to generate fuel products such as biodiesel, biogas and bioethanol, they can also serve as potential source for food supplement and livestock feed production (Figure 3-1).

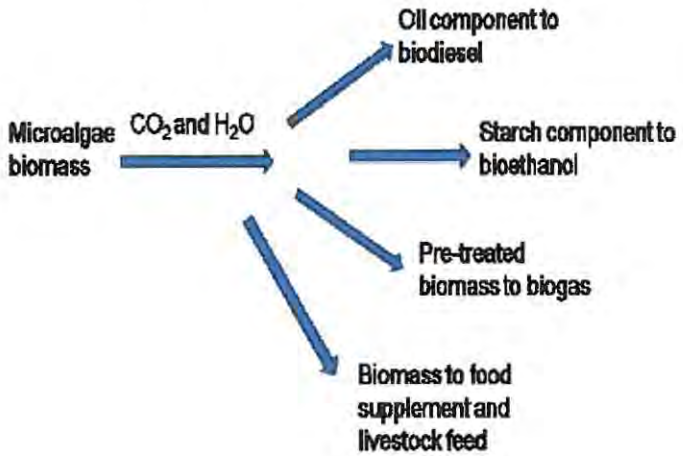


Figure 3-1: Potential pathways for conversion of microalgae to fuel and non-fuel products

All of these end products of algae biomass fermentation arise as a result of the protein, lipid and carbohydrate components of microalgae biomass. Anaerobic fermentation of microalgae not only yields biogas and bioethanol but also reduces the amount of volatile solids in wastes by about 70 % (Horan, 1991).

However, there are many disadvantages associated with microalgae fermentation, one of these is a rigid cell wall designed to protect its cellular content. Due to the recalcitrant nature of the cell wall in some algae species biogas yield from the algae may be low. Another is the high protein content of the algae These shortcomings can be counteracted by rupturing the cell wall to liberate the polysaccharides and by co-digesting algae with high carbon content wastes or waste water sludge to improve C/N ratio of the feedstock (Parkin and Owen, 1986; Salerno *et al.*, 2009). Examples of these scenarios include the demonstration by Choi *et al.*, (2010) that pre-treatment of microalgae biomass is required in order to release fermentable sugars whereas Samson and Lebuy (1983) improved the methane yield and productivity by co-digesting waste water sludge and *Spirulina* biomass (Samson and LeDuy, 1983). Similarly, Yen and Brune (2007) demonstrated a significantly higher methane output following the addition of waste paper to microalgae sludge. Another example of co-digestion is seen when Salerno *et al.*, (2009) added a high strength and high carbon waste to balance the high nitrogen content of waste grown algae (Salerno *et al.*, 2009).

In waste water systems that utilise algae as a polishing step for treatment of water, for example the IAPS, substantial biomass is produced on an annual basis. For effective treatment of the waste water and efficient nutrient removal continuous harvest of the biomass is required. The harvested biomass can be put to use as illustrated in Figure 3-1 where algae is used to generate many downstream products. In this study, the potential of algae as fermentation feedstock will be investigated. Of all the fermentation processes that give rise to biofuel, anaerobic digestion of microalgae for biogas production and alcohol fermentation are perhaps the two most important.

In this chapter, the suitability of microalgae biomass as fermentation feedstock for biogas and ethanol production was determined. The suitability of microalgae biomass generated in the IAPS was also studied as a co-substrate for anaerobic digestion to improve total energy output from the plant.

3.2 Materials and Methods

3.2.1 Source, Preparation and Culture Conditions

For alcoholic fermentation, strains of pure culture of brewer's yeast (*Saccharomyces cerevisiae*) were sourced locally from 'The Little Brewery' in Port Alfred Eastern Cape. The strains were cultured in yeast peptone dextrose broth (YPD) (Appendix 3-1) and maintained at 26 °C.

To generate an active and robust anaerobic microbial consortium for biogas generation, sludge samples containing established microbes was acquired from the Makana municipal waste water treatment works Grahamstown, Eastern Cape. The active culture was harvested from the bottom of the anaerobic digesters used for the treatment of domestic waste water. A modified basal medium (Appendix 3-2) was used to sustain the anaerobic consortium and comprised the sludge medium for methanobacteria adapted from Atlas (1993).

3.2.1.1 Scanning Electron Microscopy for Microbial Growth Studies

In order to confirm the presence of anaerobic bacteria in the sludge sample acquired from Makana municipal waste water treatment works, scanning electron microscopy was carried out. Aliquots of sludge were transferred to agar plates which contained sludge medium for methanobacteria (Atlas, 1993). The plates were incubated in an air tight anaerobic jar (Merck, South Africa) at 35 °C for 2 d. After the incubation period, colonies were sub-cultured onto fresh sludge medium for methanobacteria agar plates and re-incubated in an air tight anaerobic jar at 35 °C for 2 d. Visible colonies on the agar plates were removed by cutting out 5 mm squares of using a sterile razor blade. The excised colonies were fixed overnight in 0.1 M phosphate buffer at pH 7, containing 2.5% v/v glutaraldehyde solution (Merck, South Africa). Thereafter, the sample was washed twice in 0.1 M, pH 7 phosphate buffer solution for 10 min and dehydrated using a graded ethanol series (30%, 50%, 70%, 80% and 90%) at 15 min interval, followed by drying in a polaron critical point dryer. The critically dried sample was mounted on a 12 mm diameter aluminium slab, fixed with 12 mm carbon conducting adhesive slates and transferred into a Balzers union sputtering device before final gold coating for 2 min at 45 mA. Samples were examined using a VEGA TESCAN scanning electron microscope at 10-20 μm.

3.2.2 Feed Stock Preparation

Ethanol Fermenter

Glucose was used as the substrate in the control fermenter and microalgae in the test fermenter for ethanol fermentation. Microalgae was obtained from the HRAP of the EBRU IAPS and concentrated to 30 g/L by centrifugation at 3500 × g for 20 min prior to cell rupture using an ultrasonic dismembrator (Fisher Scientific, model 500). The substrate used in the control fermenter was prepared by dissolving 30 g of glucose in 1 L of dH_2O.

Biogas Fermenter

Waste water influent which served as substrate in the anaerobic digesters for biogas generation was obtained from Makana municipal waste water treatment works Grahamstown, Eastern Cape. The microalgae used as co-substrate was obtained from HRAP of the EBRU IAPS and concentrated to 50 g/L by centrifugation at 3500 × g for 20 min. Fermenter A served as the control, microalgae biomass was not introduced into the fermenter. In fermenter B, the concentrated microalgae biomass was introduced into the fermenter without further treatment. In fermenter C, the concentrated microalgae biomass cells were ruptured using an ultrasonic dismembrator (Fisher Scientific, model 500) prior to introduction into the fermenter. In fermenter D, the concentrated microalgae biomass was freeze dried using a bench top Freeze drier (VIS-TIR Bench top SLC) and re-suspended in dH_2O at 50 g/L followed by rupturing using an ultrasonic dismembrator (Fisher Scientific, model 500).

Chemical and biochemical analyses of the fermenter mixture were carried out according to sections 2.2.5.1 and 2.2.9.

3.2.3 Setup and Operation of Anaerobic Fermenters for Ethanol Fermentation and Biogas Generation

For ethanol production by fermentation, an anaerobic fermenter with a water trap attached to let off excess CO_2 while still maintaining a seal was used (Figure 3-2). A one litre round flat bottom flasks served as the parent fermenter (Figure 3-2) and was inoculated with 10% v/v brewers' yeast. In the control fermenter, 500 mL of growth medium and 400 mL of the dissolved glucose (Section 3.2.2) was added to the brewers' yeast. In the test fermenter, 500 mL of growth medium and 400 mL of treated microalgae biomass (Section 3.2.2) was added to the brewers' yeast. The

fermenters for ethanol fermentation were operated at a constant temperature of 26 ±2 °C and continuously agitated for uniform distribution of reactor components. Ethanol generated in the fermenters was analysed using an Agilent 6820 GC equipped with FID.

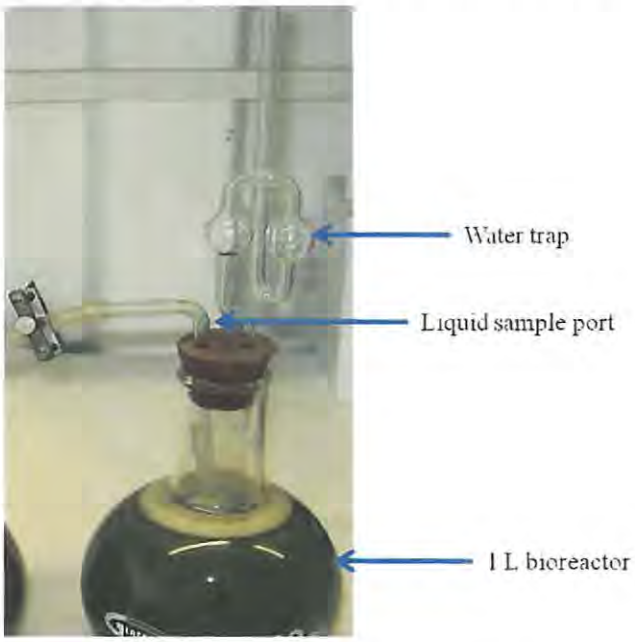
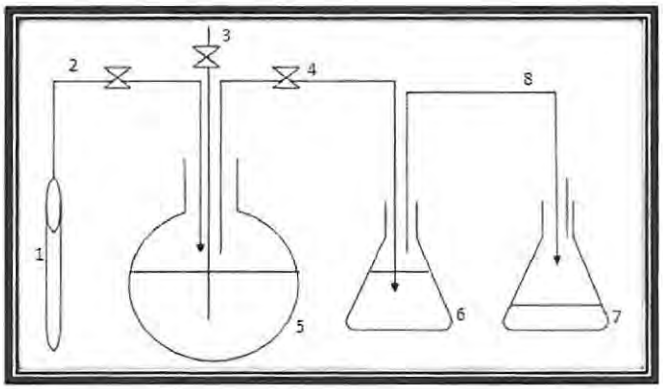


Figure 3-2: Laboratory scale bioreactor for ethanol fermentation

For biogas generation, laboratory scale fed batch reactors were established using 6 L round bottom flasks as the parent fermenter, with an effective volume of 4 L and a gas phase of 2 L (Figure 3-3). The reactors were maintained at constant temperature of 30±1 °C with no illumination and continuously mixed to ensure uniform distribution of reactor components. Inoculation of the fermenters was carried out using 10% v/v active anaerobic consortium obtained from the Makana municipal wastewater works. One litre of the basal medium prepared as described in section 3.2.1 and the content presented in appendix 3.2 was added to the fermenter followed by the addition of 2.6 L domestic waste water obtained from the Makana municipal wastewater treatment works. The lab scale fed batch reactors were sealed after feeding and connected to a gasometer (volumetric method for biogas measurement under constant pressure) (Figure 3-3). Acidic water was used in the gasometer in order to avoid solubilisation of CO_2. The fermenter headspace was then purged with 100 % nitrogen gas (Afrox South Africa) for 15 min in order to evacuate O_2 from the headspace to maintain an anaerobic

environment (Wang *et al.*, 2009). All openings on the reactor were sealed with silicone gel so that O_2 could not enter the system. Biogas from the fermenters was analysed using an Agilent 6820 GC equipped with TCD.



1. Nitrogen gas for sparging
2. Sparging port
3. Liquid sample port
4. Biogas sample port
5. 6 L bioreactor
6. Volumeteric measurement of biogas
7. Flask for displaced water
8. Connecting gas tubing from 6-7

Figure 3-3: Schematic laboratory scale continuously agitated fed batch reactor for anaerobic digestion process and biogas production and quantification.

3.2.4 Quantification of Ethanol and Determination of Biogas Composition

Ethanol was detected and quantified by gas chromatography using a fused silica capillary column (30 m × 0.25 mm I.D) fitted to an Agilent 6820 GC instrument operated in splitless injection mode. The initial oven temperature was 250 °C, isothermal for 2 min, and then temperature reduced to 240 °C at 5 °C per min. Total run time was 12 min per sample. A flame ionisation detector (FID) set at 350 °C was used for quantification with helium as the carrier gas at a flow rate of 10 $mL.min^{-1}$. Pure grade ethanol (Merck, South Africa) was used as a chromatographic standard.

Biogas composition was determined using an Agilent 6820 GC equipped with TCD as described in Section 2.2.4 of the previous chapter.

3.2.5 Statistical Analysis

STATISTICA version 9.0 software (Stat Soft, Inc. 2008) was used for statistical analysis of data generated. Due to independence of samples and equality of variances, one way analysis of variance was adopted as the method for statistical analysis. The one way ANOVA tested the

mean of the treatment against each other and the level of significance used throughout the study was 5%.

3.3 Results

3.3.1 Anaerobic Consortium

Scanning electron microscopy was carried out in order to confirm the presence of anaerobic microbial consortium in the sludge samples obtained from the Makana municipality's anaerobic digesters. Cultures were grown on agar plates using sludge medium for methanogens to sustain their growth. The colonies observed on the plates were sub-cultured and grown under anaerobic conditions at 35 °C for 2 d with no illumination. Following incubation, colonies were isolated and fixed as described in 3.2.1.1. The colonies were viewed using a VEGA TESCAN scanning electron microscope at 10-20 μm and the result is shown in Figure 3-4.

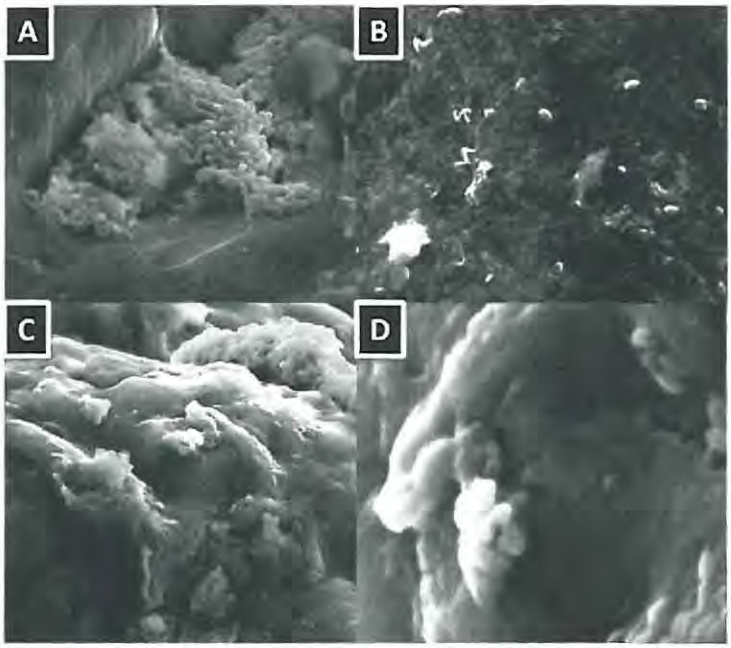


Figure 3-4: Scanning electron micrographs of anaerobic microbial consortia present in sludge samples obtained from Makana municipality. Micrograph A – organisms present in clusters with cocci and rod shapes, micrograph B – single cells, width ranging from 0.5 – 10 μm, micrograph C – uniform rod shaped microorganisms and micrograph D – cell width of 0.5 – 10 μm.

As can be seen in Figure 3-4, visible growth is present as single cells (0.5 - 10 μm) and as colonies. Thus, this indicates that the sludge sample obtained from Makana municipal contained an active consortium that may be used for biogas generation.

3.3.2 Fermentation of Microalgae to Ethanol

In an effort to demonstrate fermentation of microalgae to ethanol, several fermenters were set-up. Of three attempts only one demonstrated a positive result in terms of alcohol generation. From the control fermenter, about 385 $mg.g^{-1}$ ethanol was recovered from glucose and as low as 115 $mg.g^{-1}$ was recovered from microalgae.

The amount of alcohol recovered was inordinately low and as a consequence this section of the experiment was discontinued, concentration was diverted to microalgae fermentation to biogas. Low yield of ethanol was generated from microalgae biomass resulting in the discontinuation of this part of the experiment.

3.3.3 Anaerobic Fermentation for Biogas Generation

Four laboratory scale fed batch reactors were established for anaerobic fermentation to biogas. A stable environment was obtained by sustaining the microbial anaerobic consortium with growth medium (sludge medium for methanobacteria). A stable environment was created for the anaerobic consortium to acclimatise to the conditions of the fermenter. COD, sulphate, TC, TOC and TN analyses were carried for all four fermenters in order to monitor substrate depletion and the results are shown in Figure 3-5.

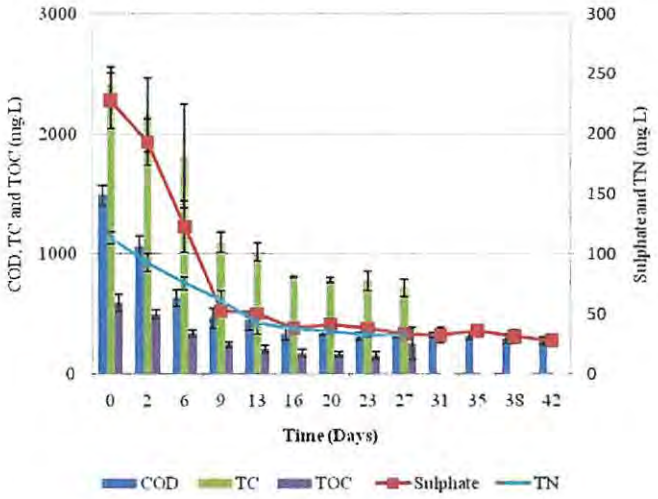


Figure 3-5: Characteristics of substrate utilization by a methanogenic consortium supplied sludge medium for methanobacteria. COD, TC, TOC, sulphate and TN depletion were monitored for 40 d as described in materials and methods. Each data point is the mean of 4 replicates ±SD.

As might be expected the concentration of COD, TC, TOC, sulphate and TN declined over the 40 d incubation period. This result indicated the presence of active anaerobic consortium which consumed the substrate via methanogenic pathway into biogas.

This was further confirmed by quantification of biogas during the 40 d incubation period and the result is shown in Figure 3-6. The highest biogas generated within the 40 d period in the fermenters was 600 $mL.d^{-1}$ on day 1 of the experiment, and the average biogas measurement recorded was 170 $mL.d^{-1}$ throughout the 40 d experimental period.

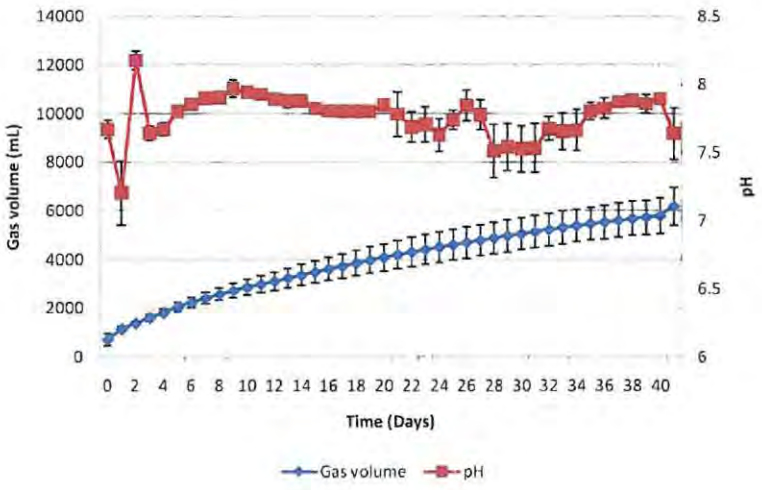


Figure 3-6: Cumulative volume of gas produced by the methanogenic consortium and change in pH over the course of 40 d. Volumetric measurement of gas was carried out by gasometer method; each data point is the mean of 4 replicates ± SD.

The pH remained within a constant range which is to be expected in a functional anaerobic digester. Biogas generation in the fermenters indicated the functionality of the system and confirmed activity of the anaerobic consortium. There was no significant difference between the four fermenters after the 42 d experimental period (ANOVA, $p>0.05$, $n=4$).

Final confirmation of the conversion of substrate to biogas was established by measuring the composition of biogas generated and the result is shown in Figure 3-7.

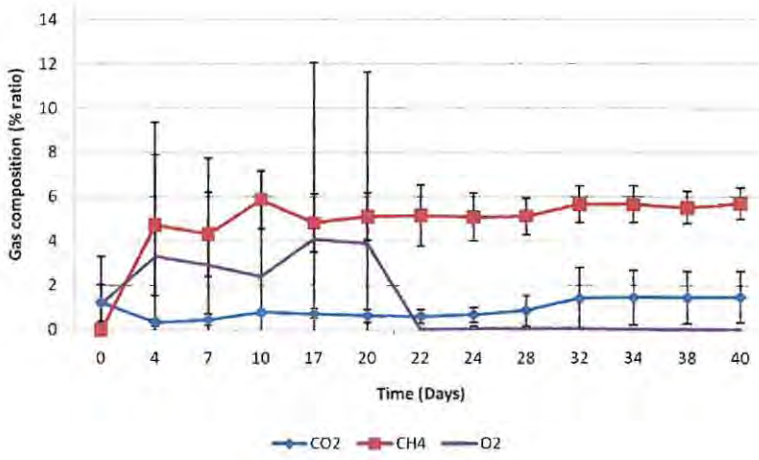


Figure 3-7: Composition of biogas generated in an anaerobic digester containing a consortium of methanobacteria supplied with sludge medium. Biogas was harvested using a 250 µl air tight syringe (Hamilton, South Africa) and analysed by gas chromatography as describe in Materials and Methods. Data are presented as % ratio of CO_2, O_2 and CH_4 for each of the four fermenters monitored over a period of 40days.

3.3.4 Microalgae Biomass Pre-treatment and its Effect on Anaerobic Digestion and Biogas Yield

Using the four fermenters established in section 3.3.3, the effects of various pre-treatment of HRAP biomass on the fermentation process was studied. It order to achieve this concentrated microalgae were prepared as described in section 3.2.2 and provided to each of the fermenter as a co-feedstock. The primary feedstock in each case was domestic waste water. The characteristics of feedstock utilised including COD, TC, TOC, sulphate and TN were monitored over the course of 62 d and the results are shown in Figure 3-8.

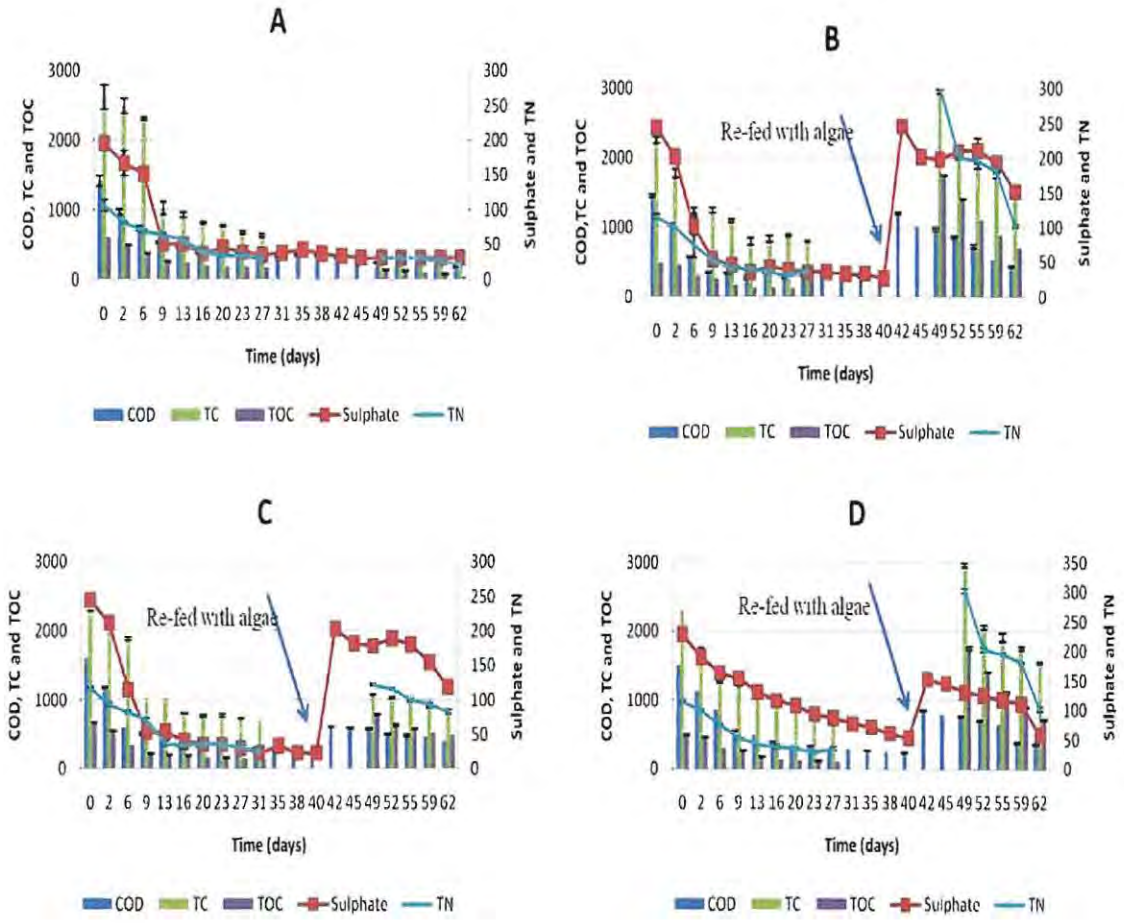


Figure 3-8: Characteristics of substrate utilization by a methanogenic consortium supplied sludge medium for methanobacteria. COD, TC, TOC, sulphate and TN depletion were monitored over the course of 62d as described in materials and methods. Each data point is the mean of 3 replicates ±SD. The pointer is an indication of when fermenter was re-fed with microalgae biomass

Each of the fermenters was allowed to acclimate for 40 d during which the largest decline in COD, TC, TOC, sulphate and TN occurred. Thereafter microalgae biomass with different treatment was introduced into each fermenter. The immediate response was a substantial increase in COD, TC, TOC, sulphate and TN as expected.

To further confirm pre-treatment of microalgae biomass as a co-substrate, the volume of biogas generated in each fermenter was quantified and the results are shown in Figure 3-9. The figure shows the cumulative volume of biogas in the fermenter over the course of 62 d.

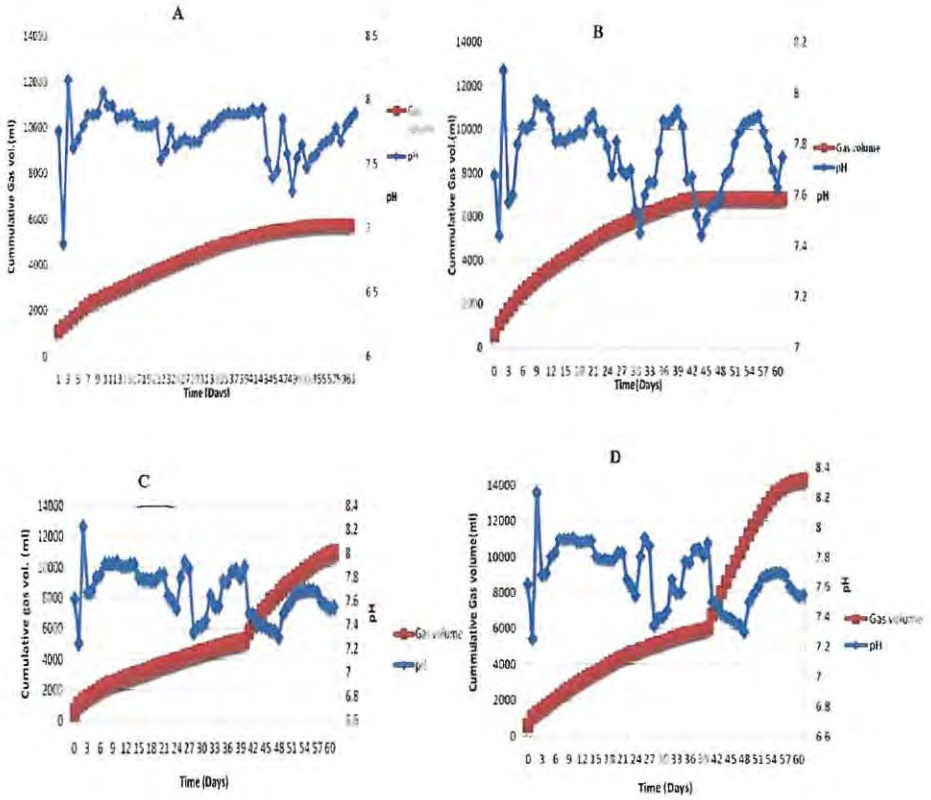


Figure 3-9: Cumulative volume of gas produced by the methanogenic consortium and pH readings in the fermenter over the course of 62 d. Volumetric measurement of gas was carried out by gasometer method; each data point is the mean of 4 replicates ±SD. The fermenters were re-fed with microalgae biomass on experimental day 42.

Biogas generation in the fermenters indicated the functionality of the system and suitability of microalgae biomass as co-substrate due to increase in volume after the addition of microalgae. Experimental pH remained within anaerobic range over the course of 62 d.

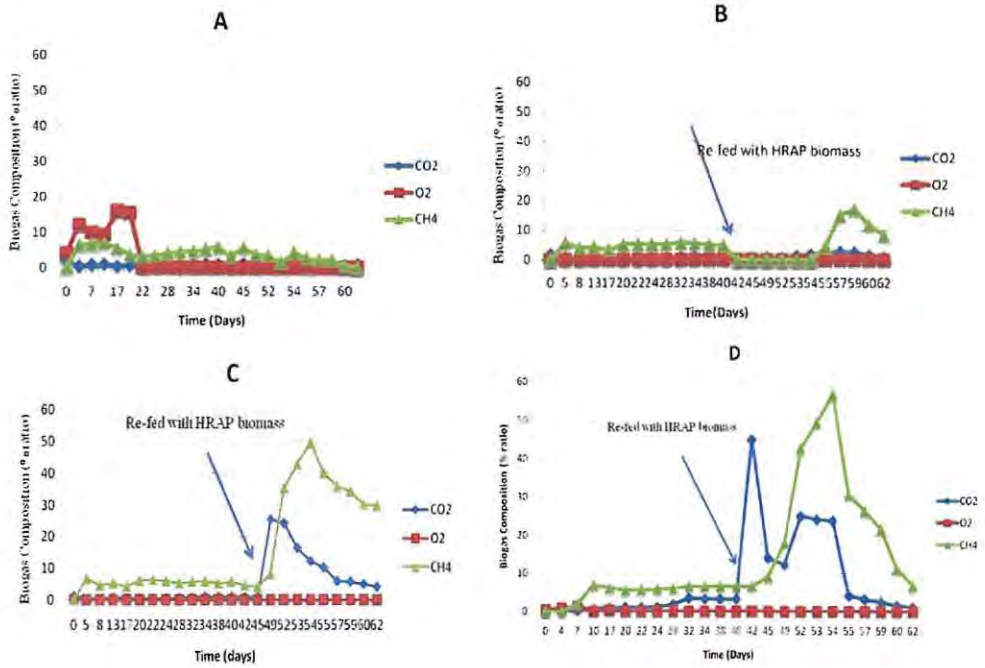


Figure 3-10: Composition of biogas generated in an anaerobic digester containing a consortium of methanobacteria supplied with sludge medium. Biogas was harvested using a 250 µl air tight syringe (Hamilton, South Africa) and analysed by gas chromatography as describe in Materials and Methods. Data are presented as % ratio of CO_2, O_2 and CH_4 for each of the four fermenters monitored over a period of 62days. The pointer is an indication of when fermenter was re-fed with microalgae biomass

3.4 Discussion

Anaerobic digestion is a multistep process with a number of microbial dependences and interrelationships. Scanning electron microscopy was used to determine the presence of an anaerobic microbial consortium obtained from the wastewater treatment anaerobic digesters. The micrographs indicated much similarity with methanogens which are mostly coccoid and rod shaped (Mardigan and Martinko 2006). These organisms also displayed the major characteristic of methanogens by generating biogas containing methane as an integral part of their energy metabolism.

In the anaerobic fermenters for biogas generation inoculation was carried out with 10% v/v of anaerobic consortium in order to avoid system overload and resultant volatile fatty acids (VFA) accumulation. As the microorganisms present in the fermenters act upon the nutrients available in form of carbon, organic carbon, nitrogen, sulphate in the substrate and enrichment media, end products in form of biogas were generated. The C/N ratios of the fermenters were at an average of 25:1 which is considered optimal in anaerobic fermentation process. Parkin and Owen (1986) stated the optimum C/N ratio for anaerobic digestion processes as 20:1-30:1(Parkin and Owen, 1986). Sulphate levels in the anaerobic fermenter were monitored in order to avoid accumulation of sulphate, sulphite and sulphide leading to high H_2S in the biogas. Since methanogens are the organism of choice for this experiment, the environment in the fermenter was made conducive for their growth.

The purpose of initially stabilizing the anaerobic system before the addition of microalgae biomass was to establish the anaerobic process for the anaerobic consortium to acclimatize. Prior to addition of microalgae into the anaerobic fermenters similar trend was noted in all fermenters. The first category of figures showed the chemical analyses carried out on the fermenter feedstock confirming the availability of nutrients required for microbial growth. The second category of graph is the quantification of gas generated with the 40 d experiment the highest gas volume recorded was 600 $mL.d^{-1}$ and the average gas measurement was 170 ±5 mLd^{-1} within the 40 d period. The experimental pH was recorded at a constant anaerobic pH of 6.8-7.9. The last category of graph illustrates biogas composition of the anaerobic fermenters. In all the fermenters, establishment of methanogenesis were evident between day 3 and 5 characterised by

the presence of methane in the biogas. The statistical analysis showed prior to addition of microalgae biomass to the fermenters, there was no significant difference amongst the four laboratory fed batch anaerobic reactors ($p > 0.05$).

Fermenter A was used as the control fermenter with no addition of microalgae biomass with CH_4:CO_2 ratio at a steady rate of 3.72:0.79%.

With the introduction of untreated concentrated microalgae collected directly from the HRAP, it was observed that after the reactor was re-fed, there was no remarkable difference in both volume of gas generated and CH_4 composition of biogas. The gas generation increased on day 42 at 120 mL/day and then stabilised only to resume gas generation on day 55 and presence of CH_4 was also detected by GC on the same day. Peak CH_4 ratio was recorded at 17.06% on day 59 and gas volume and CH_4 composition started to decline afterwards, the CH_4:CO_2 ratio was 17.06:2.52%.

When ruptured and concentrated microalgae biomass collected from the HRAP was used as co-substrate it was observed that after the reactor was re-fed, there was a remarkable and immediate increase in gas generation were noted coupled with gradual increase of CH_4 after day 42 of the experiment. Peak CH_4 was recorded at 49.56% on day 54, ~50% response in comparison to the control fermenter the average gas volume observed was 273 ±5 mLd^{-1} after addition of microalgae. The CH_4:CO_2 ratio on day 54 was 49.56:12.18%.

Use of microalgae harvested and concentrated and freeze dried using a bench top Freeze drier (VIS-TIR SLC, England) followed by rupture, it was observed that the response shown in the fermenter was over 100% compared to the control fermenter. Peak CH_4 was recorded at 56.55% on day 54 and the average gas volume observed was 394 ± $5mL.d^{-1}$ after addition of microalgae. The CH_4:CO_2 ratio on day 54 was 56.55:23.64%.

The results obtained indicate that addition of microalgae as a co-substrate increased biogas yield but that rupturing of the cell also increased CO_2 ratio of the biogas as seen from fermenters fed with ruptured microalgae. Although in fermenter B the volume of gas was not high but the quality of biogas yielded was better than that of fermenters C and D. The statistical analysis

proved that there was significant difference between the four fermenters after addition of microalgae (ANOVA, $p < 0.05$, $n=4$).

Thus, biomass generated from the EBRU HRAP can be used as a co-substrate as demonstrated above. However, more importantly when microalgae biomass was used as a co-substrate with domestic waste water, it had the potential to increase methane production by 600%. Furthermore, the use of the microalgae as a co-substrate in a 5 to 7 ML plant treating a domestic effluent of 75,000 – 80,000 L on a daily basis; there is potential to increase the methane generation from the waste stream and ability to unlock the potential by co-digestion with microalgae.

Chapter 4: Stoichiometry of Methane Production Potential by the Belmont Valley Waste Water Treatment Plant

4.1 Introduction

Globally the high dependence on fossil fuel coupled with political instability in oil producing countries is responsible for escalation in the fuel price (Yangin *et al.*, 2011). In view of the fact that most of the energy in use today is generated from fossil fuels and 60% of this accounts for the greenhouse gas emissions (Zamalloa *et al.*, 2010; Pöschl *et al.*, 2010), it is necessary to seek alternative sustainable energy that is environmentally friendly. Several efforts are being made towards seeking alternative energy. Geothermal energy, wind energy, kinetic energy, nuclear energy, solar energy and anaerobic digestion of wastes to generate biogas are examples of efforts that have contributed to seeking a cleaner and more sustainable supply of energy.

In anaerobic digestion, the waste is treated; organic matter reduced with no energy consumption, and biogas is generated (Foresti *et al.*, 2006). Biogas can be generated in various types of waste water facilities, some instances could be in independent fermenters like batch reactors, continuous stirred tank reactors, up flow packed bed reactors, fluidized bed reactors (Speece, 1983), and in fermentation pits which is a component of integrated algae ponding systems (IAPS). The Integrated algae ponding system is an emerging technology in South Africa and anaerobic digestion as a component can be applied in small rural communities to treat waste water and to harvest energy. The IAPS adopts a simpler, safer and less expensive method for domestic waste water treatment than the conventional mode of waste treatment (Oswald, 1989; Green *et al.*, 1995). The IAPS converts organic wastes into protein rich algal biomass by mainly solar energy producing a high quality effluent which is not greatly different from conventional waste water treatment plants for the treatment of domestic wastes (Oswald, 1989; Green *et al.*, 1995). Microalgae is utilised as a polishing stage in the HRAP component of the IAPS. As water is being treated, biogas is generated, by combusting the biogas the CO_2 released into the atmosphere will be further taken up by microalgae during photosynthesis (Pöschl *et al.*, 2010) consequently reducing the carbon footprint.

Fehrenbach *et al.* (2008) evaluated the production of biogas by anaerobic digestion and determined it to be one of the most environmentally beneficial technologies and energy efficient

bioenergy production systems (Weiland, 2010). The resourcefulness of biogas makes it a suitable renewable energy source which can be used for the replacement of fossil fuels in power and heat generation as well as transportation fuel (Weiland, 2010).

The generation of biogas from anaerobic digestion for combined generation of heat and power is a familiar technology that has been in existence for a long time. Pioneering work was done on farm scale for the upgrade of biogas for use as a vehicle fuel (Lampinen, 2004; Rasi *et al.*, 2007). An example is the Erkki Kalmari farm in Finland where a self-constructed biogas reactor system has been in operation since 1998 (Lampinen, 2004). The farm benefits economically by offsetting its own energy and fertilizer costs, and generates automotive fuel. Also in Germany, the number of biogas plants has increased by almost 2000 in the last 5 years, with an average output of 500 KW (Pöschl *et al.*, 2010). The government has developed an integrated energy and climate program for structured greenhouse gas reduction which focuses on increasing renewable energy utilization from 9.1% to 20% by the year 2020.

Biogas can serve as an economically sustainable fuel with a potential to drastically reduce emissions generated in road transport. Sweden is the first country in the world with a national standard for biogas as a vehicle fuel; this standard essentially states that the methane content of biogas must be higher than 95% with a set limit for dew points, sulphur content and other minor elements (Petersson, 2008). In 2006, Sweden had 12333 cars, 357 heavy cars, 787 buses that utilised upgraded biogas and the volume of biogas sold was as high as 54% (Figure 4-1) (Petersson, 2008).

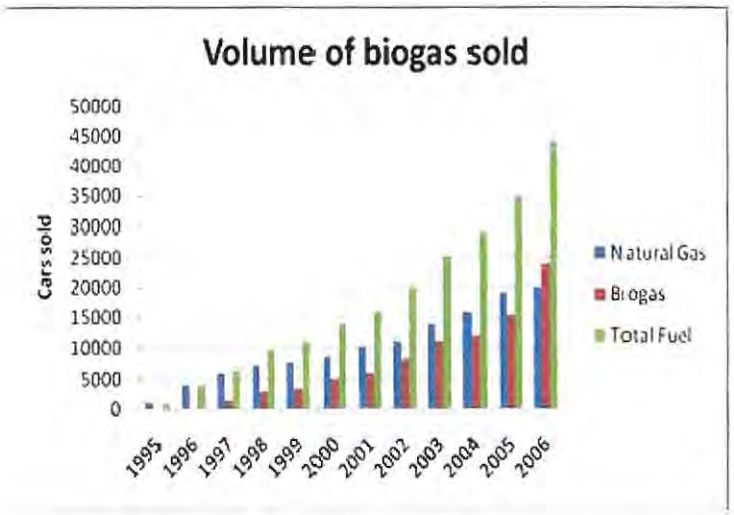


Figure 4-1: Increase in the sale of vehicles using natural gas, biogas and both by 2006 in Sweden (Petersson, 2008)

Apart from Sweden the successful use of biogas as a vehicle fuel has also been demonstrated in countries like France, Italy, Iceland, Switzerland and Germany (Petersson, 2008).

In this chapter, studies were carried out to evaluate the potential of the Belmont valley waste water treatment plant to produce sufficient CH_4 to power a fleet of vehicles. First, in view of the planned construction of a 0.5 ML IAPS by Makana municipality and funded by Partners for Water (The Netherlands), this project set out to determine the biomethane potential of an IAPS treating domestic waste water. Second, the total methane potential of the Belmont valley waste water treatment plant treating 5 ML of domestic effluent per day was estimated. Third, an evaluation of the potential to utilise the total biomethane produced at the Belmont valley plant as a transport fuel was carried out using data obtained from the transport division of Rhodes University, Grahamstown, South Africa. Results are discussed in the context of seeking a renewable fuel for transportation and the provision to municipalities of a viable means of reducing the annual cost of operating and maintaining a fleet of fossil fuel powered vehicles.

4.2 Materials and Methods

4.2.1 Elemental Analysis of Microalgae Obtained from the IAPS

Microalgae biomass yield of the EBRU pilot scale IAPS with details described in chapter 2 was quantified and its composition evaluated by elemental analysis. Waste water containing various

microalgae species was harvested from the EBRU HRAP pond and allowed to settle for 2 d using settling cones followed by harvest of the microalgae slurry by centrifugation at 3500 × g for 20 min. The biomass was dewatered by freeze drying using a bench top Freeze drier (VIS-TIR Bench top SLC). A powdery residue was obtained from the process.

Elemental analysis was carried out by loading 1 mg of the sample onto a boat sampler. The boat sampler was then moved into an Elementar vario micro cube bio-analytical instrument to determine the C, H, N, and O content. Helium served as the carrier gas in separating the analyte gases, moving the gases from the combustion zone at 1150 °C to the reduction zone at 850 °C.

4.2.2 Determination of Theoretical Methane Production Potential

In order to determine anaerobic biodegradability of some organics amendable to fermentation, their theoretical methane potential was calculated using Equation 4-1. Prediction of biogas (methane) potential is an important issue in anaerobic digestion, since the economy of existing biogas plants is dependent on the methane potential of the biomass used (Angelidaki *et al.*, 2011). Biochemical methane potential of a waste stream is the ultimate specific methane production possible from that stream and depends on the characteristic degradability and carbon oxidation state of the feedstock. Since different wastes have different methanogenic potential, the degradation time is considered indefinite (Angelidaki and Sanders, 2004; Angelidaki *et al.*, 2011).

Angelidaki and Sanders (2004) stated that methane potential can be expressed in different ways, per amount of wastes (L.CH_4/kg-waste), volume of wastes (L.CH_4/L-waste), per mass volatile solids added (L.CH_4/g-VS) or COD added (L.CH_4/kg-COD). The more reduced the organic carbon is, the more CH_4 will be produced depending on the degradability of the substrate (Equation 4-1) (Angelidaki and Sanders, 2004; Sialve *et al.*, 2009).

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

---Equation 4-1

The specific methane potential of some organic materials expressed in L.CH_4/g-VS and L.CH_4/g-COD was calculated (Equation 4-2 and 4-3) using 22.4 L as the volume of 1 mole of gas under standard conditions (273 K and 1 atm pressure). This exercise was carried out in order to

compare methane potential of microalgae to various organic materials. The results are presented in Table 4-4.

$$\text{STP}\frac{LCH4}{g-VS} = \frac{\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{3c}{8}\right)\ 22.4}{12n+a+16b+14c} \quad \text{---------- Equation 4-2}$$

$$\text{STP}\frac{LCH4}{g-COD} = \frac{\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}\frac{3c}{8}\right)\ 22.4}{\left(n+\frac{a}{4}-\frac{b}{2}+\frac{3c}{8}\right)\ 32} \quad \text{---------- Equation 4-3}$$

4.2.3 Data Collection

Data on vehicle usage was obtained from Rhodes University transport division for the financial year 2010 as a case study for evaluation of a vehicle fleet powered by biogas (Table 4-3). The data included number of vehicles, gasoline versus diesel consumption and distance travelled per annum in km/yr.

Data was provided on a per vehicle basis for petrol driven light motor vehicles and diesel driven commercial vehicles. Price of diesel and petrol used in this study was the respective average retail price in South Africa for the year 2011, June which was R10.07 per L petrol and R9.32 per L diesel.

4.2.4 Calculation of Maximum Theoretical Biogas Yield from the Belmont Valley Waste Water Treatment Plant

In the course of this study, it was impossible to obtain actual biogas yields from the Belmont Valley waste water treatment plant (WWTP) due to lack of biogas capture equipment on the independent anaerobic digesters and due to inconsistencies in operation and the maintenance thereof. In fact, for the duration of 2010 the digesters were decommissioned and in a state of repair. For these reasons, theoretical biogas yield from the WWTP was estimated. Equation 4-4 was developed for the calculation of maximum theoretical potential biogas yield from the

Belmont Valley WWTP treating a domestic waste water stream of 5 $ML.d^{-1}$ of which 0.08 $ML.d^{-1}$ diverts as influent for the EBRU IAPS. Secondly, maximum biogas yield from the EBRU IAPS with a waste stream of 0.08 $ML.d^{-1}$which yields 30 t/ha/yr biomass was calculated using Equation 4-5. Both equations were used to derive the total potential biogas yield from the waste water treatment facility. The equations were developed from the organic half reaction and Gibb's free energy for domestic waste water as shown in Equations 4-4 and that of microalgae biomass as shown in Equation 4-5 (Rittman and McCarty, 2001). The empirical formula of microalgae was obtained from the results of the elemental analysis carried out as described in Section 4.2.1. Parameters used for the calculation of maximum biogas potential of the Belmont Valley WWTP are presented in Table 4-1.

$0.02C_{10}H_{19}O_3N + 0.101H_2O \rightarrow 0.002C_5H_7O_2N + 0.115CH_4 + 0.051CO_2 + 0.018NH_4^+ + 0.018 HCO_3^-$ ----------**Equation 4-4**

$0.196CH_2O_{0.3}N_{0.1} + 0.092\ H_2O \rightarrow 0.0156C_5H_7O_2N + 0.115CH_4 + 0.047CO_2 + 0.018NH_4^+ + 0.018HCO_3^-$ ----**Equation 4-5**

Table 4-1: Parameters used in the calculation of maximum theoretical potential biogas yield from the Belmont Valley WWTP treating 5 $ML.d^{-1}$ of domestic waste water (Rittman and McCarty, 2001; Rose, *et al.*, 2002).

Parameters	Values
Generic empirical formula of domestic waste water	$C_{10}H_{19}O_3N$
Capacity of WWTP	5 ML
Organic loading (Maximum COD)	1000 $mg.L^{-1}$
IAPS influent	80 $m^3.d^{-1}$
Quantity of organic matter removed (80% efficiency)	64 $kg.d^{-1}$
Energy conversion efficiency (*f e*)	1
Cell synthesis (*f s*)	0.008

4.2.5 Energy Component and Economic Evaluation of Biogas Utilization.

For the calculation of energy equivalent of biogas to petrol, Equation 4-6 was used and for the calculation of energy equivalent of biogas to diesel Equation 4-7 was used (Yangin *et al.*, 2011). This was done in order to determine the energy component of biogas in comparison to petrol and diesel. Table 4-2 presents the parameters used for these calculations.

$[V \times t \times E_{biogas} \times (e \div \text{travel distance})] \div E_{petrol}$ ---- **Equation 4-6**

$[V \times t \times E_{biogas} \times (e \div \text{travel distance})] \div E_{diesel}$ ---- **Equation 4-7**

Table 4-2: Parameters and resulting values used in the calculation of energy equivalent of biogas to petrol and diesel.

Parameter	Values
Annual operation time of the WWTP	365 d
Energy value of biogas at a ratio of 70% (E_{biogas})	26.03 $MJ.kg^{-1}$
90% efficiency of petrol combustion (*e*)	0.048
90% efficiency of diesel combustion (*e*)	0.042
Energy equivalent of petrol (E_{petrol})	32.23 $MJ.L^{-1}$
Energy equivalent of petrol (E_{diesel})	40.70 $MJ.L^{-1}$
Energy equivalent of biogas to petrol	0.861 L/m^3
Energy equivalent of biogas to diesel	0.686 L/m^3

4.3 Results

4.3.1 Derivation of the Empirical Formulae for Microalgae Biomass

In order to calculate theoretical methane potential of feedstock amendable to anaerobic fermentation, empirical formulae were determined. For the study of anaerobic digestion of microalgae biomass and its suitability as feedstock, elemental analysis using atomic absorption spectrometry was carried out and the results are shown in Table 4-3. From the values obtained the empirical formula describing the microalgae biomass generated in HRAPs of the EBRU pilot scale IAPS was determined as $C_{1.0}H_{2.23}N_{0.095}O_{0.33}S_{0.003}$ based on the molar mass of the organic elements present.

Table 4-3: Percentage dry weight of C, H, N, S, O and the ash content of microalgae biomass. Analyses were carried out in triplicate, and recorded data are the mean of replicates (± SD).

Carbon Source	C (%)	H (%)	N (%)	S (%)	O (%)	Ash (%)
Microalgae	55 ±0.5	10 ±0.2	6.0 ±0.15	0.5 ±0.05	24 ±0.25	8.5 ±0.5

4.3.2 Calculation of the Theoretical Methane Production Potential by IAPS

Adopting Equation 4-1, theoretical product (H_2O, CH_4, CO_2) potential yields and ammonium released during anaerobic fermentation of some organic materials were determined. This was done in order to compare theoretical methane yield of other organic materials to microalgae. Furthermore, Equations 4-2 and 4-3 were used to calculate specific methane potentials of some organic materials in terms of $L.CH_4/g\text{-}VS$ and $L.CH_4/g\text{-}COD$, using 22.4 L as the volume of 1 mole of gas under standard conditions (273 K and 1 atm pressure). The empirical formulae,

potential product yields and specific methane production of the organic materials are presented in Table 4-4.

Table 4-4: Theoretical methane potential yield of some organic materials and the methane yields of microalgae biomass obtained from the EBRU HRAPs, expressed as $g.VS^{-1}$ and $g.COD^{-1}$

Organic matter	Empirical formulae	H_2O moles	CH_4 moles (%)	CO_2 moles (%)	NH_3 moles (%)	CH_4 yield $B_{0,th}$ ($STPgVS^{-1}$)	CH_4 yield $B_{0,th}$ ($STPgCOD^{-1}$)
Glycerol trioleate	$C_{57}H_{104}O_6$	4.675	6.6625 (70)	2.837 (30)	0 (0)	1.013	0.35
Ethanol	C_2H_6O	0	1.5 (75)	0.5 25)	0 (0)	0.730	0.35
Cellulose	$C_6H_{10}O_5$	1	3 (50)	3 (50)	0 (0)	0.414	0.35
Gelatine	$C_5H_7O_2N$	2.25	2.5 (42)	2.5 (42)	1 (16)	0.495	0.35
Acetate	$C_2H_4O_2$	0	1 (50)	1 (50)	0 (0)	0.373	0.35
Propionate	$C_3H_6O_2$	0.5	1.75 (58)	1.25 (42)	0 (0)	0.529	0.35
Microalgae [a]	$CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$	0.303	0.568 (51)	0.432 (39)	0.11 (10)	0.551	0.35
Waste water [b]	$C_{10}H_{19}O_3N$	3.75	6.25 (57)	3.75 (34)	1 (10)	0.696	0.35
Microalgae [c]	$C_{1.0}H_{2.23}N_{0.095}O_{0.33}S_{0.003}$	0.27	0.66 (60)	0.33 (31)	0.09 (9)	0.710	0.35

Note : Microalgae [a] – standard formula of microalgae (Grobelaar, 2004); waste water [b] – representative empirical formula of waste water (Rittman and McCarty, 2001); microalgae [c] – developed empirical formula of microalgae biomass obtained from the EBRU HRAP.

The characteristic of organic materials listed in Table 4-4 predicts potential biogas production and quality of waste's suitability for anaerobic degradation. This prediction evaluates the potential efficiency of the anaerobic process for wastes. The ratio of CO_2 and CH_4 is dependent on the oxidation state of the carbon present in the organic material. In most biological organic materials the CH_4 potential ranges from 40 to 70% (Table 4-4). This result shows the potential degradability of organic materials and their CH_4 yield, and it is deduced that microalgae from the HRAP can theoretically yield about 60% CH_4 as compared to other organic materials.

4.3.3 Biogas Yield from the Belmont Valley Waste Water Treatment Plant and its use as a Transportation Fuel

- Theoretical biogas yield

Equation 4-4 was used to estimate the theoretical biogas yield from 5 $ML.d^{-1}$ waste water treatments and Equation 4-5 was used for the estimation of biogas yield from microalgae biomass generated from the IAPS and the results are presented in Table 4-5. Since biogas yield could not be obtained directly from the Belmont Valley waste water treatment plant, the anaerobic digester was simulated in the laboratory as described in chapter 3. Microalgae biomass was used as co-substrate in these laboratory scale digesters for the purpose of increasing methane yield, 25% v/v microalgae slurry with 75% waste water was utilised to achieve this increase. An increase in biogas output of approximately 100% was achieved after concentrated microalgae biomass was added to the laboratory scale anaerobic fermenter and an increase of more than 500% was achieved following rupture of the microalgae before addition to the fermenters. In order to obtain the maximum theoretical yield of the IAPS, the same ratio (3:1) as described in chapter 3 was applied and the results are shown in Table 4-5.

Table 4-5: Maximum theoretical biogas yield from a WWTP treating a waste stream of 5 $ML.d^{-1}$

	Estimated value (m^3/yr)
Theoretical biogas yield from the Belmont valley waste water treatment plant	1,020,689.276 m^3/yr
Theoretical biogas yield from the IAPS where 25% microalgae biomass was used to supplement waste water	16,653.130 m^3/yr
Total theoretical energy yield from the WWTP	1,037,342.400 m^3/yr

The Belmont valley WWTP treats a waste stream of approximately 5 $ML.d^{-1}$ of which 0.016% goes to the EBRU IAPS as influent, Table 4-5 shows the total biogas that can be obtained from the WWTP.

- Biogas as transportation fuel

Data obtained from Rhodes University transport division was summarised and is presented in Table 4-6. The table shows the number of vehicle on petrol and diesel, including kilometres travelled per annum by the vehicles.

Table 4-6: Summarised data obtained from Rhodes University transport division for both petrol (105 vehicles) and diesel (31 vehicles) usage for the year 2010, including the energy values and densities of petrol, diesel and biogas.

	Petrol	Diesel	Biogas equivalent for petrol consumed	Biogas equivalent for diesel consumed
Volume of fuel consumed (L/yr)	113,227.10	46,076.60	131,506.50 m^3/yr	67,167.05 m^3/yr
Travel distance (km/yr)	2,103,555.00	983,628.00	3,051,007.06	987,945.14
Total cost (R/yr)	1,140,196.90	429,433.91	-	-
Energy value (MJ/L)	32.23	40.70	26.03 MJ/m^3	
Density (g/m^3)	0.673	0.850	0.97 kg.m^3	

Given that 0.34 m^3 of biogas travels 1 km in petrol powered vehicles and 1.05 m^3 of biogas travels 1 km in diesel powered vehicles.

From Table 4-2, 0.861 L of petrol is equivalent to 1 m^3 of biogas. Since 113,227.10 L of petrol was used to drive the University's vehicle, hence, 131,506.50 m^3/yr of biogas is required to substitute for the petrol used in petrol driven vehicles. Similarly, 0.686 L of diesel is equivalent to 1 m^3 of biogas as shown in Table 4-2. Thus, and from Table 4-6, 46,076.60 L of diesel was used to drive the University's diesel powered fleet, hence, 67,167.05 m^3/yr of biogas would be required to substitute for the diesel used by these vehicles. In total, 198,673.55 m^3 of biogas will be required to fuel the Rhodes University's fleet vehicles. From Table 4-5, the total potential biogas yield from the WWTP was estimated to be 1,037,342.40 m^3/yr. Thus, more biogas than is required to power the university vehicle feet can be produced on site at the Belmont Valley WWTP. Furthermore, by offsetting the biogas needed to drive the University's vehicles there will be a residual of biogas stream of 838,668.85 m^3/yr.

From Table 4-6, total cost of fuelling the university's fleet of vehicle is R1,569,630.812 per year, therefore, if the use of biogas were adopted by the University, this amount in Rands/yr will be saved on fuel cost. Since the biogas is essentially free, only the cost of installing a capture and storage device should be put into consideration. The residual biogas from the WWTP can be considered for combined heat and power of the WWTP site, electricity generation or it can be sold to generate additional income.

The domestic waste water treatment in Belmont Valley will allow for the production of biogas where by only 25% of microalgae biomass is needed to boost biogas generation. The total

amount of energy that can be obtained was determined to be 1,037,342.40 m^3/yr, and the distance that can travelled on biogas in light duty cars was 3,051,007.06 km/yr and 987,945.14 km/yr in heavy duty commercial vehicles.

4.4 Discussion

In South Africa, current energy production is heavily dependent on imported natural gases and locally mined low grade coal, consequently resulting in the urgent need for environmentally friendly and sustainable energy. Biogas which is vented to atmosphere is a by-product of anaerobic digestion, a process commonly practised at WWTP in South Africa and one of the most significant renewable energy sources. The practical biogas yield obtained in an anaerobic digester will always be lower than the theoretical values due to many reasons, but under favourable temperatures and high COD concentrations in wastewater a degree of conversion as high as 90-95% can be achieved. The reasons for low practical yield might be that some of the substrate remains unconverted in the effluent, there may be nutrient limitation, some CO_2 component of the biogas could dissolve in water to form bicarbonate and a fraction of the substrate can be used to synthesise biomass (Franco *et al.*, 2007).

Focusing on biodegradability of some organic materials, specific methane potential of these organic materials was predicted. The use of biogas generated for the IAPS was evaluated as transportation fuel. Methane yield was determined in terms of COD based on the fact that 1 kg of COD destroyed yields 0.35 m^3 at standard temperature and pressure. From empirical formula of microalgae the methane yielded at STP $g.COD^{-1}$ is 0.35 for both carbon sources and the same value even in some of the organic materials studied. From the elemental analysis, the empirical formula of microalgae yielded $C_{1.0}H_{2.23}N_{0.095}O_{0.33}S_{0.003}$ which is similar to the Formula of microalgae generated by Grobbelaar (2004). This microlaage was predicted to yield 60% CH_4 which is a good comparison to other organic materials. Methane potential in some organic materials was predicted, ethanol had the highest methane potential of 75% followed by lipid with 70% and the remaining ranged between 42 – 58%. This was achieved by in-cooperating their empirical formula into the modified formula of Symons and Buswell (1933) adapted by Angelidaki (2004) and Sialve (2009).

Energy equivalent of biogas in comparison to petrol and diesel was calculated; 0.861 L of petrol and 0.686 L of diesel is equivalent to 1 m^3 of biogas. Using the Rhodes University fleet of vehicles, the use of petrol and diesel were substituted for biogas usage. This was achieved by theoretical biogas yield from the WWTP with a waste stream of 5 ML and a biogas yield of

1,037,342.40 m^3/yr. From the data obtained from the University's transport division and the total biogas yield from the WWTP, the use of petrol and diesel can be substituted for biogas in the Rhodes University fleet of vehicle. The domestic waste water treatment in Belmont Valley will allow for the production of biogas where by only 25% of microalgae biomass is needed to boost biogas generation. The total amount of energy that can be obtained was determined to be 1,037,342.40 m^3/yr, and the distance that can travelled on biogas in light duty cars was 3,051,007.06 km/yr and 987,945.14 km/yr in heavy duty commercial vehicles.

Owing to its renewable energy sources biogas has a real potential to replace fossil fuels. Biogas utilisation as vehicle fuel proves to be the best environmentally means of biogas combustion. Theoretically, the biogas generated by the Belmont valley WWTP has the potential to fuel the University campus fleet as demonstrated in this chapter. The use of biogas by the University fleet of vehicle will not only cut cost but also reduce carbon footprint provided the system is in full function.

Chapter 5: General discussion and conclusion

Since the beginning of the industrial era in the late 18th century, the global demand for energy has been on the rise. The demand for energy at present supersedes its rate of supply due in part to depletion of available fossil fuel resources. Increasing threat of global climate change is attributed to greenhouse gas (GHG) emissions from fossil fuel usage creating uncertainty regarding sustainability of these conventional fuels (Brennan and Owende, 2010; Rao *et al.*, 2010). This uncertainty is further compounded by finitude of the resource coupled with negative effects of the liberated CO_2. With the increase in GHG emissions mainly due to combustion of fossil fuel, it has become increasingly important to develop techniques for reprieving the excess CO_2 and minimise the impact of global warming (Mussgnug *et al.*, 2010). The alternative to fossil fuel needs to be sustainable and environmentally friendly.

In recent years, numerous ideas have been considered in order to develop environmentally friendly and cleaner energy alternatives. Amongst these are: geothermal energy, wind energy, kinetic energy, nuclear energy, solar energy (Mussgnug *et al.*, 2010) and anaerobic digestion of wastes. Based on anaerobic digestion of organic wastes, a most important renewable energy resource which is biogas is produced. Emerging technologies produce biogas in stand-alone fermenters and as component of a waste water treatment facility. An example of biogas production by digesters that are part of a whole system is an Integrated Algae Ponding System (IAPS).

In a world of ever increasing population, deteriorating environment, and vanishing resources, the IAPS could play a vital part in the management of liquid waste. Environmental concerns are further complicated by limited availability of arable land, fossil fuel depletion and disposal of generated wastes. This multi-faceted treatment reclamation process provides a solution to waste disposal in conjunction with the production of an alternative energy source without necessarily using arable lands (Oswald, 1995). Eutrophication is avoided because treated water is discharged after passing through the biological water treatment. Considering biomass derived energy as an alternative is a favourable option due to its renewable concept. This process not only tackles the problem of climate change by CO_2 capture, provision of energy for power, heating and transportation, and liquid waste treatment, it also curbs the use of conventional fossil fuel (Sterling *et al.*, 2001).

In countries like Turkey, where energy production is heavily dependent on imported gasoline and coal resulting in high prices of gasoline and diesel, renewable energy recovery by anaerobic digestion is gaining vital importance (Yangin *et al.*, 2011). The need for clean renewable energy is more acute in developing countries. An Agama energy sheet estimates that in South Africa, there are 400,000 households with no electricity, 27% of the population have no access to clean water and 66% have poor sanitation facilities. The key drivers for the renewable energy market are the social and economic imperatives of access to affordable, safe and sustainable energy sources for effective operation of households and communities at large by creation of job opportunities. Although the market for renewable energy is not well defined in the Eastern Cape Province of South Africa (Agama energy, 2009), by installation of integrated algae ponding system plants in local communities, these problems can be tackled, sanitation will be improved, clean water will be provided and energy will be generated at the same time. This intervention provides in addition significant benefits to human and ecosystem health.

From the investigation, it was deduced that the cumulative biogas measured was 2340 $L.d^{-1}$ with a CH_4 composition of 1.590 $kg.d^{-1}$ measured as yield from the IAPS fermentation pit. Biogas production was quite stable with the highest CH_4: CO_2 ratio of 85:7% and the lowest ratio was 60:7%. The methane ratio shows that this system was producing a significantly higher percentage of methane than similar systems (Rasi *et al.*, 2007). The results obtained indicate that addition of ruptured microalgae biomass as a co-substrate with domestic waste water increases biogas yield by 600%. Theoretically, the biogas generated by the Belmont Valley WWTP has the potential to fuel the Rhodes University fleet. The total amount of energy that can be obtained was determined to be 1,037,342.40 m^3/yr, and the distance that can travelled on biogas in light duty cars was 3,051,007.06 km/yr and 987,945.14 km/yr in heavy duty commercial vehicles.

The study of the IAPS is very important for full understanding of the functioning of the system. It is recommended that the activity of a digester should be investigated before further studies continue especially in long dormant or exposed reactors. The water treatment capacity and the nutrient removal trend of the IAPS was also investigated, increase in biomass is linked to a 60% decline in nutrients that is acceptable for treatment of water under South African standards. The result confirms the efficiency of IAPS in the treatment of domestic waste as well as generation of

biomass. Results from the characterisation of the biogas obtained from IAPS shows an average ratio of 75 ± 3% CH_4, which indicates a good biogas yield from an IAPS.

In order to enhance the biogas generating process, microalgae biomass was used as a co-substrate in anaerobic fermentation of waste water. Microalgae with their simple growth requirement, rich composition (lipid, protein and carbohydrate) and lack of competition for arable land are the biomass of choice as co-substrate. Yen and Brune (2006) achieved significant increase in methane production by adding microalgae biomass to waste paper. The production rate doubled when 50% microalgae was added to 50% waste paper (1170 ±75 $mL.L^{-1}$ day, in comparison to 573 ±28 $mL.L^{-1}$ of algal sludge alone) (Yen and Brune, 2007). In the present research, maximum methane yield was observed when ruptured microalgae was added to laboratory scale fed batch anaerobic fermenters to supplement the use of waste water as substrate. The average biogas production before addition of microalgae was 170 ±5 $mL.d^{-1}$ in comparison to an increase in yield of 262.33 ±5 $mL.d^{-1}$ after addition of concentrated microalgae biomass with a 100% increase in CH_4 production. The highest increase in yield was observed when concentrated microalgae biomass cells was ruptured before addition into the fermenter, over 500% increment in CH_4 ratio was observed.

Methane potential of 60% was derived from the empirical formula of microalgae ($C_{1.0}H_{2.23}N_{0.095}O_{0.33}S_{0.003}$) produced in the EBRU HRAP. Methane potential of some organic materials amendable to fermentation was also predicted, with CH_4 ratio ranging from 75 – 40%. Theoretically, the biogas generated by the Belmont valley WWTP has the potential to fuel the University campus fleet of vehicle as shown in chapter 4. The use of biogas by the University fleet of vehicle will not only cut cost but also reduce carbon footprint provided the system is in functional. The residual biogas from the WWTP can be sold for extra income, and also to generate electricity, heat and power on site, which will eventually reduce the overall cost of running the WWTP.

Biogas is an attractive alternative to fossil fuels, the use of fossil fuel cannot be completely eliminated but it can be supplemented by the use of biogas. Figure 5-1 represents a proposed pathway for the integration of other processes into the IAPS in order to improve its output. By passing the biogas through an upgrading device, CO_2, H_2S, moisture and other impurities will be

removed from the gas resulting in high methane content. The upgrade step is important for use in vehicles designed to function on natural gas so as to strip H_2S in order to avoid corrosion of engines. In the proposed pathway, algae biomass harvested from the HRAPs can be used as fertilizer. An ultra sonicator will serve as an onsite mechanical treatment for microalgae biomass harvested from the HRAP. This machine will effectively rupture the microalgae biomass to liberate the cell content and in the presence of an active catalyst generate a large amount of ethanol and other organic solvents like acetone and butanol. The biogas and ethanol generated can be used as transportation fuel by the municipality. The final effluent from the system can be used for irrigation and recycled for domestic use by the community (Figure 5-1).

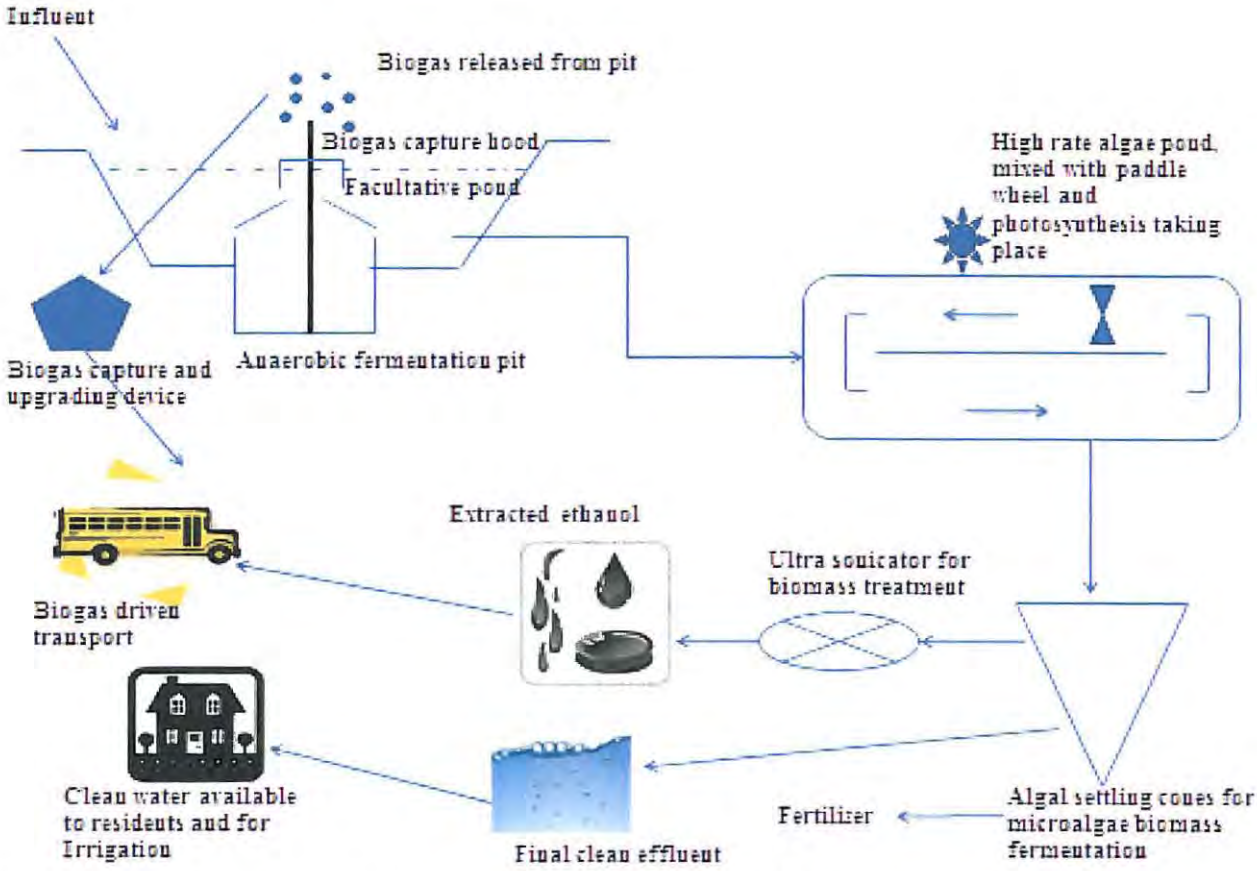


Figure 5-1: A proposed integration process for capture and upgrade of biogas, ethanol generation as well as treatment of waste water for domestic use.

5.1 Future recommendation

The present research focuses on biomass to energy, the aspect of optimisation of biogas yield involved supplementing the influent with microalgae. This objective was achieved on a laboratory scale judging by the positive response recorded. Although this can be taken further by actual incorporation of harvested microalgae biomass into the fermentation pit and utilization of concentrated microalgae as the sole substrate for biogas generation. For the ethanol fermentation phase, it was demonstrated that HRAP biomass can yield very little quantity of ethanol; this response can be increased by employing a wider range of catalysts sourced from different origins. It is recommended that ethanol generation should be integrated into the whole process for full effective conversion potential of biomass to energy. This integration should also include an on-site industrial ultra sonicator for pre-treatment of biomass. Future work on biogas generation from the IAPS should involve capture and combustion of biogas generated since it has been demonstrated that the biogas can drive the campus fleet. Methods for upgrading the biogas in order to upgrade it to an acceptable standard should also be given consideration.

5.2 Conclusion

Judging by the huge carbon foot print generated from the utilization of conventional fuels, renewable energy sourced from biomass plays a vital role in the reduction of this pollution. The viability of the process has been demonstrated in this research, and use of the proposed integrated process (Figure 5-1) should be adopted by developing countries in order to tackle both sanitation and energy problems. South Africa is faced with the problem of potable water, by adapting this process, not only would there be access to clean water, renewable energy will be simultaneously generated resulting in a cleaner environment leading to better health.

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Appendices

Appendix 1

Appendix 1-A: Examples of some organic materials amenable to anaerobic biotechnology (Speece, 1983)

Acetaldehyde	Formic acid	Isopropyl alcohol	Giant kelp
Acetic anhydride	Fumaric acid	Propionate	Animal wastes
Acetone	Glutamic acid	Propylene glycol	Cheese whey
Acrylic acid	Gluteric acid	Protocatechuic acid	Pear wastes
Adipic acid	Glycerol	Resorcinol	Pectin wastes
Aniline	Hexanoic acid	Sec-butanol	Meat packing
1-amino-2-propanol	Hydroquinone	Sec-butylamine	Corn milling
4-amino butyric acid	Isobutyric acid	Sorbic acid	Dairy
Benzoic acid	Isopropanol	Syringaldehyde	Brewery
Butanol	Lactic acid	Syringic acid	Rum distillery wastes
Butyraldehyde	Maleic acid	Succinic acid	Wine distillery wastes
Butylene glycerol	Methanol	Tert-butanol	Guar gum wastes
Catechol	Methyl acetate	Vanillic acid	Water soluble polymers
Cresol	Methyl acrylate	Vinyl acetate	Bean blanching
Crotonaldehyde	Methyl ethyl ketone	Corn	Pulp mill evaporate
Crotonic acid	Methyl formate	Potato	Coking mill
Diacetone gulusonic acid	Nitrobenzene	Sugar cane	H_2-CO pyrolysis
Dimethoxy benzoic acid	Pentaerythritol	Bagasse	Wool scouring
Ethanol	Pentanol	Peat	Tannery wastes
Ethyl acetate	Phenol	Wood	Yeast
Ethyl acrylate	Phthalic acid	Corn stover	Heat-activated sludge
Ferulic acid	Propanal	Straw	
Formaldehyde	Propanol	Water hyacinth	

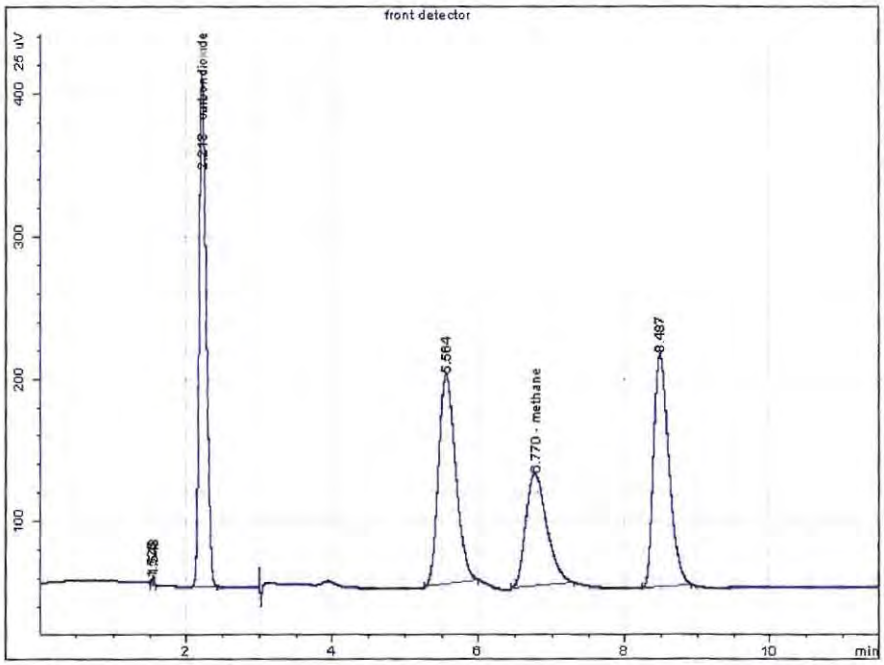
Appendix 1-B: Characteristics of some methanogenic *Archaea* (Madigan and Martinko, 2006)

Methanobacteriales	Morphology	Substrates for methanogenesis	DNA (mol %)
Methanobacterium	Long rods	H_2+CO_2, formate	30-55
Methanobrevibacter	Short rods	H_2+CO_2, formate	27-31
Methanosphaera	Cocci	Methanol + H_2	23-26
Methanothermus	Rods	H_2+ CO_2	33
Methanothermobacter	Rods	H_2 + CO_2, formate, thermopiles	32-61
Methanococcales			
Methanococcus	Irregular Cocci	H_2+CO_2,pyruvate+CO2, formate	29-35
methanotermococcus	Cocci	H_2+CO_2, formate	31-34
Methanocaldococcus	Cocci	H_2+CO_2	31-33
Methanotorris	Cocci	H_2+CO_2	31
Methanomicrobiales			
Methanomicrobium	Short rods	H_2+CO_2, formate	49
Methanogenium	Irregular cocci	H_2+CO_2, formate	47-52
Methanospirilum	Spirilla	H_2+CO_2, formate	45-50
Methanoplanus	Plate shaped cells	H_2+CO_2, formate	39-50
Methanocorpusculum	Irregular cocci	H_2+CO_2, formate,alcohols	48-52
Methanoculleus	Irregular cocci	H_2+CO_2, alcohol,formate	49-61
Methanofollis	Irregular cocci	H_2+CO_2, formate	54-60
Methanolacinia	Irregular rods	H_2+CO_2, alcohol	38-45
Methanosarcinales			
Methanosarcina	Large irregular cocci in packets	H_2+CO_2,methanol,methylamines, acetate	36-43
Methanolobus	Irregular cocci in aggregates	Methanol, methylamines	39-46
Methanohalobium	Irregular cocci	Methanol, methylamines:halophiles	37
Methanococcoides	Irregular cocci	Methanol, methylamines	42
Methanohalophilus	Irregular cocci	Methanol, methylamines,methylsufides;halophile	39-41
Methanosaeta	Long rods to filament	Acetate	52-61
Methanosalsum	Irregular cocci	Methanol,methylamines,dimethylsulfide	38-40
Methanopyrales			
Methanopyrus	Rods in chains	H_2+CO_2:hyperthermophile,growth @110^{0}C	60

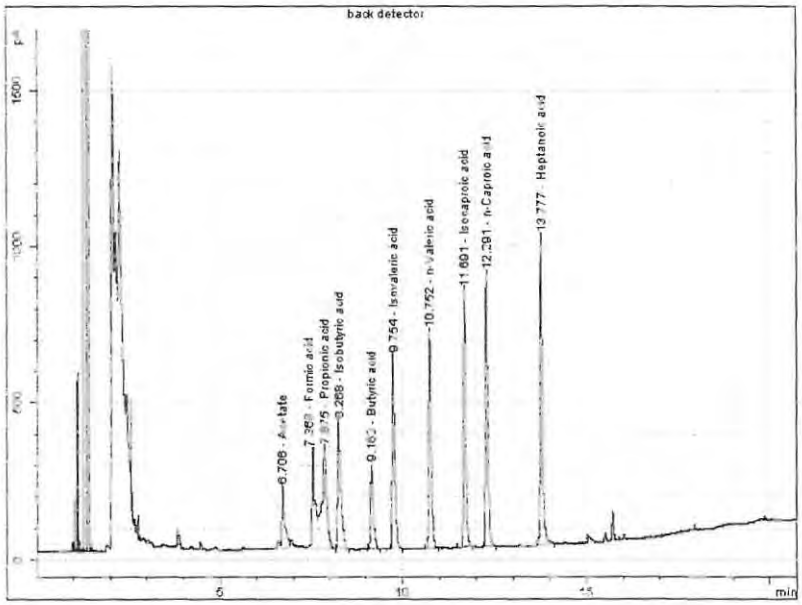
Appendix 1-C: Biochemical Composition of several species of microalgae (Sialve *et al.*, 2009)

Species of microalgae	Lipids (%)	Carbohydrates (%)	Proteins (%)
Euglena gracilis	14-20	14-18	39-61
Chlamydomonas reinhardtii	21	17	48
Chlorella pyrenoidosa	2	26	57
Chlorella vulgaris	14-22	12-17	51-58
Dunaliella salina	6	32	57
Spirulina maxima	6-7	13-16	60-71
Spirulina platensis	4-9	8-14	46-63
Scenedesmus obliquus	12-14	10-17	50-56

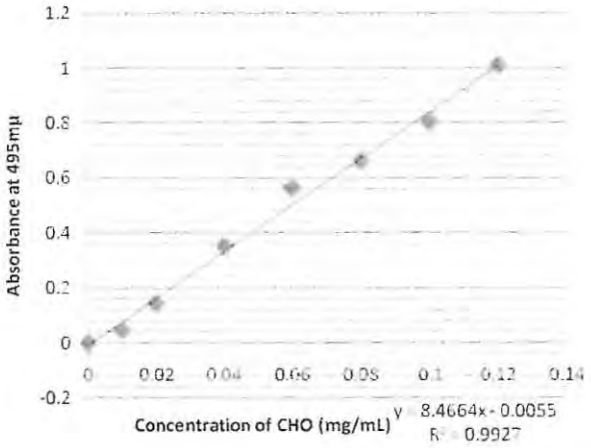
Appendix 2



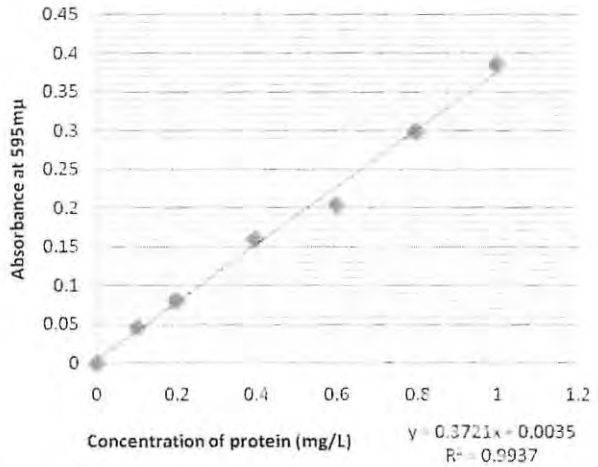
Appendix 2-A: Chromatograms of standard mix (Afrox South Africa) used in the identification of gases generated in the anaerobic fermenters. The STD gas mix contains 20% CO_2 (first peak); 20% N_2 (second peak); 20% CH_4 (third peak); 20% CO and 20% H_2S. The GC elutes the H_2S in the gas in order to avoid corrosion in the equipment.



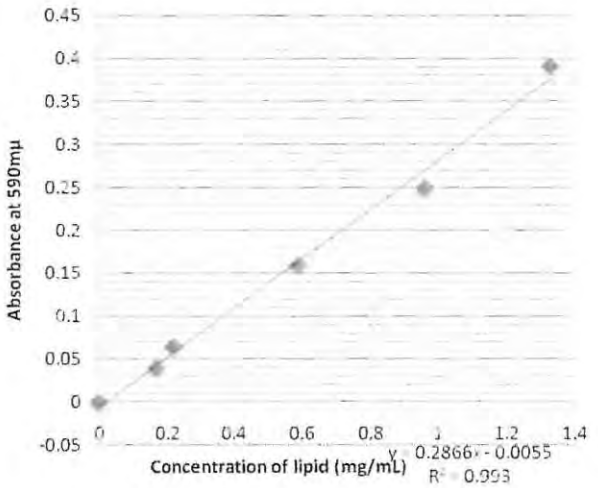
Appendix 2-B: Volatile fatty acid standard, the chromatograms shows fatty acid from C1-C7 acid used in the identification of VFA generated in the fermenter



Appendix 2-C: Carbohydrate standard curve, D-glucose was used as the standard



Appendix 2-D: Protein standard curve, bovine serum albumen (BSA) was used as the standard



Appendix 2-E: Lipid standard curve, palmitic acid was used as the standard

Appendix 3

Appendix 3-A: Composition and quantity per litre of Yeast peptone dextrose broth used to culture pure strains of brewers' yeast.

Composition	Quantity (g/L)
Yeast extract	10
Peptone	20
Glucose	20

Appendix 3-B: Quantity per litre of composition of Sludge Media for Methanobacteria and their function in the cell (Atlas, 1993)

Composition	Quantity per Liter	Function in the cell
$NaHCO_3$	4.00g	Contains a source of organic carbon
Sodium formate	2.00g	Necessary for enzymes and also carbon source
Sodium Acetate	1.00g	Necessary for enzymes and also carbon source
Yeast extract	0.50g	Growth factor
L-Cysteine.HCL.H_2O	0.50g	Source of vitamins
KH_2PO_4	0.50g	Contains P for nucleic acid and phospholipids
$Na_2S.9H_2O$	0.40g	Contains S source of vitamins
$MgSO_4.7H_2O$	0.40g	Mg stabilizes ribosome, cell membrane and nucleic acids
NaCl	0.40g	Necessary for enzymes
NH_4Cl	0.40g	Major element in nucleic acid and amino acids
$CaCl_2.2H_2O$	0.05g	Stabilizes bacterial cell wall
$FeSO_4.7H_2O$	0.002g	Present in cytochromes
Resazurin	0.001g	Absorbs O_2 to create an anaerobic environment
Sludge Fluid	50.0mL	Growth factor
Trace element solution.SL-6	1.0mL	Micronutrients necessary for specific enzymes