

Enhancing Methane Production in the UK WWTP via Co-digestion of Microalgae and Sewage Sludge

by

Oladipo Adebayo Adewale

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Dedication

This work is dedicated to all my colleagues who dropped out of PhD for several reasons.

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Abstract

The study investigated the possible integration of microalgal digestion into existing WWTP configurations to benefit from the existing infrastructure. This was with the overall aim of increasing energy production. In particular, it was aimed at plants that utilise advance anaerobic digestion with the thermal hydrolysis process (THP) in place, as this is hypothesized to enhance algal degradability and overall CH₄ yield while simultaneously producing biomass suitable for co-digestion with sewage sludge.

The effect of the existing THP was studied on the *Chlorella vulgaris* adopted for the study and results showed a 35% increase in methane yield from 0.265 to 0.357L CH₄ /g VS_{added} suggesting the possibility of this approach to upgrade microalgae to a competitive standard with the likes of food waste and FYM. It was then proposed for the first time, the co-digestion of pre-treated microalgae with sewage sludge thus, several co-digestions (0%, 25:75, 50:50, 75:25 and 100%) alga: sludge respectively were studied. Results showed the addition of pre-treated alga to sludge had a linear relationship to methane yield obtained up to a ratio of 75% algae with a corresponding methane yield of 0.369L CH₄ /g VS_{added}.

Based on this, laboratory scale CSTR were developed to identify the possible operational parameters and challenges that can favour and be encountered during the continuous running of these substrates. A co-digestion ratio of 75:25 with an OLR of 4 g VS L⁻¹d⁻¹ and HRT of 20 days produced the highest methane yield of 0.434L CH₄ /g VS_{added} suggesting a balance between substrate thus favouring methanogenic activities.

The study goes further to investigate alternative biofuel production using the carboxylate approach under which the influence of iodoform, pre-treatment and retention time on the possible VFA yield and production was studied. During the study, optimum iodoform for complete methanogenic inhibition in order to avoid conversion of the produced VFA into methane gas was reported at 10mg/L. Experiments were then carried out to investigate the respective co-digestion studied in this research under the carboxylate platform experiment, peak VFA concentrations ranged between 6.01 and 6.94 g/L, highest VFA concentration was produced in the

50:50 (alga:sludge) fermenter with a corresponding yield of 0.992 g total acid/g VS fed and a retention time of 11 days, this was seconded by 25:75 (alga:sludge) with a yield of 0.952 g total acid/g VS fed and a varying retention time of 17 days.

Comparing both approaches investigated in this study, the carboxylate study proved a significant increase in VFA yield over the AD experiments with an increase between 92 and 166% for all tested co-digestion ratios. Conclusively, it could be inferred from the results in the study that the anaerobic fermentation of WAS and microalgae at several co-digestion ratios to produce VFAs may be an alternate option to methane production. While 75:25 proved to be the most optimal at an OLR of 4 g VS L⁻¹d⁻¹ and HRT of 20 days in the AD experiments, 50:50 (alga:sludge) proved to be the most effective co-digestion for carboxylate production. Retention time under this platform however ranged between 11 and 20 days.

The anaerobic digestion of microalgae using the existing facilities installed in the WWTP presents a positive energy balance for a WWTP about the size of Esholt UK, serving population of about 700,000 and currently treating about 80 tonnes dry sludge daily, where each possible tonne of microalgae produced and digested within the system would gain 1926 MJ (535 kWh). Not only will this implementation of technology help produce additional revenue from the extra biogas produced, it is also expected to offset some of the energy expended in wastewater treatment via utilization of the nutrients in the digestate and reducing nutrient load being recycled back for wastewater treatment.

The production of alternative products such as acetic acid and ethanol using a modified AD system will lead to an energy profit of 3536 MJ/tonne (982 kWh) and 6654 MJ (1848 kWh) respectively per ton of microalgae, suggesting an improvement by 1.8 – 3.4 times the methane production. The study concludes with some possible areas recommended for further study to achieve full exploitation of these techniques/resources at a commercial level.

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List of Acronyms

AD	Anaerobic digestion
BMP	Biochemical methane potential
BMW	Biodegradable Municipal Waste
BNR	Biological nutrient removal
BVS	Biodegradable Volatile solids
CHP	Combined heat and power
CNG	Compressed natural gas
COD	Chemical oxygen demand
CST	Capillary Suction Time
CSTR	Continuous-stirred tank reactor
DEFRA	Department of Environment Food and Rural Affairs
EU	European Union
FIT	Feed in Tariffs
FW	Food waste
FYM	Farm Yard Manure
GC	Gas chromatography
GHGs	Greenhouse gases
HAc	Acetic acid
HRT	Hydraulic retention time
IPCC	Intergovernmental Panel on Climate Change
LATS	Landfill Allowances Trading Scheme
MAD	Mesophilic anaerobic digestion
MAS	Microalgae Systems
MSW	Municipal solid waste
MR	Methanogenic reactor
NER	Net energy ratio
OFMSW	Organic fraction municipal solid waste
OLR	Organic loading rate
RHI	Renewable Heat Incentives
ROC	Renewable Obligation Certificate
RPM	Rotations Per Minute

RVS	Refractory Volatile solids
SCOD	Soluble Chemical Oxygen Demand
SD	Solubility Degree
SELR	Specific Energy Loading Rate
SEM	Scanning Electron Microscopy
SRT	Solid retention time
STP	Standard temperature and pressure
TAN	Total Ammonia Nitrogen
TCOD	Total Chemical Oxygen demand
TEP	Total Energy Potential
THP	Thermal Hydrolysis Pre-treatment
TKN	Total Kjeldahl Nitrogen
TS	Total solids
UK	United Kingdom
UN	United Nations
VFA(s)	Volatile fatty acid(s)
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant(s)

Chapter 1 Introduction

1.1 Background

The UK water industry is currently facing a series of challenges driven mainly by stringent water quality regulations, population growth, climate change and its mitigation, and an increasing debt in order to meet such commitments (Palmer, 2010). Over the last 20 years, UK water companies have accrued debt to around £33bn; as a consequence, water bills have also increased up to 45% in real terms (Ofwat, 2011). Greenhouse Gases (GHG) produced in the sector on the other hand are estimated to contribute up to 1% of the national emissions and in order to meet current targets set by the UK government (i.e., reducing GHG emissions by 34% based on 1990 levels by 2020), water companies in England and Wales are expected to reduce their annual GHG emissions to approximately 3.5Mt CO₂ by 2020 (Ofwat, 2011).

In terms of energy requirements, up to 3% of the UK's total energy is consumed by the water industry. This energy demand is not negotiable as the society increasingly demands intensive treatment to remove nutrients and chemicals from wastewater, before it is discharged back into water bodies or is reused. The industry is regulated to meet increasing stringent water quality and reliability standards but as a result of energy use scrutiny, financial cost and environmental cost, there is need to advance into more sustainable wastewater treatment with process options aimed at reducing carbon footprint, energy use and optimizing renewable energy production (STW, 2010).

One of the solutions to the stipulated problems could be the generation of more renewable energy within the sector, which has the potential to reduce GHG emissions. Even though strategies in that line have already been implemented, wastewater companies currently provide just 8.5% of their energy use from renewable sources, primarily via sludge combustion and anaerobic digestion. With innovations, wastewater treatment companies could increase renewable energy generation to produce 25% of their energy needs from renewable sources (STW, 2010).

Presently in the UK, anaerobic digestion infrastructure is used to treat up to 66% of the total sewage sludge produced. These digesters in operation are underexploited having an overcapacity of at least 20% (WMW, 2012), which is not utilized as well as a poor feed source (sludge) with a high nitrogen and low carbon content (Kim *et al.*, 2004) leading to a reduced methane yield. Thus, to increase renewable energy generation within the wastewater treatment works, utilising the digester capacity with the use of additional sources of feedstock as well as optimizing the quality of the feedstock used to one which is more readily biodegradable and with a high carbon content (the process of co-digestion) with sewage sludge will be a potential pathway to enhance methane yield.

Algal biomass could be considered as an alternative for co-digestion with sewage sludge as it can be grown well in wastewater and has additional benefits such as carbon capture and nutrient uptake. Microalgae are photosynthetic microorganisms which are termed a third generation bio-fuel with a noticeable feature as a result of their high lipid, starch and protein content, and it is one that does not have its growth linked to human food or land consumption.

This recognition as a potentially good source for bio-fuel production, ability to grow in wastewater and most importantly its ability to convert carbon dioxide and water into biomass in a relatively short time, highlights its many environmental credentials.

The incorporation of algal biomass production into wastewater treatment seems to be a “win-win” situation as microalgae are able to sequester carbon i.e., taking up the CO₂ produced by the industry, remove nutrients from wastewater, as well as simultaneously producing biomass which can be used for methane production. This technology would also fit into the existing treatment plant flow train without any major modifications. Challenges in the use of microalgae as feedstock for anaerobic digestion exist, for instance cell wall resistance which is dependent on the microalgae species, ammonia toxicity and sodium toxicity from marine species; all of which limit the possible energy gain (biogas yield) from the digestion of microalgae. Thus there is a need to research ways to improve possible biogas production from these microalgae.

Co-digesting algae with sewage sludge in anaerobic digesters at WWTPs would be a possible option to achieve energy increase from microalgae. Thus it is hypothesized that using the existing facilities of a typical UK WWTP with advanced digestion i.e. thermal hydrolysis, will increase algal solubility by destroying the resistant cell wall. This will help achieve increased energy and resource recovery from microalgae in subsequent anaerobic digestion.

In order to verify these hypotheses, the thermal hydrolysis process was mimicked in the laboratory to observe the effects on microalgae solubility and biogas yield. The commercially exploitable methane potential obtained from the study warranted further experiments to verify the effect of co-digesting pre-treated microalgae with sewage sludge. Several batch experiments of algae with different proportions of sewage were also performed to see the effect of co-digestion on biogas yield. Independent digestion of microalgae and sewage sludge as sole feedstocks was performed as a control for comparing results. The positive results obtained led to the experiments in a semi CSTR in order to identify optimum operational conditions and possible operational problems with the digestion at a full scale.

Subsequently, the novel carboxylate platform was explored for the anaerobic co-digestion ratios studied with the experimental objective of possible production of more useful biofuels such as ethanol, butanol and propanol, which are more economically valuable than methane, simply by making slight modifications to the digester conditions. Finally, through a comprehensive analysis, tools to evaluate the technical and economic feasibility of the full-scale were developed.

For the integration of microalgae into a typical wastewater treatment plant to achieve bioenergy generation and greenhouse gas mitigation, several design concepts have been proposed (Pittman *et al.*, 2011; Boelee *et al.*, 2012). Figure 1.1 shows the system/concept adopted for this research where the digester effluent and CO₂ from combined heat and power (CHP) units are recycled for algae cultivation. The sludge dewatering liquor is used for algae cultivation in a photobioreactor (PBR), the harvested algae are digested in the anaerobic digester alongside sewage sludge for bioenergy recovery. Biogas produced is used for cogeneration of energy and heat on

site. The large amount of N and P produced from anaerobic digesters can be fixed in algal biomass

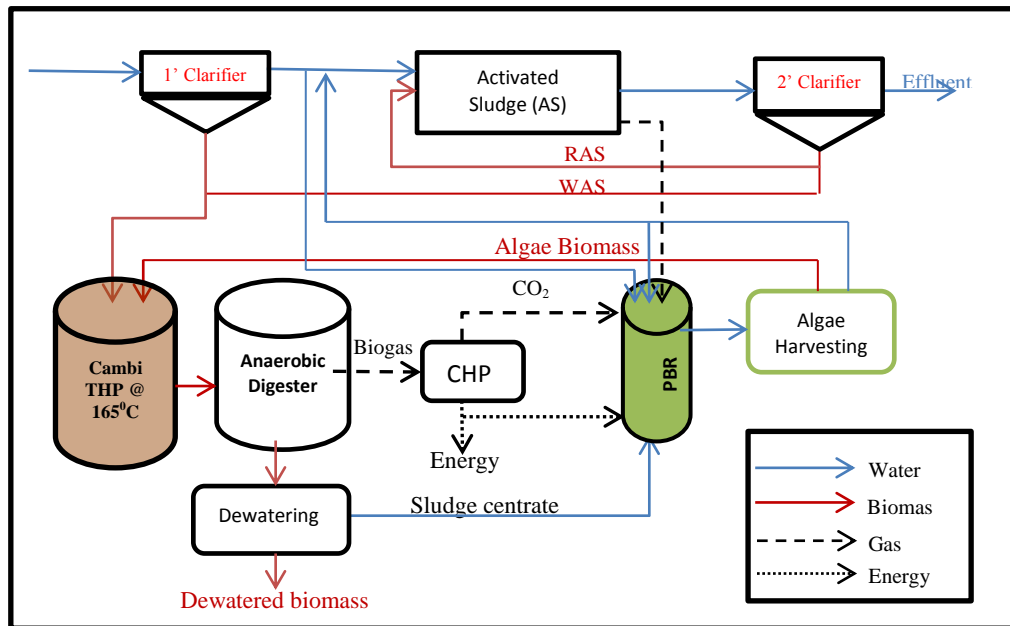


Figure 1.1 Proposed integration of algae cultivation in typical WWTP for enhanced nutrient recovery and bioenergy generation

Theoretically, the integration of microalgae into the existing WWTP looks practical and balanced, however there are concerns about successful application of algal based wastewater treatment. This ranges from the existence of bacteria in wastewater to other environmental factors including light supply, carbon sources and nutrients which will simultaneously affect metabolism and morphological characteristics of microalgae biomass produced. This research moves downstream to explore how feasible energy recovery might be using this approach with the focus on the effects of the existing THP on algae solubility/hydrolysis and biogas production as well as the optimal configuration for the co-digestion of pre-treated microalgae with sewage sludge to produce enhanced methane yield and other biofuels that may be of more economical benefits.

1.2 Scope, aim and objectives

The research aims to evaluate the feasibility of enhancing energy production in typical wastewater treatment plants (WWTP) via microalgae integration with the

ultimate aim of benefiting from the nutrients in the wastewater for microalgae growth and biomass production as well as achieving increased energy production while benefiting the existing facilities (i.e. the unutilized digester head space and the THP) already setup in the WWTP.

Specifically, the objectives of this research project are to:

1. Identify the potential of microalgae (*Chlorella vulgaris*) to produce methane under anaerobic conditions.
2. Investigate the effect of the Thermal Hydrolysis Process on microalgae (*Chlorella vulgaris*) cell wall disintegration, solubility, hydrolysis and overall methane yield.
3. Evaluate co-digestion of microalgae with sewage sludge with the purpose of finding the best co-digestion ratio that favours optimal energy production, process performance and resource recovery.
4. Enhance engineering and process control factors such as organic loading rate (OLR), hydraulic retention time (HRT) and pH to achieve increased biogas production from selected co-digestion ratios.
5. Explore alternative technological approaches (carboxylate platform) to provide more economical and valuable fuels such as ethanol, butanol and propanol from the co-digestion of microalgae and sewage sludge.
6. Carry out an integral analysis of the processes including the energy balance of the proposed processes (AD route and Carboxylate alternative) that allows the study of possible scenarios and their feasibility at full scale.

13 Organization of Chapters

This thesis is organized in thirteen (13) chapters with Chapter 1 being the introductory chapter highlighting the background, research hypotheses and objectives. Chapter 2 focuses on water and energy within UK wastewater treatment plants (WWTP), as well as the current/future challenges faced by the system. Chapter 3 focuses on anaerobic digestion processes, stages, biochemistry and limiting factors. Other end-products of anaerobic digestion are also discussed prior to the review of the engineering factors that can influence the process.

Chapter 4 details the exploitable opportunities from algae; possible application in wastewater treatment and opportunities for energy recovery from microalgae biomass. This includes an extensive review of previous studies on anaerobic digestion of microalgae for energy production. From the review, multi criteria decision analysis (MCDA) is used to select the algae specie for this research. Criteria considered include: nutrient uptake ability, high growth rate (biomass productivity), methane potential, short retention time and a high loading rate. All this are weighted as necessary and best specie was selected. Chapter 5 gives full details of the materials and methods used all through the study.

Chapter 6 is the first of the results and discussion chapters, which aims to justify the intended *Chlorella* source for the research and demonstrate the feasibility of microalgae to produce a substantial amount of methane enough to justify it as a co-substrate to co-digest with sewage sludge. The challenge encountered at this stage is the presence of a tri-laminar cell wall which inhibits the hydrolysis step prior to methane production thus limiting achievable methane yield from the substrate of interest.

In Chapter 7, the research objective is to by-pass the resistant cell wall of microalgae. The chapter presents results on the effectiveness of Thermal Hydrolysis Process on disintegrating the microalgae cell wall, this is then compared to other pre-treatment options to evaluate effectiveness.

In Chapter 8 the possible results of co-digesting pre-treated microalgae and sewage sludge were investigated. The aim at this experimental stage is to find out the best co-digestion ratio between sewage sludge and microalgae to achieve the highest methane production using the Biochemical Methane Potential (BMP) test.

This is further scaled up to laboratory scale anaerobic digesters in Chapter 9 where the results obtained from enhancing engineering and process control factors such as organic loading rate (OLR), hydraulic retention time (HRT) and pH to achieve increased biogas production from the selected co-digestion ratios are reported.

Chapter 10 explores alternative routes of energy production by making minor modifications to the anaerobic digestion process (pH and methanogenic bacteria

inhibition). At this stage, the optimal performance for carboxylate production is investigated. The feasibility of producing more useful biofuels (ethanol, butanol and propanol) which have higher calorific value compared to methane in Chapters 8 and 9 are explored.

In Chapter 11, mass and energy balances to see the feasibility of co-digestion using the traditional AD route and the Carboxylate alternative are compared to see the suitability of the suggested approaches.

In Chapter 12, a general discussion of the findings and contradictory evidence obtained throughout the experimental work and its analysis were analysed. Finally, conclusions of the study and recommendation for further studies are summarised in Chapter 13.

Chapter 2 Wastewater, sludge and energy

2.1 WWTP and sewage production in the UK

Wastewater arriving at a typical treatment plant varies depending on the source of pollution, while its composition is a reflection of lifestyles and technologies practiced in the society that produces these pollutants (DEFRA, 2012). Wastewater can be termed as a complex mixture of natural/man made organic and inorganic materials with source pollutants such as industrial effluent discharge, runoff from agricultural land, leachates from disposal of solid wastes, discharge of raw/treated sewage from towns and villages amongst many others (Horan, 1990; Abdel-Raouf *et al.*, 2012).

In recent times, the need to exploit the potential of this wastewater is driven by the scarcity of water and need for re-use, as well as energy and food requirements in order to achieve sustainability. As a result of this, the role of a typical wastewater treatment plant is to remove pollutants in order to protect the receiving watercourse. These pollutants are typically BOD, suspended solids and ammonia-N. Occasionally phosphorus removal is required and in some situations where the treated effluent discharges to a bathing water, shellfish water or is used for crop irrigation, then pathogen removal is also required. The challenge is to achieve this using the most energy efficient, cost effective and optimal technologies.

Using the UK as an example, on a daily basis more than 11 billion litres of wastewater is produced all of which are collected in the sewerage network. Effective treatment of this wastewater typically requires treating it to meet a discharge consent of 20 mg/l BOD, 30 mg/l suspended solids and < 1 mg/l ammonium nitrogen, measured with a 95 percentile compliance, thereby nullifying any threat it may pose to human health and the environment as a whole before discharging into any receiving water body (Ruiz-Marin *et al.*, 2010). For particularly sensitive waters additional nutrient removal is required to achieve <10 mg N/L and < 1 mg P/L.

The origin of these nutrients can be traced back to the nutrients present in human food which we consume and subsequently excrete, as well as household detergents, and a contribution from industry. The accumulation of these nutrients (nitrogen and

phosphorus) if not properly treated prior to discharge in water bodies may lead to eutrophication and damage the ecosystem (Christenson and Sims, 2011).

The problem of eutrophication is a major global challenge with up to 54% of lakes/reservoirs in North America, Asia, Pacific, Europe and Africa classed as eutrophic (Defra, 2012). An insight into eutrophication in the UK shows estimates England to have 33 waters under threat of eutrophication with a shoreline stretching up to 4,338km², 11 have been reported for Scotland with a shoreline stretching up to 2,246km² and the least to be in Wales, with 120km eutrophic water (DEFRA, 2012). This means that water authorities need to strengthen regulations and discharge standards to minimize the eutrophication effect caused by the discharge of nutrient rich wastewater into water bodies.

In reducing the eutrophication tendencies, an economic/sustainable approach is always a priority. The European Community (EC) is responsible for producing legislation, such as the Water Framework Directive to protect the quality of watercourses and these lead to the promulgation of discharge standards by the relevant national authority, that the wastewater treatment plant must meet. In England and Wales this is the responsibility of the Environment Agency to enforce stringent standards that arise, as a consequence, water companies have invested in energy intensive processes that remove nutrients from wastewater (STW, 2010). As a result as well as additional energy usage, it is anticipated that larger quantities of sludge and GHG emissions will be produced as a result of the adoption of additional processes. Thus it is important to continue the search for the most feasible approach, with improved operational efficiencies.

2.2 Conventional wastewater treatment

It is documented that no single process can lead to the successful and efficient removal of all the pollutants mentioned above (Horan, 1990), thus a combination of several approaches is required by the wastewater industry. In a typical UK WWTP, a 5 stages treatment design is generally adopted; this includes: preliminary treatment, primary treatment, secondary biological treatment, tertiary treatment and very occasionally, disinfection.

- Preliminary stage:

This is basically a screening stage where all large solid materials that could obstruct flow or damage downstream equipment are removed. Screening involves the use of bars or perforated plates with spacings of 6 mm to remove large floating materials such as rags, wood and plastics. The flow velocity is then reduced to < 0.3 m/s under which conditions, grit will settle out whereas the organics matter remains in suspension. It is also essential to note that this stage has no significant benefit on improving the biological or chemical characteristics of the water to be treated.

- Primary Stage

Following the removal of grit and floating materials, a sedimentation tank is applied to remove the settleable solids under gravity. These solids account for up to 60% of the total solids and with good design up to 40% of BOD in the form of settleable solids may be removed (Horan, 1990).

- Secondary/Biological stage

This stage is aimed at BOD and ammonia removal via oxidation. BOD removal is achieved by a mixed population of heterotrophic bacteria that utilize the organic constituents for energy and growth, whereas ammonia oxidation to nitrate is undertaken by autotrophic nitrifying bacteria. For the aerobic oxidation of BOD, two main systems are generally applied: fixed film where biofilms are attached to a fixed surface where organic compounds are absorbed onto and the suspended growth (activated sludge) where microorganisms mix freely with the wastewater and are kept in suspension via mixing or agitation (Horan, 1990).

- Tertiary stage

This stage employs the use of chemical or biological systems primarily to improve suspended solids removal but also to remove nitrogen and phosphorus where required. The tertiary process aimed for example at the removal of ammonium, nitrate and phosphate is estimated to be four times as expensive as a primary treatment costs (Noûe *et al.*, 1992). In scenarios where a higher quality effluent is intended, additional polishing processes such as sand or gravity filters may be employed (DEFRA, 2012). Following the successful tertiary stage, a clear and

apparently clean effluent is produced however this contains pathogens which still require inactivation.

- **Disinfection**

This is an important stage in the treatment process aimed to minimize pathogens which are microorganisms with capacities to negatively impact human health. This is achieved using UV light, chlorination or occasionally by ozonation.

Overall, the treatment steps have proven effective however, they give rise to sewage sludge which contains substantial amount of biodegradable material which may be beneficial if sustainably harnessed. This sewage sludge is of particular interest in this study.

2.3 Sewage/Sludge (Production and disposal)

Over 90% of the wastewater from both domestic and industrial sources receives treatment in the UK. Sewage sludge is a by-product produced during water treatment in the process of separating solids from the liquid phase. Sewage sludge generally possess a solid content <2% solids and is basically the residual particulate organic matter and dead bacteria which are employed for the biological break down and reduction of residual organic matter.

Up to the early 1990s, a great proportion of the sludge produced in the UK (997,673 tonnes per annum) was discharged to the marine environment. This route was banned in 1998 under the Sludge Directive (86/278/EEC) as a result of the UK becoming a signatory to the North Sea Convention on Sludge Disposal. Since that time increases in water quality standards as well as a rising population, has led to more sludge being produced and this requires re-using or disposing through alternative routes. In 2010, sludge production had almost doubled (1,412,836 tonnes) with agriculture accounting for the recycling of 80% of the produced sludge.

Sludge recycled to land must be stabilised prior to application in order to reduce nuisance, principally from odour production. This was usually achieved by anaerobic digestion (AD) which destroys much of the volatile organic material that is responsible for odour production. AD has been used in this role for over 100 years. Although a by-product of AD is the production of methane, an energy source, the

value of the methane produced would not cover the capital cost of the equipment necessary to collect and use it. Spiralling energy costs (Figure 2.1), together with government subsidies (in the form of Renewables Obligations Certificates, or ROCs) means that this methane gas is now a valuable income stream.

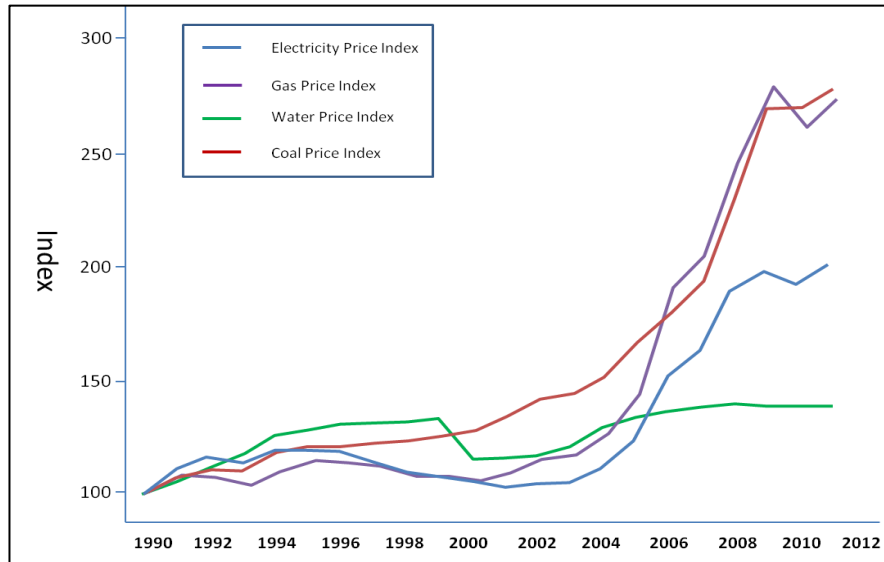


Figure 2.1 Changing cost of energy prices in the UK over time

AD is currently employed in the treatment/processing of about 75% of sludge produced in the UK (DEFRA, 2012). Biogas produced is now being processed in CHPs to produce high grade heat which can be used to heat the digesters in small scale treatment plants to achieve improved digestion or conveyed off site in larger facilities. The electricity produced can also offset energy usage within the sector with potential for export to the national grid (DEFRA, 2012).

AD typically destroys 60% of the volatile organic material and thus other recent/novel technologies, are under investigation to improve this destruction and energy recovery. They include: gasification to produce syngas, pyrolysis to produce biooil, fermentation by bacteria to produce hydrogen and microbial fuel cells (POST, 2007). Some of these have been operated at a pilot scale and they include a gasification scheme set up within the Anglian Water plant sited in Wellingborough as well as a pyrolysis scheme at a water corporation's plant in Perth Australia (EA, 2009).

2.4 Effectiveness of the existing approach for wastewater treatment

The current approaches used in the treatment of wastewater are very effective, for example biological nutrient removal (BNR) in the secondary stage treatment can achieve over 90% removal with regards to nutrient recovery. It however remains a linear approach with limited opportunity for recovery and possible use of the nutrients as fertilizer (Booker *et al.*, 1999).

Furthermore, organic carbon, which provides the electrons that drive N and P removal, is a major limitation in wastewater and it reduces the effectiveness of bacteria responsible for nitrogen and phosphorus removal. To overcome this limitation, supplemental carbon source in the form of methanol and acetate is often added to wastewater. This is bound to increase the overall wastewater treatment cost (Ra *et al.*, 2000).

In respect to cost, treatment using the existing flow sheet is also of concern as the units for nutrient removal demands a large energy input and are capital intensive, They also carry a risk of emission of N₂O, a potent GHG which is emitted in the process of nutrient removal.

Other concerns and challenges associated with the existing techniques can be related to sustainability in terms of GHG emission (Figure 2.2), energy consumption (Figure 2.3), and meeting the stringent environmental targets set for the industry.

Current estimations in regards to Greenhouse Gases (GHG) produced in the wastewater treatment sector account for up to 1% of the national emissions. This requires reducing to meet current targets set by the UK government (Ofwat, 2011), thus there is the need to combine approaches which include adoption of new technologies, optimization of existing technologies and a paradigm shift in systems to achieve these set targets (EEWWT, 2008).

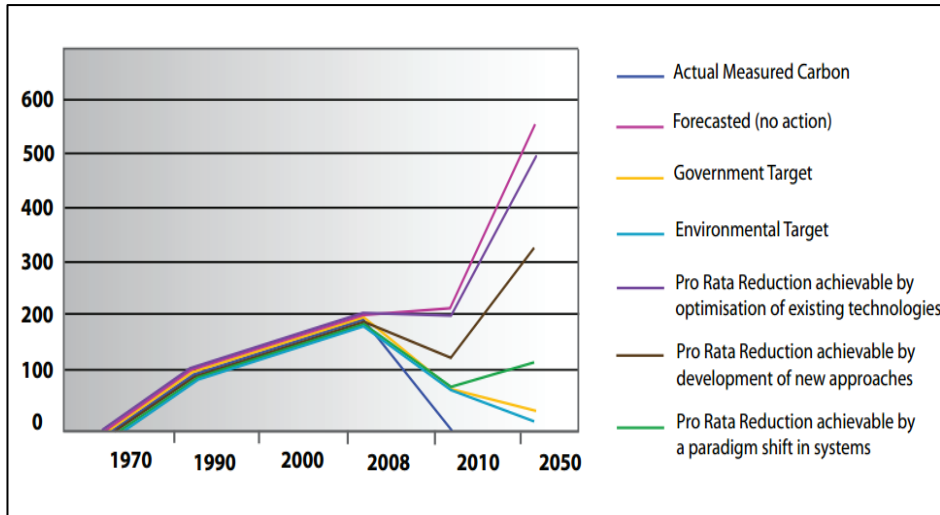


Figure 2.2 Carbon Expressed as a percentage on 1990 Levels adopted from (EEWWT, 2008)

Energy consumption in the water industry (Figure 2.3) is currently estimated as 3% of the UK’s total energy usage. This energy demand is not negotiable as society increasingly demands intensive treatment to remove nutrients and chemicals from wastewater. Further projections however show a significant increase in energy consumption in the coming years (EEWWT, 2008) thus, the industry is regulated to meet increasing stringent water quality and reliability standards at a sustainable rate. To achieve this sustainability and meet up government targets, a combination of optimized existing techniques and adoption of new technologies will have to be implemented.

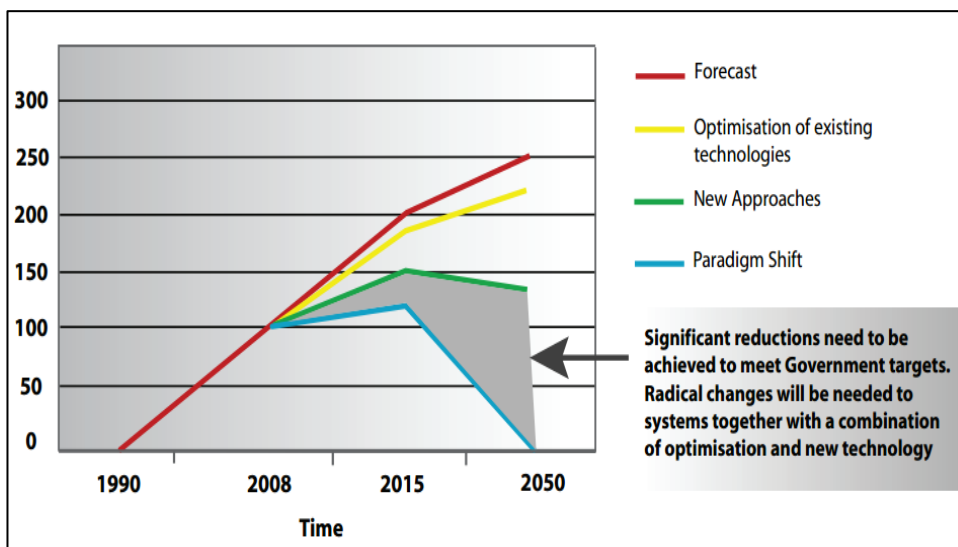


Figure 2.3 Forecasted Energy Usage % adopted from (EEWWT, 2008)

2.5 WWTP and Energy

The UK Wastewater Industry has advanced in its treatment approach over the past 20 years, providing clean and safe water for the increasing population as well as improving river water quality and ensuring adequate protection of the environment. To maintain this pace, a tremendous amount of energy is required by the industry thus ranking it as 4th most energy-intensive sector in the UK (POST, 2007)

Individual use of water is estimated at about 150 litres per day, with the same volume of water used in industrial activities as used by the domestic population. This means daily production of about 17 million m³ of clean water and collection of 16 million m³ of wastewater, both which are treated to a very high standard. These amounts to an annual usage of about 8,290.1 GWh to collect, treat and discharge the sewage. This maybe expressed in terms of units of water supplied and treated as 1.4kWh/m³, a daily per capita use of 0.2kWh (Horan, 2014)

The opportunity to exploit wastewater as a resource for energy, nutrients and treated water exists. For example, reclaimed water may be used for irrigation and domestic purposes amongst many other opportunities. Likewise, the sludge produced may be digested anaerobically to produce biogas which is a form of renewable energy. Also, the use of combined heat and power (CHP) may be used to produce on site electricity and heat from the produced biogas reducing overall energy costs, with savings of CO₂ emissions up to 102,000 tonnes a year assuming a 50% optimization in the industry.

The water companies also take advantage of government incentives aimed around renewable energy production and GHG mitigation. The main incentives benefited from include: Renewables Obligation Certificates (ROC), the Government Renewable Heat Incentives (RHI), Carbon Emission Reduction Target (CERT), Community Energy Savings Programme (CESP) and the Carbon Reduction Commitment (CRC).

Hence, the opportunity to utilize wastewater as a renewable resource is anticipated to be an appropriate solution for managing the increasing wastewater successfully so as to meet the environmental quality standards (Tyagi *et al.*, 2013).

Chapter 3 Anaerobic Digestion

3.1 Anaerobic Digestion in the UK

In the past, the majority of organic wastes were sent to the landfill. As a result of the biodegradation of such wastes to methane within the landfill, a number of government driven initiatives have been undertaken to divert such wastes away from landfill. Several methods have also been developed and employed successfully in the treatment of these wastes amongst which the biological and thermal options are outstanding in achieving energy from waste (Shanmugam and Horan, 2009). Fiscal rewards in the form of Renewables Obligation Certificates (ROCs) for energy generated in this way have also played a major role in encouraging diversion.

Anaerobic digestion is one of the technologies that can deliver a positive net energy output as well as allowing for closing energy, water and nutrient cycles. It makes use of microorganism (mainly archae) that can grow in anaerobic conditions, to break down organic solid waste such as organic farm wastes, sewage sludge, green wastes, energy crops amongst many others into simpler chemical components with the generation of methane gas.

As a result of the flexibility in substrate required for anaerobic digestion coupled with its implementation scale, varying from very small to very large and its relatively higher net energy yields per acreage, anaerobic digestion is most preferred amongst other bioenergy forms for its biogas productivity. This technology has been successfully used in the treatment of sewage sludge, industrial wastewater treatment (Lier, 2008), as well as for the stabilization of solid waste slurries, energy crops, crop residues and municipal solid waste (Mata-Alvarez *et al.*, 2000).

Controlled AD of organic materials can be environmentally beneficial in two ways. Firstly, by means of managing waste in a sealed environment thus reducing potential greenhouse gas emissions. Secondly, to produce renewable energy i.e. biogas which contains up to 60 percent methane and 40 percent carbon dioxide (CO₂) (FOE, 2007) and can be further burnt in a combined heat and power (CHP) unit to produce heat and electricity which can be injected into the grid or upgraded to a higher quality gas which is suitable for vehicular fuel. Additional benefits of the anaerobic digestion

(AD) process also include the recovery of nutrients in the digestate which may be used as an alternative for chemical fertilizers.

Anaerobic digestion is expected to play a major role in: achieving the UK's share of the EU's binding target for renewable energy proposed to be 15% by 2020; increase energy recovery from waste; help the water industry achieve their target, which requires at least 20% of energy usage within the company to be from renewable sources by 2020 (DEFRA, 2009). In terms of biofuel production, AD is perhaps better than other popular biofuel options including bioethanol and biodiesel, in the sense that its resource demand such as fossil energy, water and nutrients is less and also it has the potential to re-use plant nutrients (Fredriksson *et al.*, 2006).

AD currently attracts 2 ROCs with a value of £44 - £50/MWh and an estimated annual market value of £400 million (Hopwood, 2011). Although anaerobic digestion could be used either in the treatment of biodegradable wastes or in the production of heat and electricity which have monetary / economic value, it would be more valuable if these two aims could be simultaneously achieved.

3.2 Biochemistry of the process

Anaerobic digestion is a technologically simple process which involves decay and decomposition in which anaerobic microorganisms digest the organic matter from a wide range of waste water types, solid wastes and other types of biomass found within the environment. In the absence of oxygen it produces methane and carbon dioxide as well as small amounts of hydrogen sulphide (H₂S), ammonia (NH₃) and trace amounts of other gases.

A solid / liquid nutrient rich digestate is also produced. This by-product corresponds to the input material not converted to gas, the newly grown bacterial mass residue as well as the mineralized fraction and may be used as a soil conditioner to fertilize land.

The energy conservation in anaerobic digesters is through fermentation or anaerobic respiration. While fermentation processes occurs when an organic compound is both the electron donor and acceptor and ATP is produced by substrate level phosphorylation, in anaerobic respiration, there is a transfer of electrons to an

external acceptor other than O₂ (for instance nitrate), and ATP production is by oxidative phosphorylation (Madigan, 2012; Rodriguez, 2012).

For example, fermentation of compounds such as glucose (simple sugar) via glycolysis breaks down glucose into two molecules of pyruvate. This is a pivotal compound in anaerobic digestion that is reduced by the coenzyme NADH to fermentable products. The NADH is further oxidized back to NAD⁺ thus enabling glycolysis and other reactions that depend on NAD⁺ to continue (Madigan, 2012). Since it is a necessity to achieve a redox balance in fermentations, the main avenue for discarding the excess electrons comes via a range of bacteria that are able to catalyse the reduction of pyruvate, resulting in a number of distinctive fermentation compounds including lactate, propionate, etc.

Based on the characteristic of these bacteria and the important conversions which take place in anaerobic digestion, the anaerobic digestion process could be subdivided in 4 phases (Figure 3.1) for easy understanding. These stages include: hydrolysis, acidogenesis, acetogenesis and methanogenesis

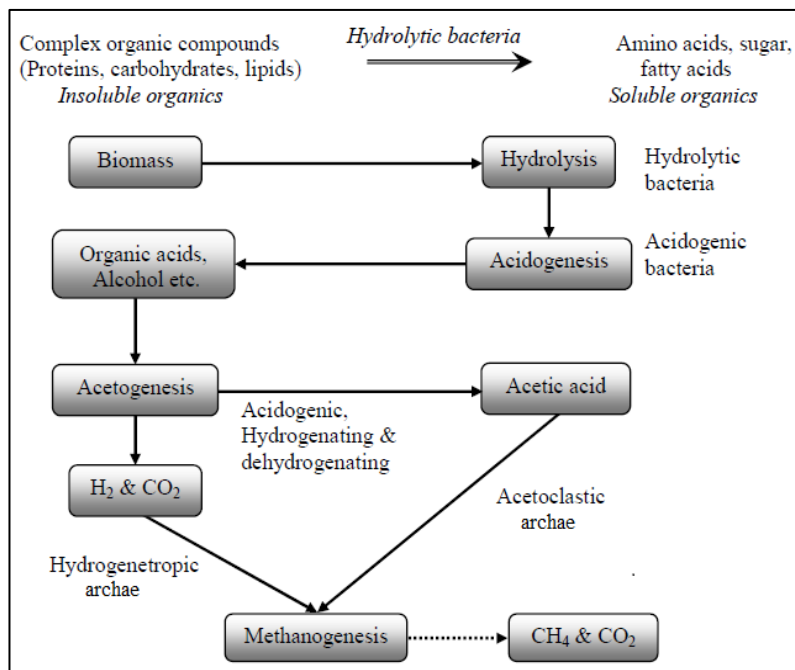
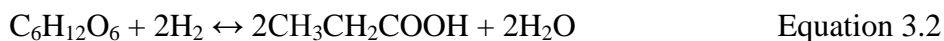
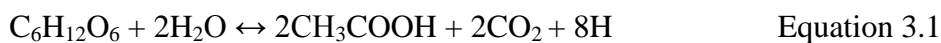


Figure 3.1 Degradation steps of anaerobic digestion (Chaudhary, 2008)

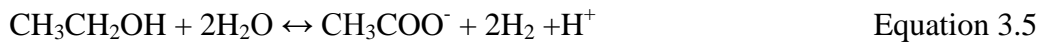
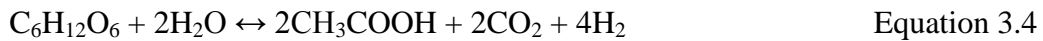
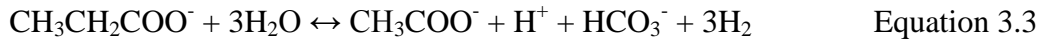
Hydrolysis: Hydrolysis is a chemical reaction which inserts a water molecule (H+ and OH-) between two large molecules to cleave them into two. This is the first stage where microorganisms excrete enzymes which hydrolyse particulate material and colloidal matter (carbohydrates, proteins and fats) to their monomeric or dimeric components such as glucose, amino acid and long chain fatty acids (LCFA). This stage is often the rate limiting step during anaerobic digestion of waste containing lipids or a high amount of particulate matter (Haandel and Lettinga, 1994). Chemicals may be added in this stage to influence digestion time or methane yield (Ostrem, 2004).

Acidogenesis: in this stage, the monomers (glucose, amino acids, fatty acids and glycerol) formed in the hydrolysis phase are fermented by acidogenic bacteria which transform them into short chain volatile acids, ketones, alcohols, hydrogen and carbon dioxide. Products from this stage include propionic acid (CH₃CH₂COOH), butyric acid (CH₃CH₂CH₂COOH), acetic acid (CH₃COOH), formic acid (HCOOH), lactic acid (C₃H₆O₃), ethanol (C₂H₅OH) and methanol (CH₃OH), amongst others. Acidogenic bacteria grow rapidly and double their number in as little as 20 minutes thus in the case of overloading a reactor, accumulation of carboxylates can occur thereby causing a pH drop and may eventually lead to a digester failure. Equations 3.1 and 3.2 (Ostrem, 2004) show the conversion of glucose to acetate and propionate respectively.

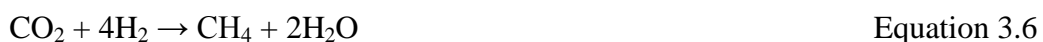


Acetogenesis: the hydrogen, carbon dioxide and acetic acid produced from the previous stage skip this phase however, other products including propionic acid, butyric acid and alcohols produced in the previous stage are converted by acetogenic bacteria into hydrogen, carbon dioxide and acetic acid. The role of hydrogen in this stage cannot be neglected as the reaction occurs only when the hydrogen partial pressure is low (below 10⁻³ atm), enough to thermodynamically allow the conversion of all the acids. This is carried out by hydrogen scavenging bacteria. Under standard conditions, the presence of hydrogen in solution inhibits the oxidation thus, the hydrogen concentration measured by partial pressure of a digester could be used to

extrapolate its health (Mata-Alvarez, 2003). Equation 3.3 shows the conversion of propionate to acetate only achievable at low hydrogen pressure. Equations 3.4 and 3.5 respectively show conversion of glucose and ethanol to acetate during the acetogenic stage



Methanogenesis: this is the final stage where methanogens (anaerobic archaea) convert the products of the acetogenesis stage (hydrogen and acetic acid) to methane and carbon dioxide. The methanogens can be separated into 2 groups consisting of the acetate consumers (acetoclastic methanogenesis), which are the most significant producers of methane accounting for up to 70-75% of overall methane formed in the reactor by utilizing acetic acids and the hydrogen/carbon utilizers known as hydrogenotrophic methanogenesis, which reduce carbon dioxide by dihydrogen, producing the remaining amount of methane. These methanogens grow slowly compared to the bacterial growth in the previous stages and are very sensitive to pH. pH values below 6.5 cause their inhibition. Equations 3.6, 3.7 and 3.8 show the conversion of the resultant hydrogen and acetic acid formed in the earlier stage to methane.



3.3 Biodegradability and Methane Potential

Biodegradation basically gives an account of the extent of degradation of an organic material. It is a function of the intrinsic properties of the material as well as the microorganisms involved (Bisaria and Ghose, 1981). According to Mata-Alvarez, (2003), two types of substrate biodegradability can be calculated; the ultimate biodegradability and the biodegradability under the applied reactor conditions.

3.3.1 Biochemical Methane potential

The BMP test is a simple and rapid method developed to examine gas production and the biodegradation of an organic waste under optimal anaerobic conditions as well as the possibility of toxicants in feedstock used in the anaerobic treatment process (Owen *et al.*, 1979). The BMP test enables determination and comparison of organic carbon content in a given material that can be converted to methane anaerobically as well as an evaluation of biogas production efficiencies between different substrates. Its design makes it an inexpensive and more flexible alternative compared to the continuous techniques used for biodegradability evaluation (Gunaseelan, 1997).

BMP experiments require incubating the substrate of interest under stringent anaerobic conditions in the presence of excess inoculum and nutrients. A summary of its assay description as well as factors that may influence its performance is summarized by Chynoweth *et al.*, (1993).

Since the BMP was proposed by Owen *et al.*, (1979), modifications to this process have been adopted by several researchers e.g. a modified process was used by Chynoweth *et al.*, (1993), where the initially proposed medium was modified with addition of trace amounts of H_2WO_4 and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. The addition of these trace compounds was justified as a necessity required by key-coenzymes in methane production. These key co-enzymes include coenzyme M, which is utilized to reduce carbon dioxide to methane, and the nickel-containing coenzymes (F_{420} ad F_{430}), which are important carriers in methane forming bacteria (Gerardi, 2003).

Besides nutrients being modified for the BMP test, the concentrations of sample fed into the reactors also vary. An initial proposal of 2g/L degradable COD was adopted (Owen *et al.*, 1979) however, further work by the same researchers (Chynoweth *et al.*, 1993) showed they opted for a concentration of 2g VS/L. Nevertheless, a more recent study by Hansen *et al.*, (2004) proposed the benefits of a higher concentration of sample in generating increased methane production.

For BMP experiments, seed inoculum, incubation time and temperature are very important parameters. While it is essential to acclimatise the inoculum prior to the

experiments in order to avoid toxicity and to optimize methane production, the inoculum: substrate ratio is also necessary for optimal results. Varying inoculum:substrate has been tested (Raposo *et al.*, 2009; Eskicioglu and Ghorbani, 2011) with a ratio of 2 suggested by Chynoweth *et al.*, (1993). This may however be higher for readily degradable substrates.

An incubation time of 30 days is the most common in BMP experiments however; some authors have carried out experiments with longer incubation times up to 100 days. Temperature on the other hand is not as important and BMP experiments may be carried out under mesophilic or thermophilic conditions. The preference of the mesophilic over the thermophilic includes its use at full scale.

Overall, while some researchers embrace the same nutrient media, some have modified the solution and some have neglected the use of the nutrient solution in the first place. Also the concentrations fed into the reactor as well as the inoculum to substrate ratio was also found to vary in different research. Thus, the Table 3.1 below highlights some of the technical approaches to develop a BMP experiment in terms of nutrients, seed, inoculum, concentration and pre-treatment of sample as well as the mixing volume and incubation time.

In summary, BMP like other tests has its limitations and questions exist on how reliable the results can be when used in the performance projection of an actual digester in operation. Therefore, it is essential that all the conditions that facilitate anaerobic digestions are optimised during the test and that the results are carefully evaluated (Angelidaki and Sanders, 2004).

Table 3.1 Description of BMP test from different researches adopted from Rodriguez, (2012)

Description of BMP test from different researches	Seed inoculum	Sample conditions	Effective Volume	Conditions of the test	Reference
Nutrients solution Defined media with nutrients and vitamins for mixed anaerobic cultures. Resazurin and sodium sulphide.	Acclimated inoculum from a semi continuous seed digester working with a low organic loading	Sample concentration of 0.5 and 2 g TS/L Alk 2500 mg l-1 as CaCO ₃	Total volume 200 ml. Inoculum at 20% by volume	Temperature 35°C Incubation period: 30 days	(Owen <i>et al.</i> , 1979)
As outlined by Owen <i>et al.</i> 1979, but adding trace amounts of H ₂ WO ₄ and NiCl ₂ .6H ₂ O	Acclimated inoculum from a mesophilic CSRT reactor fed with primary sludge	Sample concentration of 2 g VS/L	Total volume 100 ml	Temperature 35°C Incubation period: 30 days	(Chynoweth <i>et al.</i> , 1993)
As outlined by Owen <i>et al.</i> 1979	Acclimated inoculum from a mesophilic CSRT reactor (OLR 2 g VS/l/d, HRT 20 days)	Sample concentration of 6.67 g TS/L	Total volume 75 ml. Inoculum at 20% by volume	Temperature 35°C Incubation period: 100 days	(Gunaseelan, 2004)
No nutrient solution used As outlined by Hansen <i>et al.</i> , 2004 Owen <i>et al.</i> , 1979 modified. One ml nutrients solution per litre reactor	Inoculum from a thermophilic plant, partially acclimatised	Sample concentration of 20 g VS/L	Total volume 500 ml Inoculum at 80% by volume Total volume 400 ml	Temperature 55°C Incubation period: 50 days Temperature 35°C Incubation period: 28 days	(Hansen <i>et al.</i> , 2004) (Shanmugam and Horan, 2009)

In addition, protocols to complement and aid the interpretation of BMP results have been developed. These approaches include the use of stoichiometric methane potential (SMP) and ATP analysis for evaluating the suitability of a range of organic solid wastes as substrates for anaerobic digestion (Shanmugam and Horan, 2009).

The extensive use of BMP has helped in the delineating and comparison of the methane potential and anaerobic degradability of different waste materials. Depending on the kind of waste evaluated, a different methane yield is obtained. Table 3.2 below highlights a summary of BMP reports for different kinds of wastes in the literature.

Table 3.2 BMP report for different wastes adapted from (Rodriguez, 2012)

Substrate	BMP reported	Reference
Thermochemical pre-treated peat.	0.041 – 0.116 m ³ /kg TS 9 – 26% Conversion efficiency	(Owen <i>et al.</i> , 1979)
MSW	0.186-0.222 m ³ /kg VS _{applied}	(Chynoweth <i>et al.</i> , 1993)
Yard wastes	0.155 m ³ /kg VS _{applied} (on average)	
Paper samples	0.084-0.369 m ³ /kg VS _{applied}	
Food packaging	0.318-0.349 m ³ /kg VS _{applied}	
Chemically pre-treated municipal sludge	0.350 m ³ /kg COD _{removed}	(Lin <i>et al.</i> , 1999)
Fruits and vegetables	0.180-0.732 m ³ /kg VS _{applied}	(Gunaseelan, 2004)
Source-separated organic household waste	0.495 m ³ /kg VS _{applied}	(Hansen <i>et al.</i> , 2004)
Source sorted OFMSW	0.300-0.570 m ³ /kg VS _{applied}	
Chemical sludge	0.36 m ³ /kg VS _{removed}	(Shanmugam and Horan, 2009)
Activated sludge	0.52 m ³ /kg VS _{removed}	
MSW	0.36 m ³ /kg VS _{removed}	
Primary sludge	0.38 m ³ /kg VS _{removed}	
Microalgae	0.09 to 0.45 m ³ /kg VS _{applied}	(Sialve <i>et al.</i> , 2009; Zamalloa <i>et al.</i> , 2012)

3.4 Other products from anaerobic digestion

The anaerobic digestion system is a sustainable biological treatment traditionally used for the stabilization of primary and secondary waste sludge with the main benefit of biogas production. More recent development in this approach has revealed the possibility of producing other valuable products one of which includes the production of hydrogen (Chinellato *et al.*, 2013). Biological production of hydrogen via anaerobic fermentation is now gaining importance since its capability as an ideal fuel and its high energy yield has been identified (Hwang *et al.*, 2004; Antonopoulou *et al.*, 2008). Other valuable products that may be obtained from anaerobic digestion include solvents and acids which may be of economic/financial benefits (Dogan *et al.*, 2009).

3.4.1 Hydrogen

In the effort to find alternatives to fossil fuels, special considerations have been put on fuels that not only supply the world with energy but also offer a cleaner alternative. One which has received attention in recent times is hydrogen, since it is termed as a clean and sustainable energy source with minimal or zero use of hydrocarbons. The benefits of hydrogen as an energy source includes its high gravimetric energy yield (122 kJ/g which is 2.75 times greater than gasoline) (Antonopoulou *et al.*, 2008), the production of water as the only reaction product (Koutrouli *et al.*, 2009), and its application for electricity production using fuel cells.

Biologically, hydrogen may be produced from a wide range of organic substrates using two processes i.e. the fermentative and the photosynthetic, however, anaerobic fermentation is a simpler process in the sense that it proceeds at higher rates and does not require light sources like photosynthesis (Han and Shin, 2004).

Research on the preferred route for hydrogen production has been carried out using different types of substrate. Some of the studies investigated the use of pure cultures of bacteria on pure substrates including glucose, starch and cellulose in batch processes (Hawkes *et al.*, 2002). These revealed high hydrogen yields achievable with the highest conversion efficiencies at 2.6 mol H₂/mol glucose using *Clostridium* (Zhang *et al.*, 2006). Other studies investigating the use of mixed cultures revealed

achievable hydrogen yields up to 1.9 mol H₂/mol glucose and 0.37 mol H₂/mol hexose for synthetic wastewater and sewage sludge respectively (Zhang *et al.*, 2006; Massanet-Nicolau *et al.*, 2008).

For a more optimized process and enhanced hydrogen production, the benefits of co-digestion have been identified as this process can bring about pH control and nutrient supplementation which is beneficial to the process (Zhu *et al.*, 2008). Nevertheless, for the commercial exploitation of this approach, a series of obstacles must be eliminated with the first being the need for a procedure that inhibits methanogenic bacteria in a (scaled up) continuous process over time while still remaining economically efficient. Another problem may be as a result of pH control in a scaled up process (Khanal *et al.*, 2004).

3.4.2 Carboxylates and Solvents

In addition to the production of biogas from anaerobic digesters, other intermediaries (Figure 3.2) such as VFAs may be produced and may be marketable along with methane (Dogan *et al.*, 2009). VFAs are carboxylic acids possessing a carbon chain of six carbon atoms or fewer. Acetate, propionate, lactate and n-butyrate are the primary high value intermediates produced in the carboxylate platform. This product may be obtained from the acidogenesis stage of AD and its production from several wastes including sludge has been successfully documented (Lee *et al.*, 2014)

During the anaerobic digestion of wastes, total VFA production reaches its peak after 5 days and then it begins to decline. This trend is however assumed by acetic formation which is a short chain VFA. Subsequent declination of VFA indicates that short chain VFA are easily used up by methanogenic bacteria to produce CO₂ and CH₄.

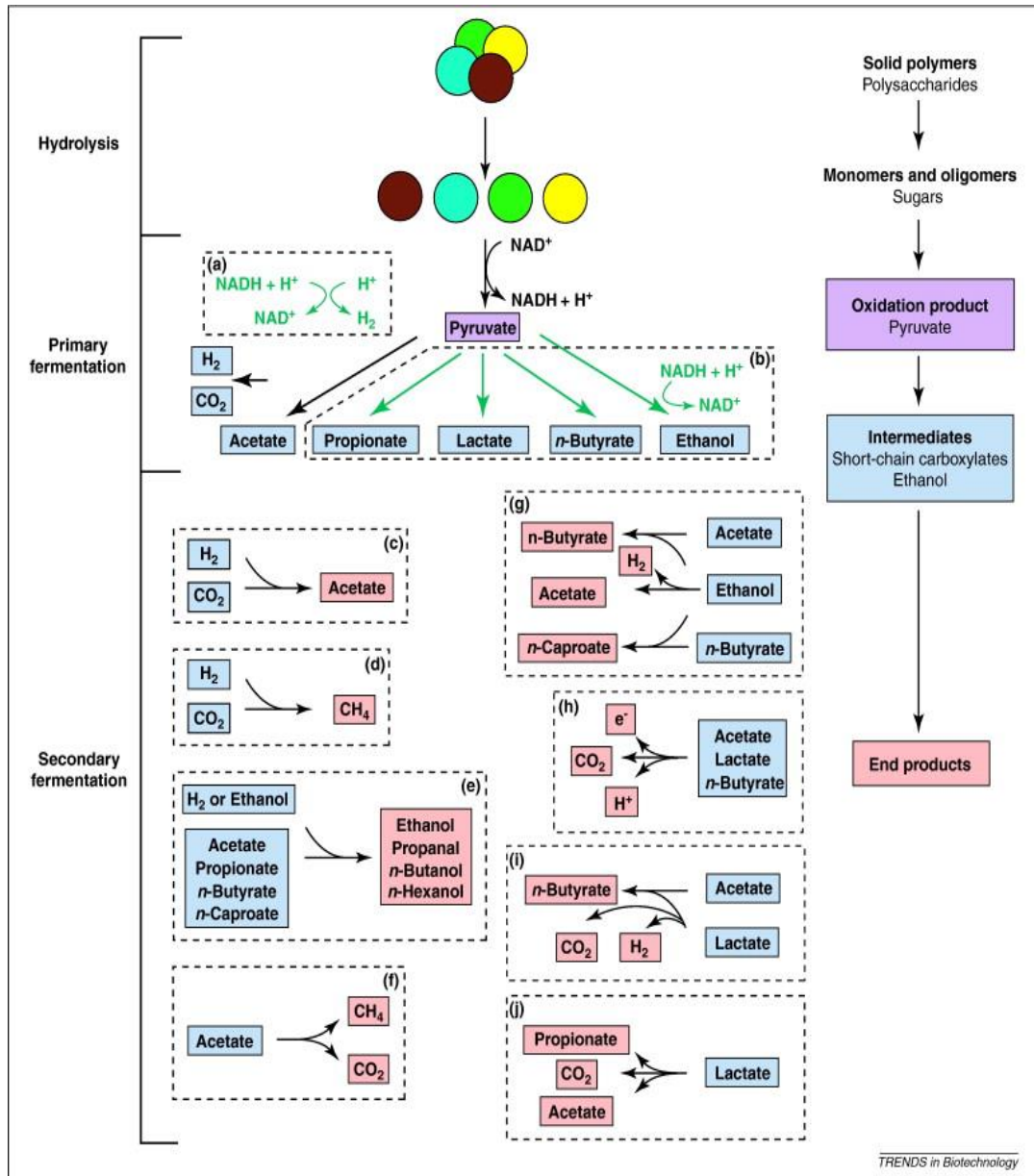


Figure 3.2 Carboxylate platform for production of more economical biofuels adopted from (Agler *et al.*, 2011)

VFA may be fermented using either undefined mixture or in pure cultures via routes including biochemical, electrochemical and thermochemical post processes to yield more valuable bio products of interest such as alkane and alcohols (Agler *et al.*, 2011). Depending on the substrate biodegradability, lactate fermentation may dominate primary fermentation because the lactate pathway permits a rapid disposal of the reducing equivalents. Other compounds that can be produced in the carboxylate platform are n-valerate, n-caproate, n-caprylate, iso-butyrate and biopolymers, such as poly(lactic acid) (ibid). To achieve further processing which

may lead to production of fuels, these short chain carboxylates may require separation from the undefined mixed culture broth, which is one of the barriers in the carboxylate platform.

In an attempt to produce a mixed and variable product spectrum as an alternative to the production and separation of a single carboxylate, several systems have been developed (Kleerebezem and van Loosdrecht, 2007; Agler *et al.*, 2011). Some of these systems which enable conversion of carboxylates into blends of chemicals or fuels by processing include the MixAlco process.

- **Mix Alco Process / Review**

The Mix Alco process is a mixed fermentation process which is aimed at conversion of biodegradable substrates to alcohols such as 2-propanol, 2-butanol and higher alcohols. The process employs a variety of steps with the first being a pre-treatment step aimed at increasing the substrate degradability and amenability to anaerobic/fermentation bacteria.. This may be achieved by lime treatment and 0.1g Ca(OH)₂/g dry solid appears to be the most effective dose, at a temperature ranging between 85 - 135°C for 1-3hrs (Chang *et al.*, 1997; Kaar and Holtzapple, 2000).

This step is subsequently followed by introducing the pre-treated substrate into a mixed-culture of fermentative bacteria which utilize these substrates to produce a mixture of carboxylic acids. In the process, it is essential to maintain the pH, thus CaCO₃ is added to react with the acids to produce carboxylate salts, such as calcium acetate which are then dewatered and dried. The last step on the multi-step approach is the thermal conversion of salts to ketones which may be further hydrogenated to alcohols.

To achieve a high concentration of the final/expected product, a counter current fermentation is recommended as this enhances substrate conversion via reducing the possible inhibition from carboxylate salts due to the new media addition. This enables the freshest biomass contact with the highest carboxylate concentration, which allows for higher product concentration (Chan and Holtzapple, 2003).

Some of the substrates explored under this platform include MSW and MSS, whilst the MSW possess a high carbohydrate concentration, it lacks adequate nutrients,

which are essential for effective performance of fermentative microorganisms. MSS on the other hand possess essential nutrients but lack adequate carbohydrate thus, literatures have found a combination of these approach beneficial for fermentation experiments with the most optimal ratio to be 80% MSW and 20% MSS (Chan and Holtzapple, 2003)

In the Mix Alco process, inhibiting the methanogens is a priority to prevent conversion of the produced carboxylic acid into methane. In light of this, methanogenic inhibitors (e.g iodoform or bromoform) may be utilized. While it is essential to inhibit the methanogen, it is necessary to use the optimal iodoform concentration as this can impact on acetate selectivity as methane inhibition generates excess reducing power thus eliminating methane as a potential hydrogen “sink” thereby causing the production of more reduced products such as propionate and butyrate (Russell and Martin, 1984)

Temperature is also a vital parameter that should be carefully selected in the conversion of substrates to carboxylic acids. The main temperature ranges are the thermophilic ranging between 50 – 60°C and the mesophilic within the range of 30 and 40°C. Both temperatures control the acetic acid selectivity and fermentation activity. While some studies identify an improved performance at mesophilic, some other studies show thermophilic fermentation gives a better yield and performance, this lack of agreement for the optimal temperature is however likened to the different microbial consortium in the studies (Lee *et al.*, 2014).

The use of anaerobic, undefined mixed-culture is a relatively new approach however and it is an inexpensive methods, capable of handling the complexity and variations in organic wastes to produce carboxylates that may be converted to more useful bioproducts. Other benefits of the approach are that it requires no sterilization, no enzyme inoculation and it allows a high rate of biomass conversion.

Some studies have been carried out to determine the achievable yield under this platform (Table 3.3). Despite the promise of this platform, some hurdles however have to be overcome technically to benefit from the full exploitation of this approach

Table 3.3 Batch studies for volatile fatty acids production (Ramírez-Sosa, 2014)

Studies	Parameter													
	Feedstock	Inoculum	Feedstock pre-treatment	Fermentation temperature (°C)	Substrate's concentration (g/L)	Iodoform (mg/L)	Total VFAs produced (g/L)	Productivity (g total acid/Lliq/day)	Yield (g acid/g VS fed)	Acetic acid (wt%)	Propionic acid (wt%)	Butyric acid (wt%)	Fermentation days	
Golub <i>et al.</i> , (2013)	Paper	Soil	No treatment	55	8.97 (as VS)	1.6	2.31	--	0.04	72	1	26	30	
Datta, (1981)	Corn stover	Cow manure/SS	Lime/ Na ₂ CO ₃	25	--	Low T	--	--	0.55	--	--	--	12	
Pham <i>et al.</i> , (2012)	Macroalgae	Sewage Sludge	0.5 N NaOH	35	50	30	15.2	-	0.30 – 0.41	52	36	11	5	
Pham <i>et al.</i> , (2013)	Macroalgae	Sewage Sludge	Biologic 0.5 N NaOH	35	40	30	15.6			53	27	15	5	
Forrest <i>et al.</i> , (2010)	Glycerol	Marine sediment	--	55	80	2	24.0	0.75	0.29	61.6	1.8	36.5	30	
Rughoonundun <i>et al.</i> , (2010)	WAS	Marine sediment	Ca(OH) ₂ /100°C	55	50	0.016	10.72	0.34	0.34	65.9	8.76	12.8	28	
Forrest <i>et al.</i> , (2010)	Water hyacinths	Marine sediment	Ca(OH) ₂ /100°C	40	100	1.6	19.93	--	0.30	73.81	14.48	9.90	30	
Ross and Holtzapple, (2001)	80 MSW/ 20 SS	Rumen fluid	Ca(OH) ₂ /121°C	40	88	0	30	-	0.219	-	-	-	12	
	Cattle manure	Rumen fluid	Ca(OH) ₂ /121°C		105	2	20		0.158				5	
Smith and Holtzapple, (2011)	Paper	Marine sediment	--	40	93	3-1.6	30.02	0.84	0.239	-	-	-	32	
Lee <i>et al.</i> , (2014)	Macroalgae	SS	--	37	92	?	29.17		0.35	40.4	18.3	26.0	60	
Rughoonundun <i>et al.</i> , (2012)	70 Sugarcane bagasse/ 30 SS	SS	Lime	55	50	0.016	15.1		0.36	79	2	17	30	
Nachiappan <i>et al.</i> , (2011)	Paper	Marine sediment	Lime/ 50°C	55	80 (as VS)	3	18.4	--	0.23	90.0	1.58	7.40	20	
	Sugarcane bagasse				63 (as VS)		19.7	--	0.31	85.7	1.99	11.1		

3.4.3 Digestate

Besides the aforementioned resources that can be recovered from the anaerobic digestion process, the effluent stream of AD is potentially rich with nutrients, which may be extracted and used as liquid fertilizers. The main nutrients found in the digester effluent are nitrogen and phosphorus, which may be recovered via crystallization in the form of magnesium ammonium phosphate hexahydrate ($\text{MgNH}_4\text{PO}_4\cdot 6\text{H}_2\text{O}$, struvite or MAP) after which the recovered struvite may be applied to land as agriculture fertilizer. Struvite serves as a valuable fertilizer, which releases nutrients slowly and has non burning features as a result of its low water solubility. Struvite crystallizes when the stoichiometric ratio of $\text{Mg}:\text{NH}_4^+:\text{PO}_4^{3-}$ is greater than 1:1:1 according to the following reaction (Equation 3.9):



The benefits of struvite formation from anaerobic effluents could reduce the cost associated with chemical or biological nutrient removal in wastewater treatment works and also help meet policy requirements for phosphorus recovery due to chemical precipitation of phosphorus. According to Shu *et al.*, (2006), more than 90% of dissolved phosphorus may be recovered from anaerobic digester effluent via struvite crystallization thus achieving up to 80% total phosphorus recovery. It is speculated that the technical constraints and financial costs of these approaches can be likened to why this recovery techniques are still undergoing research and development.

Perhaps, a more effective utilization/recovery of the nutrients in anaerobic digester effluent may be achieved via utilization by microalgae for biomass production. This uptake is however dependent on the chemical forms and speciation of these nutrients. The possibility of microalgae using this nutrient source for growth has been studied whilst highlighting some preference such as the utilization of nitrogen in the form of ammonium over nitrate (Yuan *et al.*, 2010). Overall findings showed that microalgae can play the perfect role of nutrient recovery/utilization from digester effluent with the added advantage of producing microalgae biomass which may be co-digested with the produced sewage sludge to increase biogas yield.

3.5 Engineering factors affecting performance of anaerobic digestion

Anaerobic digestion is a complicated process as a result of the complexity of the bioconversion process and several engineering factors that play a significant role in influencing the digester performance and stability. These factors can be classified into 3 main groups: (i) kinetics, (ii) operational conditions, and (iii) substrate characteristics.

3.5.1 Process Kinetics

The importance of process kinetics on AD performance cannot be neglected particularly from the engineering point of view as this is a main determinant for analysis, control and design of digesters (Eastman and Ferguson, 1981). In this light, enormous research has been carried out to determine the kinetic constants of the anaerobic digestion process when being applied to a range of organic wastes. Nevertheless, the finding of the researches highlighted variations in the obtained kinetic values, which is associated with variation in operational mode/condition coupled with other environmental variations.

For a simplified kinetic determination, the overall AD process is mostly divided into two processes: the acidic phase and the methanogenic phase, with the former extensively studied and viewed as a two process reaction (hydrolysis and fermentation) occurring in series. Based on this, a model of the acidic phase was formulated (Eastman and Ferguson, 1981) using the mass balance of reactants and products in terms of COD. Findings of the study suggested the hydrolysis stage as a rate limiting step in the conversion of wastes to fermentation products in the acidic phase. This finding also aligns with several later studies (Pavlostathis and Giraldo Gomez, 1991; Vavilin *et al.*, 1996)

With hydrolysis of the utmost importance in kinetic determination, it is essential to note that while dealing with complex substrates, the hydrolysis rate may be affected by parameters including pH, particle size (Hobson, 1983; Sanders *et al.*, 2000), enzymes produced as well as diffusion and absorption of enzymes to particle (Gavala *et al.*, 2003).

Depending on the substrate of interest and the operating conditions, differing kinetics may be applied to study its hydrolysis/biodegradability. Of the numerous models

such as Monod, step diffusion, Inhibition and Shrinking core developed for modelling biodegradation (Zaman, 2010), the first order kinetic model (Equation 3.10) is the simplest model applied to describe the hydrolysis rate during anaerobic digestion with respect to the concentration of degradable particulate organic matter (Pavlostathis and Giraldo Gomez, 1991) .

$$\frac{dF}{dt} = -k_h F \quad \text{Equation 3.10}$$

Where: F= concentration of degradable, particulate organic matter (ML⁻³) and k_h =hydrolysis rate co-efficient (T⁻¹)

Although the first-order kinetic model provides a simple basis for comparing stable process performance under practical conditions, it is sometimes limited when complex substrates and systems are involved (Zaman, 2010).

A review of the possible hydrolysis kinetics of substrates (Table 3.4) particularly sewage sludge is detailed where first order kinetics was assumed (Pavlostathis and Giraldo Gomez, 1991)

Table 3.4 kinetic constant values for the hydrolysis of biodegradable substrates

Substrates	K_{hyd} (d⁻¹)	Temperature (°C)	Reference
Primary sludge	3.0	35	(Eastman and Ferguson, 1981)
Mixture of primary and WAS	0.077 – 0.150	25-35	
Primary sludge (domestic WWTP)	0.007-0.990	35-60	(Gavala <i>et al.</i> , 2003)
Primary sludge (domestic WWTP)	0.4-1.2	35	
WAS	0.168-0.60	35	
Casein	0.35	35	(Pavlostathis and Giraldo Gomez, 1991)
Gelatin	0.6	35	
Corn Protein	0.04	35	
Biowaste	0.03 – 0.47	20-40	(Veeken and Hamelers, 1999)
Screenings	0.061 – 0.1773	37	(Rodriguez, 2012)
Microalgae (raw, THP and lipid extraction)	0.21 – 0.29	38	(Keymer <i>et al.</i> , 2013)

3.5.2 Operational conditions

The performance of anaerobic digesters vary depending on the substrate to be digested, technological configuration as well as the operation of the AD unit. To increase anaerobic digestion efficiencies, microbial activities must be enhanced as their growth rate is important in the digestion process. Some factors however are significant at specific stages in the process. These factors may be used to delineate the process output while some give indication and help to understand the positive and negative effects on the whole process.

Thus this section briefly highlights the parameters which require careful monitoring and analysis for the process development as they can impact on anaerobic digestion performance.

- **pH value and Alkalinity**

These parameters are important and work hand in hand in optimising digester performance and microbial activity. Alkalinity plays the role of buffer by preventing rapid changes in the pH, since acidic conditions generally inhibit the growth of anaerobic microorganisms especially the methanogens thus, the alkalinity of the digester needs to be maintained. For biomethanization, a pH ranging between 6.8-8.5 is optimal for anaerobic digestion (Dennis A and Burke, 2001). Factors that influence the pH in the AD may vary as a result of OLR and HRT as well as process change (acidification and methanogenesis stages) since both groups of microorganisms responsible for these have different optimal pH ranges.

pH may impact the microbial consortium, for instance, the methanogens are extremely sensitive to pH and perform better with an optimum range of 6.5 – 7.2, while the fermentation bacteria are more tolerant to pH ranging between 4 and 8.5. Fermentation product is also affected as low pH produces acetic and butyric acids while the higher pH of 8 produces mainly acetic and propionic acids (Appels *et al.*, 2008). Other metabolic shifts in process as a result of pH were observed by Yu and Fang, (2002) where reduction of pH in AD from 7.2 to 5.5 and 4.4 gave rise to hydrogenesis and solventogenesis.

According to Veeken *et al.*, (2000), pH plays an anonymous role in controlling the hydrolysis rate in anaerobic solid state fermentation process; this role is however anticipated to vary depending on the waste of interest as well as possible composition.

Other factors that may influence pH include: ammonia accumulation during degradation of protein thus leading to a pH increase, or accumulation of VFA (volatile fatty acids) thus favouring a pH decrease. Maintaining the pH within the recommended range is essential for efficient gas production.

- **Volatile Fatty Acids**

High concentrations of these intermediate compounds found in the metabolic pathway of methane fermentation may cause microbial stress. This happens by inhibiting the growth of acid producing bacteria in a digester thus reducing the acidogenesis. Of the intermediate products during anaerobic digestion (Buyukkamaci and Filibeli, 2004), the propionic and acetic acid are the most active VFA present and their presence can be used to estimate digester performance. According to Pullammanappallil *et al.*, (2001), digester failure was observed when the concentration of acetic and propionic acid reached above 3000 mg/l. In relationship to pH, acetate yield is increased slightly with pH increase while no relationship can be established between the pH and propionate yield (Yue *et al.*, 2007). One of the most common factors leading to VFA accumulation is as a result of a sudden increase in organic loading rate of a digester (Wijekoon *et al.*, 2011).

- **Solids (SRT) and Hydraulic Retention time (HRT)**

SRT is the time between the solids being added to, and removed from the digester and is a very important factor that controls the conversion of solid to gas and maintains digester stability. HRT on the other hand is the amount of days in which the soluble compounds remains in the digester. It is the time between which there is no removal or addition of water to the system and is important in establishing the length of time available for bacteria growth and conversion of organic matter present to gas. The HRT equals the volume of the tank divided by the daily flow, $HRT = \text{Volume (V)} / \text{Flow (Q)}$. Some of the factors that determine the HRT of a

digester include temperature and Organic Loading Rate (OLR), substrate pre-treatment etc.

Findings have shown that a SRT shorter than 5 days may be detrimental to digester performance by inhibiting substrate degradation; whereas a SRT of 5-8 days can lead to an incomplete degradation of compounds especially lipids. While Solids retention time of 8-10 days has been reported to favour a stable anaerobic digestion process (Appels *et al.*, 2008), general HRT for solid waste degradation may however range between 8 and 25 days.

Depending on the degradability and composition of a studied substrate, the HRT may be influenced as it is expected that a substrate with low degradability will require more time in the digester to achieve maximum biogas yield. The HRT of a digester is responsible for digester stability and production of intermediary compounds; it also dictates the amount of substrates that may be processed in the digester. While a short HRT is beneficial for increased substrate per unit time, proper monitoring of this parameter is essential as a low HRT can lead to VFA accumulation and eventual failure of digester.

Finally, hydraulic retention time (HRT) and organic loading rate (OLR) work alongside each other and cannot be neglected as a high OLR implies a low HRT and *vice versa*, and this determines the rate of production of intermediary metabolites. These parameters also determine the efficiency and economy of the process e.g. a high organic loading rate (OLR) in the digester can reduce the HRT and capital cost generated by digester size.

Literatures report shows a range of optimal HRT for the anaerobic digestion of several substrates, some of this also extends to co-digestion experiments (Table 3.5). It is evident that several conditions may affect the optimal HRT of digested substrates. Such conditions include substrate type, composition, temperature, pH, digester mixing as well as pre-treatment.

Table 3.5 Optimal HRT for a range of biodegradable wastes

Substrate	Optimal HRT	Reference
Sewage Sludge	16-40	(Bolzonella <i>et al.</i> , 2005)
Sewage sludge and Grease waste	20	(Silvestre <i>et al.</i> , 2011)
Food waste and WAS	13	(Heo <i>et al.</i> , 2004)
Organic urban solid wastes	18	(Castillo M <i>et al.</i> , 2006)
Food waste	8-12	(Kim <i>et al.</i> , 2006)
Microalgae (different species and conditions)	10-28	(Ehimen <i>et al.</i> , 2011; Mairet <i>et al.</i> , 2011; Ras <i>et al.</i> , 2011; Passos <i>et al.</i> , 2014)

- **Organic Loading Rate**

OLR can be expressed in a unit weight as chemical oxygen demand (COD) or volatile solids (VS) per unit volume. This is the measure of the biological conversion capacity of the AD system and is a very important factor in the anaerobic digestion process as feeding the system above its sustainable OLR rate can result in poor conversion rates through the accumulation of inhibitory substances such as fatty acids in the digester slurry (Monet, 2003) which has been reported in many plants (RISE-AT, 1998).

The OLR determines the intermediate metabolites and may influence digester performance. For instance, a high organic loading rate (OLR) in the digester can cause excessive ammonia and VFA accumulation. Furthermore, at a high OLR, the biogas produced under these condition can cause foaming in the digesters thus negatively impacting digester performance (Ganidi *et al.*, 2011).

Optimal OLR in digesters may vary. In a typical unmixed digester treating WAS, the loading capacity may range between 0.48 – 1.6 kg VS/m³d, this may however be extended between 1.6 and 6.4 kg VS/m³d in high rate digesters (Llaurado, 2009). Nevertheless, it is essential to identify the optimal OLR and HRT of particular substrate of interest to maximise energy production and digester efficiency.

A wide range of OLRs has been reported for several substrates, e.g Sakar *et al.*, (2009) identified the optimal OLR for poultry and livestock waste to fall with 0.117

and 7.3 kg VS/m³d while the optimal OLR for cattle manure range between 2.5 – 3.5 kg VS/m³d (Burton and Turner, 2003). Other optimal OLR for co-digestion include co-digestion of sewage and high fat medium with optimal OLR ranging between 1.70 and 3.70 kg VS/m³d.

With respect to microalgae studies, varying OLR have been investigated to achieve enhanced digester products. According to the literature, an OLR between 1 and 2.5 kg COD m⁻³ may be employed (González-Fernández *et al.*, 2013). Other studies on microalgae co-digestion in terms of VS content include a study by Jegede, (2012) where co-digestion of *Cyanobacteria* and *Chlorella* in which a range of OLR between 1 and 9 g VS were studied. Findings revealed the optimal OLR to be 7 g VS . Other studies identifying optimal OLR of microalgae range between 0.91 and 6 g VS/L (Golueke *et al.*, 1957; Marzano *et al.*, 1982; Yen and Brune, 2007)

Overall, the OLR of anaerobic digesters is an important operational parameter which requires proper studying for a successful anaerobic digestion process. Although a high organic loading rate (OLR) in the digester can reduce the HRT and capital cost generated by digester size, it may also be detrimental to digester performance thus leading to failure, possible pH reduction, reduced organic content destruction and negative impact on methane production rates.

- **Mixing**

This is done inside the digester to homogenize the material, increase the mass transfer and provide an intimate contact between microorganisms and substrate for enhancing the anaerobic digestion process. It is essential to understand the impact of mixing in a digester. Slow mixing is recommended as it allows the digester to better absorb shock loading while excessive mixing disrupts the microbes thus reducing biogas production or reducing the rate of oxidation of fatty acids thus leading to digester instability. In addition, mixing may prevent scum formation as well as the development of temperature gradients within the digester.

- **Temperature**

This is the most critical parameter to maintain at a required range as digestion rates are strongly dependent on it. Basically, three temperature ranges are used in the operation of AD to provide optimum digestion for methane production. These are the

mesophilic range which is operated between the temperatures of 30 – 35°C, the thermophilic which is operated between the temperatures of 50 -65°C (RISE-AT, 1998) and the psychrophilic with temperature up to 20°C. Generally, many methanogens are very efficient in the mesophilic range and since methane production is the favoured reaction, engineered digesters usually operate at mesophilic or thermophilic ranges. Although biogas production varies depending on the feed being digested and the influence of added chemicals (Mata-Alvarez *et al.*, 2000), operating the digester at a thermophilic range can enhance microbial growth, OLR, biogas production as well as reducing the retention time (Kim and Speece, 2002). Other potential benefits include enhanced digestate dewaterability and reduction of foaming potential over the mesophilic range (Suhartini *et al.*, 2014).

3.5.3 Feedstock Characteristics

- **C/N ratio**

This is the relationship between the total elemental carbon to the total elemental nitrogen present in the organic material to be digested. The available nitrogen is utilized by the bacteria for their growth and metabolism and also contributes to the alkalinity, while the carbon fraction is converted to methane and carbon dioxide. To ensure optimal digestion, a high and low C/N ratio should be avoided as a high C/N ratio is said to result in lower biogas production due to the lack of alkalinity while low C/N ratio (signifying high level of nitrogen) can cause ammonia toxicity. A C/N ratio of 20 – 30 should be used (Fricke *et al.*, 2007). Several studies however show that the C/N ratio for microalgae species varies between 6 and 9 (Geider, 2002). Based on this, to optimize digestion of microalgae there will be a need to find a co-substrate with high carbon content or reduce the nitrogen available in the algal cells by limiting nitrogen during their growth conditions. Table 3.6 below shows the C/N values of some materials

Table 3.6 Typical C/N ratios of some materials

Raw material	C/N ratio
Duck Dung	8
Human Excreta	8
Pig Dung	18
Cow Dung	24
Water hyacinth	25
MSW	40
Maize Straw	60
Wheat Straw	90
Saw Dust	<200

Adopted from (RISE-AT, 1998)

- **Nutrients**

Nutrients are essential for the growth and survival of microorganisms for efficient biogas production. Nutrients administered should have a ratio of C:N:P:S = 600:15:5:1 to allow proper anaerobic digestion. Nutrients can be divided into two groups which are the micro and the macro depending on the quantity needed by bacterial cells to facilitate their growth. The macronutrients include nitrogen and phosphorus both of which are needed in large quantities by the anaerobic bacteria while the micronutrients are only needed in trace amounts with examples like nickel, sulphur and cobalt. The presence of these 2 groups of nutrients is essential for bacterial cell utilisation however, excessive or insufficiency of these nutrients may be toxic or inhibit the digestion process

- **Total solids (TS) and Volatile solids (VS)**

This is the degradable part of a substrate in a unit volume of slurry. The total solid (TS) concentration in a slurry can influence the pH, temperature and effectiveness at which microorganisms decompose wastes in a digester. Optimal solid concentration within a digester is mostly dependent on the reactor design and is recommended at 7 – 9% solid concentration for floating dome reactors. For a continuous stirred reactor (CSTR), a range of TS between 4% and 10% is recommended.

Volatile solids on the other hand are the amount of solids lost when heated in a furnace at 550°C and expressed as a percentage of the total solids being fed to the digester. Volatile solids are important in determining biodegradation by giving direct metabolic status of microbial groups and are in two fractions, which are

Biodegradable Volatile solids (BVS) and Refractory volatile solids (RVS). The BVS fraction of MSW has been observed to deliver a more accurate estimation of the biodegradability of the waste, its biogas estimation as well as organic loading rate and C/N ratio (Kayhanian and Rich, 1995). Generally the best waste characteristics for AD treatment are those with high VS and low non-biodegradable matter.

- **Toxicity and inhibition**

These are substances that negatively affect the inoculum or completely inhibit the inoculum growth as well as function. Some of these may be contained in the wastewater feed, which may vary in composition while others are generated in the process thus resulting in process failure or instability. Toxic wastes which inhibit the anaerobic digester performance are numerous and diverse however, Chen *et al.*, (2008) gives a review of the most common types including ammonia, hydrogen sulphide and heavy metals.

Although these wastes are toxic to the methanogens, the archaea tend to tolerate a higher rate of these toxins when they are acclimatised. A summary of some organic and inorganic toxic wastes to anaerobic digesters as well as values/ranges in which this toxicity can occur is detailed in Gerardi, (2003).

3.6 Pre-treatment/Advanced Anaerobic Digestion in the UK WWTP

Anaerobic digestion of sludge is well understood and plays an essential role as a means of sludge stabilization and energy production. A growing population and industrial expansion globally has led to an increased volume of sewage treated and sludge produced thus, the need for sustainable methods of sludge treatment and disposal. This has led to an intense research effort to explore the full potential of anaerobic digestion with main advances in the reactor design, configuration, operation and the microbial aspect of anaerobic degradation (Lettinga, 1995).

Whilst these advances in the typical AD process are aimed at dealing with issues such as energy recovery, GHG reduction and enhancement of process performance, in addition, it is anticipated to deal with more recent challenges such as:

- Reducing the digester retention time,
- Providing optimal conditions for the hydrolysis and acidogenesis stage,
- Increasing organic loading rate, and
- Achieving more effective biological pathogen inactivation

Thus the UK water industries have moved on to AAD (Advanced Anaerobic Digestion) for sludge processing and energy production to achieve maximum sludge utilization and VS destruction of waste produced. This involves the addition of a pre-treatment stage to the existing configuration to condition the feedstock/sewage sludge prior to feeding into the main AD system.

In the UK, the main preferred pre-treatment route adopted for advanced digestion processes are Enhanced Enzymic Hydrolysis (EEH) and thermal hydrolysis (TH). These routes have experienced development in recent years and have emerged as the fastest growing pre-treatment route in the UK (CAMBI, 2006). While both of these aim to achieve sludge hydrolysis and enhance degradation, different pathways are employed:

3.6.1 Enhanced Enzymic Hydrolysis (EEH) Plant:

Enhanced Enzymic Hydrolysis is a modified process of the conventional enzymic hydrolysis. In EEH, the digester feed is passed through three reactors operating in series at temperatures ranging between 32 and 42°C followed by a second stage which involves a heating reactor and 2 holding tanks all operating at 55°C prior to feeding into the parallel operated mesophilic anaerobic digesters (Leach and Edgington, 2012).

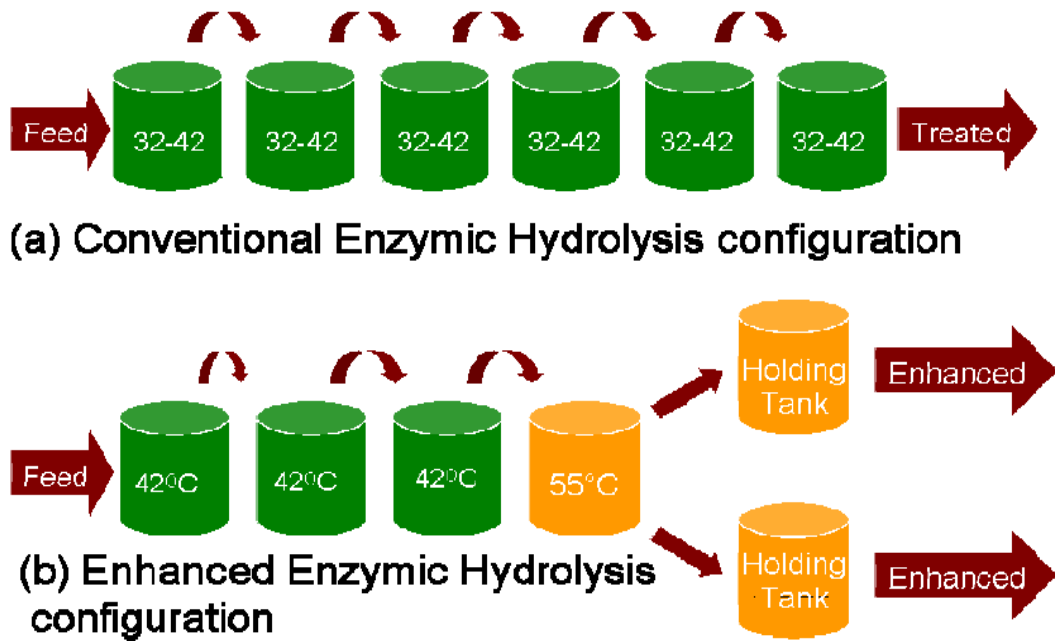


Figure 3.3 process flow conventional EH and EEH (Leach and Edgington, 2012)

This configuration of the EEH (Figure 3.3) is intended to achieve an enhanced treated sludge via passing through series of vessels at both mesophilic and thermophilic temperatures. Whilst the series of vessels operated at mesophilic temperatures are operated with an HRT of 1.5days, adopting the acid phase of a typical AD process to hydrolyse complex organic compounds and simultaneously produce VFAs; the thermophilic pasteurisation stage operates at 55°C with an HRT of 1.5 days incorporating of a 5hr holding time for methane production and process stabilization (Bungay and Abdelwahab, 2008).

The effectiveness of this approach for the treatment of wastewater sludge and cellulose rich materials has been studied and shown to positively influence the rate of particulate matter solubilisation, VS destruction, pathogen destruction, VFA production, hydrolysis rate of lignocellulose materials as well as to enhance biogas production and quality (Bochmann *et al.*, 2007; Riffat, 2012; Rodriguez, 2012).

3.6.2 Thermal Hydrolysis

The most widely adopted THP process used in the UK WWTP has been developed by Cambi (CAMBI, 2006). This comprises three stages to achieve hydrolysis of sludge (Figure 3.4). Stage one is the pulper where the dewatered sludge is heated by recycled steam from the reactors and the flash tanks. Stage 2 is the reactor in which

the sludge is pumped in batches into. The heated sludge then undergoes thermal hydrolysis at 165°C @ 8 bar pressure for 30mins where organic matter is hydrolysed into soluble compounds.

At this stage, the pre-treated sludge is homogenized using a cycle loop and macerator. In the third stage, hydrolysed sludge is rapidly pushed into a flash tank using available steam pressure. Here, steam explosion occurs as a result of rapid pressure drop thus disintegrating the cell fibres of the sludge. The sludge temperature is then decreased to approximately 102°C by flashing steam back to the pulper (Ringoot *et al.*, 2012).

The success of Cambi at UK WWTPs has been identified with benefits for plants like Thames Water's Chertsey WWTP (London, UK) where adoption of Cambi has tremendously impacted and have tuned up the plant to exceptional loading rates of up to 7 kg VS m³/d and retention times as low as 10-12 days (Ringoot *et al.*, 2012).

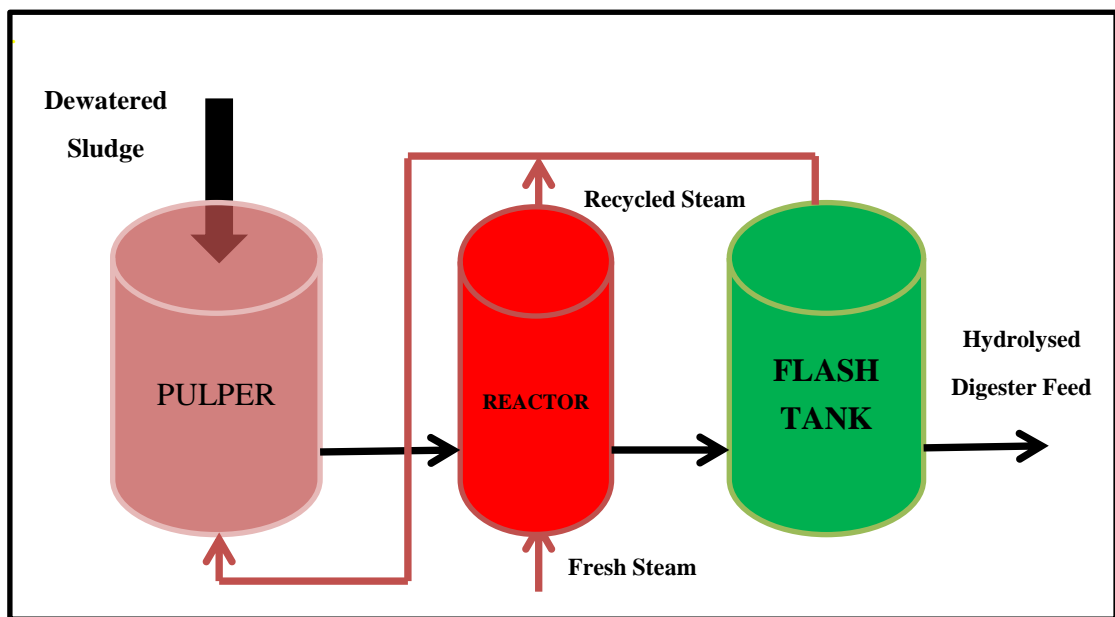


Figure 3.4 Schematic view of the Cambi (THP) Process

Chapter 4 Opportunities for Energy generation from algae

4.1 Biomass Energy

Bioenergy is renewable energy made available from materials derived from biological sources which could be living or dead organisms, including plants and animals. The fuels produced from these feedstocks could be in a gaseous or liquid state and can be used either on site to improve the efficiency of a process or used in other applications such as generation of electricity and transport fuel (Demirbas *et al.*, 2004).

Although biomass is rated as one of the most promising renewable energy resources with up to 3.7 million of TEP (total energy potential) coming from forests and 1.33 million of TEP per year coming from agricultural and urban wastes (Hall, 1997; Hoogwijk *et al.*, 2005; Thrän *et al.*, 2010), research is still being carried out to see how technically and economically viable the power generated from biomass is.

Biomass can be burnt directly or it can be converted into solid, gaseous and liquid fuels using conversion technologies such as fermentation to produce alcohols, bacterial digestion to produce biogas and gasification to produce a natural gas substitute. Industrial, agricultural livestock and forest residues can be used as biomass energy sources (Ferreira *et al.*, 2009).

Based on the production pathways of biofuels, reduction in GHG emissions is expected in the transport sector through the use of biofuels. The literatures confirms that the use of bio-fuels as gasoline and diesel fuel additives/ substitutes could positively influence the baseline total transport energy demand by 2030 with an increment of 3%. Depending on future oil and carbon prices, improvements in vehicle efficiency and the success of technologies which will utilize cellulose biomass as much as 5 to 10% increment could be achieved (Gomes and Muylaert de Araújo, 2009).

Considering the impending oil crisis, concerns about energy security, climate policy as well as the search for alternative sources of agricultural income (van Vuuren *et al.*, 2009), bio-based energy is seen as the next new wave for future businesses,

solutions to high energy cost and strategies for sustainable development (Wonglimpiyarat, 2010). For example alternative fuels such as fuel ethanol from cellulosic biomass can provide benefits in terms of environmental protection, economic development and global energy security.

4.1.1 **First generation biofuels**

These are fuels which are derived from feedstocks such as starch, sugar, animal fat, and vegetable oil (Campbell *et al.*, 2011). On the basis of conversion technologies, first generation fuels make use of technologies that utilize plants (such as cereals, sugar cane, cassava, sugar) or oil seed crops (such as sunflower, rapeseed, soybean and palmoil) as feedstock to produce ethanol and biodiesel respectively (Rutz and Janssen, 2007)

This generation of biofuel has for many years been in commercial production in several countries and is reliant on existing technologies (Damartzis and Zabaniotou, 2011). They can be added to petroleum-based fuels prior to combustion in internal combustion engines, may be used in existing vehicles with an alternative technology such as natural gas vehicles and Flexible Fuel Vehicle or circulated through an existing infrastructure (Naik *et al.*, 2010). The use the first generation fuels provides an opportunity to rectify energy security for domestic purposes and may also help attain a reduction in net CO₂ emissions. The most freely available of this generation are in the form of bioethanol and biodiesel. Currently up to 50billion litres are produced annually but as attractive as they seem, there have been some arguments as to how sustainable this generation fuel is.

Studies have highlighted the challenges facing the first generation fuel, these include its low efficiency in lowering CO₂ emissions in the process of biodiesel production (Naik *et al.*, 2010), the adverse effects it poses to biodiversity as a result of land use (causing deforestation) as well as competition with food (Scharlemann and Laurance, 2008). In terms of the energy balance, arguments on energy input compared to output also exist (Pimentel and Patzek, 2005; Farrell *et al.*, 2006). Therefore it is anticipated that increased production of this generation fuel would directly influence food prices and availability as cultivation of these crops means

large expanses of arable land, water and fertilizers would be utilized to achieve increased crop yield (Yang *et al.*, 2009).

Several factors have contributed to setbacks in the use of biofuel including the high cost associated with processing materials (Damartzis and Zabaniotou, 2011).

Although the first generation biofuels seem to be relatively more environmentally friendly compared to fossil fuels, disputes as to their competitiveness when compared with fossil fuels as well as other criticism of the sustainability of this generation fuel has raised attention to the potential of the second generation biofuels.

4.1.2 Second generation biofuels

These are basically the biofuels produced from the technological conversion of lignocellulosic materials such as agricultural and forest residues as well as advanced feed stock such as jatropha (Timilsina and Shrestha, 2011). This generation fuel tend to outperform the first generation biofuel as they are abundant, relatively cheap and non-food crops, which offer flexibility when used in the production of biofuel (Naik *et al.*, 2010) thus overcoming the problems of availability and interference with the food industry.

Conversion technologies utilize these lignocellulosic materials found in a range of biomass types like husks, stalks and waste seed all of which are plant residues and not needed for food production. Examples of biofuels in this category includes ethanol from cellulose although it has not been produced commercially, a few pilot scales and demonstration plants have been set up in recent years alongside research activities in many parts of the world including North America, Europe as well as developing countries like Brazil, China, India and Thailand (IEA, 2010; Naik *et al.*, 2010).

This generation fuel may be produced using existing infrastructure from either the petroleum industry or the sugar industries but technical barriers are still encountered during the conversion process and this must be overcome to achieve a cost effective biofuel production (Naik *et al.*, 2010).

It is anticipated that the potential of this generation fuel will shift from the first generation fuel and successful distribution will be attained based on favourable national policies.

4.1.3 **Third generation biofuels**

As a result of concerns and controversies about the first generation of liquid bio fuels particularly when considering their impact on the global food market as well as food security (in the most vulnerable regions of the world economy), there was a major interest in the search for alternative systems of biofuel production. This introduced second-generation liquid bio fuels based on lignocellulosic biomass and this was mainly limited to their large demand for land. Thus to produce a bio fuel that is economically viable, certain criteria need to be met which include: 1) little or no land use, (2) lower cost than petroleum fuels, (3) carbon sequestration, and (4) require minimal water use (Brennan and Owende, 2010)

Microalgae are termed as a 3rd generation biofuel, which has a noticeable feature as a result of their high lipid content as well as ability to convert solar energy into biomass in a relatively short time. As its growth is not linked to human food or land consumption, it is recognized as a potentially good source for biofuel production and it is expected to be the most important bio fuel source in the near future meeting future energy demand as well as benefiting the environment (Mata *et al.*, 2010).

Generally most algae are phototrophs with the ability to produce large amounts of biomass through photosynthesis and with the ability to complete their entire growth cycle in days, unlike other terrestrial energy crops that utilise a much longer growing season (Mata *et al.*, 2010; Feng *et al.*, 2011). They absorb sunlight with the help of their chlorophyll pigments under natural conditions and absorb carbon dioxide from the air and nutrients from their aquatic habitat. The absorbed solar energy is subsequently stored as a chemical energy in the form of carbohydrates and oxygen is released (Figure 4.1).

The stored carbohydrate is used as an energy source and broken down during cell metabolic activities. Due to the microalgal cell structure, access to CO₂, water and

other nutrients they have been found to be more efficient in solar energy conversion to produce biomass (Ross, 2011)

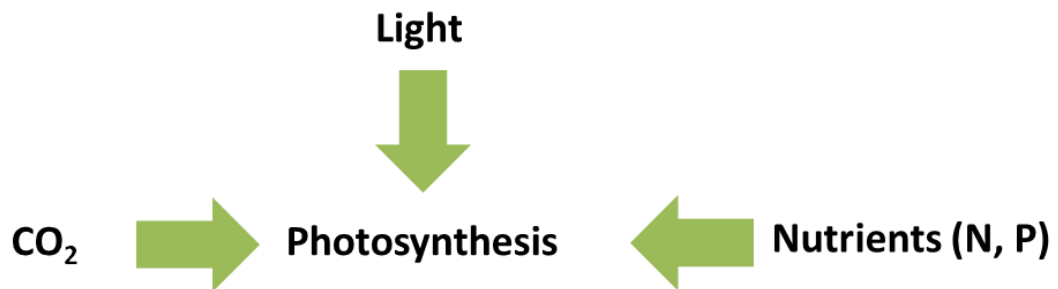


Figure 4.1 Requirements for Photosynthetic Algae Growth (Ross, 2011)

The use of microalgae for energy is potentially superior to other biological energy sources considering the fact that it has a high growth rate, requires far less space for cultivation unlike land-based plants and its ability to grow on non-arable (biodegraded/contaminated) land thus reducing its competition for resources including fresh water, with conventional agricultural products meant for food.

Microalgae as a raw material for bio-energy production could also be beneficial for carbon emission capture and re-use, as its growth can be enhanced by the use of supplemental CO₂ (1kg of dry algal mass is estimated to fix about 1.83kg of CO₂), which could be redirected from stationary industrial sources such as fossil-fired power plants, cement plants, fermentation industries etc. thus reducing the impact GHG via reduction on overall carbon emission (Chisti, 2007)

The biofuel potential of algae is outstanding considering its high biomass production and oil content which can be converted to bio-oil, bio-ethanol, bio-hydrogen, and bio-methane through the use of the thermo chemical and biochemical methods. Furthermore, after oil extraction from algae, the co-products they produce are found to be rich in protein and residual biomass which can then be used for agricultural feed or fertilizer (Oilgae, 2011)

Although algal prospects have received increasing attention in recent years, research into algae biofuel is not new as it has been explored since the 1970s as a result of the major oil crisis and for its potential to produce feedstock for advanced fuels and its

more promising source for more biomass and bio-oil in comparison to other land-based crops (Chen *et al.*, 2009). Despite the potential features and prospects of algae for energy production, recent literature explains that the process of energy generation from these algae does not appear carbon neutral or economically viable as there are many challenges (both technical and engineering) impeding its development and sustainability (Milledge and Heaven, 2014).

These include (1) identifying the algal strain (specie) that will balance biofuel production and extraction of valuable products; (2) development in production systems to attain higher photosynthetic efficiencies; (3) limitations of techniques that can cultivate single species, reduce evaporation and prevent CO₂ diffusion losses; and (4) energy requirement to power the process (water pumping, CO₂ transfer, harvesting and extraction), (5) cell wall disruption of microalgae which is energetically demanding, (6) energy conversion efficiencies.

4.2 Algae bio-products/ wastewater treatment

Throughout history, algae have found different uses such as feed for animals, agricultural fertilizers and as a source of pigment. In the mid-20th century, microalgae were examined as a possible source of protein, antibiotics and energy (Spolaore *et al.*, 2006). They have been used as emulsifiers to thicken and stabilize low fat foods e.g. the use of polysaccharides which are mainly derived from seaweeds and used as food additives, also the production of agar and alginates which are made from the red and brown algae respectively (Guiry, 2011).

The use of microalgae biomass as biofertilizers, animal feed, food soil conditioner, cosmetics as well as for waste water purification have successfully been explored (Borowitzka and Borowitzka, 1988; De-Bashan *et al.*, 2010).

Since the 1970s energy crisis, there has been increased research effort to produce algal energy. This has intensified recently as a result of insecurities surrounding the future of fossil fuels as well as the associated greenhouse gas (GHG) emissions. It was only due to the energy crisis in the 1970s that serious effort and research to produce energy from algae was initiated but this has intensified in recent years due to

concern over limited fossil fuels and the associated greenhouse gas (GHG) emissions (Chen *et al.*, 2009; De Schamphelaire and Verstraete, 2009).

The latest research development involves the use of microalgae in the production of compounds including pigments, proteins, vitamins, lipids, sterols, carotenoids, enzymes, antibiotics, polysaccharides, pharmaceuticals, hydrogen, hydrocarbons and biofuels such as methane (Borowitzka and Borowitzka, 1988; Ignacio, 2008). Microalgae species such as *Spirulina*, *Isochrysis*, *Chlorella*, *Dunaliella*, and *Chaetoceros* have been reported to be the mostly used in the large scale production of these compounds (Borowitzka and Borowitzka, 1988). Another appreciable use of microalgae may be use achieved in the aspect of wastewater treatment and possible re-use (Park *et al.*, 2011).

4.3 Microalgae and wastewater treatment

One of the many appreciable values of microalgae includes its ability to grow on wastewater. This is beneficial and of increasing interest as microalgae prospects include its application in wastewater bioremediation to enhance the removal of nutrients, organic contaminants, heavy metals, and pathogens and interestingly providing substantial amount of raw material for the production of high-value chemicals (algae metabolites) or biogas (Borowitzka and Borowitzka, 1988). Although few wastewater facilities have adopted the use of algae for wastewater treatment, the potential of algae in wastewater remediation is of much wider scope than its current role (Park *et al.*, 2011).

For sustainable production, the cost of producing microalgae biomass must be less than the value of energy obtained. Although this is yet to be realised in large scale practice, the use of waste streams such as wastewater and flue gas has great potential to reduce the cost of production as well as to minimize the dependence on freshwater for microalgae biomass production (Li *et al.*, 2008).

Wastewater contains valuable nutrients such as nitrogen and phosphorus. There is a need for the removal of these nutrients in the final effluent from wastewater treatment plants due to increasing concerns about eutrophication in receiving rivers and lakes (Braga *et al.*, 2000; Nyenje *et al.*, 2010). According to Pittman *et al.*,

(2011) concentrations of nitrogen and phosphorus in municipal wastewater range between 10 and 100 mg/L. This can lead to the production of cyanotoxins, undesirable pH shifts, low dissolved oxygen concentrations and fish kills in aquatic environments (Christenson and Sims, 2011).

The application of microalgae for wastewater treatment and mass production of strains (*Chlorella* and *Dunaliella*) spans about 75 years with more interest and development in recent years. Countries like Australia and USA amongst many others have developed interest in this approach having identified/understood the biology/ecology of large scale culture systems, engineering of large scale culture system and harvesting techniques all of which are prerequisite in designing and operating high rate algal cultures to yield the product of interest. In light of the bio-treatment potential of microalgae, more than 1000 algal taxa have been reported as pollution tolerant. This includes 240 genera, 725 species and 125 varieties out of which eight green algae, five blue-greens, six flagellates and six diatoms have been termed the most tolerant genera (Abdel-Raouf *et al.*, 2012).

Earlier attempts at bio treatment using microalgae include the work of Golueke *et al.*, (1957) where microalgae were used to remove nutrients (N & P) from wastewater and simultaneously produce oxygen for the heterotrophic bacteria. Ever since then, studies have been carried out using various versions of these systems both at laboratory scale and pilot scale.

In a study by Lavoie and de la Noüe, (1985), hyper concentrated algal cultures were adopted for the removal of N and P in wastewater. High efficiency was proven as nutrient uptake occurred in very short times (less than an hour). Sawayama *et al.*, (1995) also demonstrated the potential of microalgae for nutrient uptake in wastewater where *B. braunii* was used to remove phosphate and nitrate, present after the primary treatment of sewage. Other successful nutrient removal in wastewater using microalgae includes the works of Martínez *et al.*, (2000) and Hodaifa *et al.*, (2008) where *S. obliquus* was used to achieve 100% removal of ammonium, 98% removal of phosphorus and a high percentage removal of BOD₅.

The microalgae *Chlorella vulgaris* is one of the more successful strains studied and developed for the removal of nitrogen and phosphorus in wastewaters. Lau *et al.*,

(1996) demonstrated a removal efficiency up to 86% and 70% of inorganic N and P respectively from wastewater. The potential of these microalgae is also reported by Colak and Kaya, (1988) for the elimination of nitrogen and phosphorus in industrial wastewater at 50.2% and 85.7% respectively.

Subsequently, Martínez *et al.*, (2000) and Shi *et al.*, (2007) reported that microalgae *Chlorella* and *Scenedesmus* species in most cases are more tolerant to sewage achieving greater than 80% removal of nitrate, phosphorus and ammonia. Also, some algal systems have been recognized to have the potential to treat human sewage, livestock wastes, agro-industrial waste, piggery effluent and effluent from food waste have been studied (Abdel-Raouf *et al.*, 2012).

Microalgae for heavy metals uptake

Toxic metals including mercury, cadmium as well as other organics are often present in industrial wastewater. This is treated biologically using the activated sludge system which is effective for nutrient removal but not economically friendly in terms of cost associated with treating the generated sludge, purchasing chemical as well as the high energy demand. The use of microalgae is capable of achieving nutrient and heavy metal removal via absorption and adsorption from wastewater thus minimizing the treatment costs, as well as producing valuable by-products with no generation of pollutants, thus making it also attractive for biofuel production (Brennan and Owende, 2010; De-Bashan *et al.*, 2010).

Some of the successful work with the use of microalgae to absorb heavy metals from wastewater is reported by Darnall *et al.*, (1986) who reported the successful use of *Chlorella vulgaris* to selectively recover Cu^{2+} , Zn^{2+} , Au^{3+} and Hg^{3+} . Other successful studies on heavy metal absorption can be found from Nakajima *et al.*, (1981). It is worthy to mention that the accumulation of some heavy metals including Cd, Cu and Ni may adversely impact the anaerobic digestion process (Mudhoo and Kumar, 2013)

Microalgae for CO₂ recycling

Furthermore, biogenic sources of CO₂ exist within the wastewater treatment plants which include CO₂ emission during anaerobic digestion, post combustion CO₂ during CHP, CO₂ emission from anaerobic treatment as well as CO₂ emission during

sludge combustion (EPA, 2010). All these add up to about 40 percent of the total CO₂ emitted within the sector however, the ability of microalgae to tolerate high CO₂ concentrations such as those present in flue gas and its efficient conversion of the CO₂ into biomass makes it an ideal candidate for such applications (Li *et al.*, 2008) and the effectiveness of this has been successfully researched (Brennan and Owende, 2010). Though the extent of reduction varied depending on the microalgal species used and the CO₂ content of the flue gas, it showed that microalgae cultivation using flue gas is a possible means of carbon capture. According to Yun *et al.* (1997) up to 1.8kg of CO₂ could be fixed in the production of 1kg of *Chlorella vulgaris* in wastewater.

More recent development to enhance microalgae growth and demonstrate biofuel feasibility at industrial scale includes the FP7 Algae Cluster EU Commission Demonstration Project with programmes including ALL GAS, BIOFAT, and INTESUSAL (FP7-ENERGY, 2010). The ALL-GAS (coordinated in Spain) programmes demonstrates large scale production of biofuels based on low cost microalgae cultures by integrating and upscaling innovative systems to double algal yields. The programme incorporates a full chain of processes from algal ponds to biomass separation, processing for oil and other chemicals extraction, and downstream biofuel production, as well as the use in vehicles. This will be implemented on a 10 ha site to achieve a minimum productivity yield of 90 t/ha/yr via re-using wastewater influent and nutrients to stimulate algae growth, and the use of additional CO₂ obtained by the thermal transformation of both external and internal biomass (ALL-GAS, 2011). Similarly, the BIOFAT (BIOFAT, 2011) and INTETUSAL (INTESUSAL, 2011) project (coordinated in Portugal and United Kingdom respectively) are aimed at both biodiesel and ethanol production while integrating the whole algae process value chain from algae optimized growth and starch and oil accumulation, to downstream biofuel production processes. These programmes will demonstrate sustainability of this approach, in terms of both economic and environmental implications across the whole process; including optimum use of algal biomass resources to enable commercialisation.

4.3.1 Challenges of microalgae use for wastewater treatment:

Microalgae possess a great capacity to utilize the majority of the waste nutrients produced in typical WWTPs. Nutrient removal is basically by precipitation, stripping and biomass uptake of nitrogen and phosphorus. Considering the requirement for microalgae growth (CO₂, light, heat, N & P) and the abundance of this in WWTP, there is no doubt that integration into the wastewater treatment will be a beneficial approach especially for tropical and subtropical climates.

In addition, the associated environmental benefits from the exploitation of microalgae may be obtained during microalgae cultivation in wastewater, as large amounts of nitrogen and phosphorus can be fixed in microalgae biomass thus eliminating concerns of eutrophication, reducing cost of wastewater treatment and prevention of GHG emission such as the nitrogen gas associated with the conventional nitrogen removal (nitrification/denitrification).

One of the anticipated challenges with this approach however includes illumination, which is a necessity for microalgae efficiency considering the fact that turbidity increases simultaneously with microalgae cultivation and that turbid conditions inhibit light penetration thereby retarding algal growth. Illumination is expected to be limited in the winter seasons thus requiring the adoption of artificial lighting. Although artificial lighting on the other hand has been reported to improve effectiveness of microalgae use in wastewater treatment in areas such as Quebec, Canada during winter season it was also reported to significantly increase wastewater treatment cost (de la Noue and de Pauw, 1988).

Furthermore, selection of the appropriate strain is also a challenge as not all microalgae strains have the bio adsorption tendencies as well as the ability to thrive well using wastewater. Thus, it is essential to overcome this challenge by selecting an appropriate strain with the ability to survive in wastewater of nutrient whilst still keeping its nutrient uptake ability, high biomass productivity and good biofuel potential. Some of the well-researched strains include the *Chlorella* family: *Chlorella vulgaris*, *Chlorella kesleri* and *Chlorella pyrenoidosa* have shown great potential in wastewater and biomass accumulation, yet fallen short in commercial

biofuel production (Li *et al.*, 2011). Other strains studied include *Dunaliella*, *Spirulina* and *Scenedesmus*.

4.4 Microalgae to Energy (Anaerobic Digestion)

Although research on microalgae conversion into biogas via anaerobic digestion is not as well researched as biodiesel production from algae, which has proven to be energy intensive and expensive (Razon and Tan, 2011), some researchers in the past have carried out laboratory research on anaerobic digestion using algae as feedstock. This concept of methane production was first suggested by Meier, (1955) from the carbohydrate fraction of the cells. Advancement of this idea was however introduced by Golueke *et al.*, (1957) with a conceptual techno economic engineering analysis of digesting microalgal biomass grown in large raceway ponds to produce methane gas.

This section gives an overview of some of the previous work carried out on the anaerobic digestion of algae with the aim of identifying the methane yield obtainable from microalgae, the preferred species for digestion, challenges of digesting microalgae and the conditions that can improve the methane yield.

Most of the microalgae species used in investigating the methane potential were mostly from the cyanobacteria or the green algae group. The choice of the algal species used for each study depended on either availability such as algal species harvested from wastewater treatment ponds, or growth rate in lab scale production.

A literature review showed that this falls in the range of 0.143 to 0.450 m³/kg of volatile solids (VS) depending on the operating conditions and algal species. Most of the studies reviewed were carried out under mesophilic conditions, but at different retention times and loading rates ranging from 10 to 30 days and 0.9 to 22.5 g VS/ L, respectively. The methane yield obtained from the anaerobic digestion was low compared to the methane production from the anaerobic digestion of sewage and food waste. This was observed to be a result of the presence of the microalgal cell wall which is resistant, microalgal nutritional composition (protein, lipid and carbohydrate content) and in some cases as a result of increased ammonia concentration.

As early as 1950, the use of anaerobic digestion as an energy saving disposal route for algal biomass obtained from waste stabilization ponds was explored by Golueke *et al.*, (1957). Using a microalgae mixture dominated by *Scenedesmus* and *Chlorella* species, a performance comparison between algae and raw sewage as a substrate for digestion was made. The study also observed the impact that varying operating conditions such as temperature, retention time and loading rate could have on the anaerobic digestion of algae. Using an HRT of 30 days, mesophilic conditions (35°C) and organic loading rate of 1.44 g VS /L and 2.9 g VS /L, the methane yield obtained was 0.256 m³ and 0.512 m³ per kg of VS introduced respectively.

Increased methane yield from 0.256 m³ to 0.320 m³ per g of VS introduced (OLR of 1.44 g VS /L) was observed under thermophilic conditions as a result of increased degradation of the algal cell wall at that temperature making the algae more susceptible to bacterial activity. The study reported that HRT much less than the conventional 30 day period did not significantly reduce methane production from algae, although very low HRT such as 7 days affected methane production due to the flushing out of the anaerobic bacteria.

Also with the use of batch digesters, Sánchez Hernández and Travieso Córdoba, (1993) studied *Chlorella vulgaris* for its digestibility under anaerobic conditions using an HRT of 68 days, mesophilic conditions (28°C – 31°C) and sewage sludge as the inoculum. The experimental setup involved digesting 4 litres of *Chlorella vulgaris* under different COD concentration range between 1.73 – 4.47g VS/l. The result showed that the biogas produced from each batch was more significant within the first 4 weeks and this corresponded to a reduction in the chlorophyll and COD concentrations.

Total biogas produced was highest in the batch with the highest OLR at 4.47g/VSL with a methane yield of 0.51 to 0.54 m³/kg VS introduced (N.B: concentrations were reported in COD and were interpreted using an average biogas composition of 72.2% CH₄ and a COD/VS ratio of 1.5). An initial increase in chlorophyll concentration was observed during the first week of digestion. Although this declined with time, it confirmed algae growth, thus the need for pre-treatment to facilitate the break-up of algal cells.

In an experiment carried out by Mussgnug *et al.*, (2010) using microalgae as substrates for biogas production six dominant microalgae species including *C. reinhardtii*, *Dunaliella salina*, *Scenedesmus obliquus*, *Chlorella kessleri*, *Euglena gracilis* and *Arthrospira platensis* were investigated as alternative substrates for biogas production. These microalgal species were anaerobically digested in batch mode under mesophilic conditions (38°C) for 32 days. Loading rate was estimated to be 2.9g solids/L based on cellular material loaded per unit volume, as it wasn't clearly stated by the author. The experiment showed that *C. reinhardtii* and *Dunaliella salina* had the highest methane yield with respective values of 0.387m³ CH₄/kg VS and 0.325 m³ CH₄/kg. The overall findings of this experiment showed that methane proportion of microalgae biomass could be higher than that of maize silage by up to 7-13 % and that algal specie as well as pre-treatment were a major determinant of biogas production. Furthermore, “drying” as a pre-treatment as well as the presence of microalgal cell wall was observed to adversely affect methane production of algae.

With the aim of studying the feasibility of coupling algae (*Chlorella vulgaris*) production to an anaerobic digester unit, Ras *et al.*, (2011) carried out anaerobic digestion of *Chlorella vulgaris* using two HRTs of 16 and 28 days under mesophilic condition (35°C) and a COD loading rate of 1g COD/L. Results showed a higher *Chlorella vulgaris* degradability and a higher methane conversion efficiency was achieved under the 28 days HRT giving values 147 and 240 mLCH₄gVSS⁻¹ respectively for the 16 and 28 days HRT.

Also, 50% of the digested biomass was observed not to undergo anaerobic digestion even at longer retention times as a result of the carbon and nitrogen fractions of the microalgae and this basically highlights the need to investigate microalgae biomass digestion to achieve higher methane yield by pre-treatment to improve its bioavailability of resistant compounds in their cell wall or the selection of algal species without cell wall.

Another study on anaerobic digestion of algal species was carried out by Zamalloa *et al.*, (2012) using two algae strains consisting of a fresh water alga (*Scenedesmus obliquus*) and a marine alga (*Phaeodactylum tricornutum*) as feed substrate. The

study was carried out to determine the CH₄ production from the anaerobic digestion of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophilic conditions. Digestion was carried out in batch using a 1.15L glass bottle with a working volume of 1L. A loading rate of 2 gVS/L and inoculum (digestate from a full scale anaerobic digester treating potato-processing waste water) to substrate ratio of 3:1 was used based on VS content and this was run for 30 days.

The results showed that *Phaeodactylum tricornutum* has a higher digestibility by a factor of 1.5 compared to *Scenedesmus obliquus*, with both having a cumulative methane yield of $0.35 \pm 0.03 \text{ LCH}_4 \text{ g}^{-1}\text{VS}_{\text{added}}$ and $0.21 \pm 0.03 \text{ L CH}_4 \text{ g}^{-1}\text{VS}_{\text{added}}$ respectively. The ultimate methane yields were estimated to be $0.35 \pm 0.03 \text{ LCH}_4 \text{ g}^{-1}\text{VS}$ and $0.21 \pm 0.03 \text{ L CH}_4 \text{ g}^{-1}\text{VS}$ for *Phaeodactylum tricornutum* and *Scenedesmus obliquus* respectively. Overall result showed that algae biomass is not readily biodegradable under digestion conditions and that anaerobic digestion should be integrated in a process change either through pre- or post- treatment in order to harvest the full energetic and chemical potential of the algae biomass.

Varel *et al.*, (1988) also carried out an experiment to study anaerobic digestibility of microalgae. Their study involved the use of the cyanobacterium; *Spirulina maxima* as the feed substrate. This was carried out to determine methane production from the anaerobic digestion of *Spirulina maxima* under mesophilic and thermophilic conditions. A daily loading rate of 22.5 g VS /L (2.25 %) at retention times of 8, 12 and 16 days under both mesophilic and thermophilic conditions were observed. The methane yield ($\text{m}^3 \text{ CH}_4/\text{kg VS}$ introduced) obtained under mesophilic conditions were 0.135, 0.225 and 0.225 for 8, 12 and 16 days of retention time, respectively. Under thermophilic conditions, methane yields were 0.075, 0.165 and 0.135 respectively to the corresponding retention time tested. An ultimate methane yield of $0.33 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ introduced was obtained after 105 days under mesophilic conditions, although 90% of the CH₄ was produced in the first 20 days at the respective retention times respectively.

Results showed that no significant methane yield was achieved under thermophilic digestion and this contradicts the normal convention as methanogenic activities are

expected to increase simultaneously with temperature rise. Poor yields were suggested to be a result of a low C:N ratio, lack of nutrients, generation of toxic substances or increased instability of the methanogenic archaea under thermophilic conditions. The loading rate used in this study was also too high to benefit the methane yield of the process thus a slightly lower loading rate is expected to be more suitable.

Heerenklage *et al.*, (2010) carried out experiments using five different conditions as follows; under mesophilic conditions, under mesophilic conditions (35°C) after one of the three pre-treatment techniques tested and under thermophilic conditions (55°C). The pre-treatment techniques explored were ultrasound, mechanical compression using a French press and enzymatic and thermal decomposition. The aim of this study was to investigate the effect that different pre-treatment techniques can have on methane production from the digestion of algal biomass using *Chlorella vulgaris* as feedstock for digestion. Pre-treatment was observed to have increased methane yield although thermophilic digestion still gave a higher methane yield. Enzymatic decomposition achieved the greatest increase and was observed to be more effective at higher temperatures. The increase in methane yield obtained from pre-treatment with ultrasound and the French press was found not to be justifiable due to the high energy requirement to carry them out.

With the attempt to investigate the effect of co-digestion on the achievable methane yield from microalgae cells, Yen and Brune, (2007) used microalgae sludge containing a mixture of *Scenedesmus* and *Chlorella* species obtained from a Partitioned Aquaculture System (PAS). The microalgae sludge was digested at different daily loading rates of 2.0, 4.0, and 6.0g VS/L under mesophilic conditions for 10 days and respective methane yields attained were 0.090, 0.143 and 0.136 m³ CH₄/kg VS fed. Results confirmed that the C : N ratio of the algal sludge (5.3 :1) led to high ammonia and fatty acid concentrations thus inhibiting microalgal biodegradation and reducing the methane yield.

Increased methane yield was observed when microalgae was co-digested with waste paper. This was as a result of the increased C: N ratio as well as the fact that addition of paper provided cellulose for cellulase activity which is another possible source of

methane production. The study showed the potential of microalgae to achieve increased methane yield via increasing its C:N ratio with a high carbon source.

A methane yield of 0.31 m³ CH₄/kg VS was obtained from the anaerobic digestion of fresh *Tretraselmis* at mesophilic conditions (35°C), loading rate of 2g VS/l and a 14 days (Marzano *et al.*, 1982). Also digestion of *Spirulina maxima* under mesophilic and thermophilic conditions using a loading rate of 0.97 g/l VS and retention time of 33days was reported to give methane yield of 0.26 m³ CH₄/kg VS (Samson and Leduy, 1982).

A summary of the maximum methane yield obtained from the digestion of microalgae reviews as well as the digestion conditions is presented in Table 4.1 to ease selection of the appropriate strain required for this study.

Table 4.1 Methane yields and process conditions of experiments on anaerobic digestion of the microalgal species as reviewed 2011.

Algal Species	HRT (day)	Loading rate (g VS/l)	Temp (°C)	Methane yield (m ³ CH ₄ /kg VS)	References
<i>Chlorella-Scenedesmus</i>	30	2.89	35	0.32	Golueke <i>et al.</i> , 1957
<i>Spirulina maxima</i>	12	22.5	35	0.225	Varel <i>et al.</i> , 1988
<i>Chlorella vulgaris</i>	28	4.47	31	0.277	Sanchezhernandez and Traviessocordoba, 1993
<i>Chlorella-Scenedesmus</i>	10	4	35	0.143	Yen & Brune, 2007
<i>Chlorella vulgaris</i>	-	-	35-55	0.337-0.475	Heerenklage <i>et al.</i> , 2010
<i>Spirulina</i>	28	0.91	35	0.38	(Sialve <i>et al.</i> , 2009)
<i>Dunaliella</i>	28	0.91	35	0.45	(Sialve <i>et al.</i> , 2009)
<i>Tetraselmis</i>	14	2	35	0.31	(Marzano <i>et al.</i> , 1982)
<i>Spirulina maxima</i>	33	0.97	35	0.26	(Samson and Leduy, 1982)
<i>Chlamydomonas reinhardtii</i>	32	2.9	38	0.387	Mussgnug <i>et al.</i> , 2010
<i>Dunaliella salina</i>	32	2.9	38	0.323	Mussgnug <i>et al.</i> , 2010

4.4.1 Selection of Microalgae strain for the research

In an attempt to select a suitable strain for the research, a Multi Criteria Decision Analysis (MCDA) was used for the selection of the preferred strain based on the available literature information on the anaerobic digestion of microalgae up to the beginning of the research February 2011 (Table 4.2). The reviewed algal species were ranked based on their digestion having the following characteristics:

- 1) A high methane production
- 2) A short retention time and
- 3) A high loading rate.

The algal species were allocated in ranks according to how they match the three criteria. As shown in table 4.2, the ranking scores are then added up and the species with the lowest rank is taken as the preferred species.

Table 4.2 Ranking for determination of microalga species with the highest yields (Yusuf, 2011)

Algal Species	HRT (day)	Loading rate g VS/l	Methane yield (m ³ CH ₄ /kg VS)	Rank total
<i>Chlorella-Scenedesmus</i>	30 ⁵	2.89 ⁵	0.32 ⁴	5 + 5 + 4 = 14
<i>Spirulina maxima</i>	12 ²	22.5 ¹	0.225 ⁸	2 + 1 + 8 = 11
<i>Chlorella vulgaris</i>	28 ⁴	4.47 ²	0.277 ⁶	4 + 2 + 6 = 12
<i>Chlorella-Scenedesmus</i>	10 ¹	4 ³	0.143 ⁹	1 + 3 + 9 = 13
<i>Spirulina</i>	28 ⁴	0.91 ⁸	0.38 ³	4 + 8 + 3 = 15
<i>Dunaliella</i>	28 ⁴	0.91 ⁸	0.45 ¹	4 + 8 + 1 = 13
<i>Tretraselmis</i>	14 ³	2 ⁶	0.31 ⁵	3 + 6 + 5 = 14

<i>Spirulina maxima</i>	33 ⁷	0.97 ⁷	0.26 ⁷	7 + 7 + 7 = 21
<i>Chlamydomonas reinhardtii</i>	32 ⁶	2.9 ⁴	0.387 ²	6 + 4 + 2 = 12
<i>Dunaliella salina</i>	32 ⁶	2.9 ⁴	0.323 ³	6 + 4 + 3 = 13

Superscripts represents ranking given to individual strains.

Based on the ranking results, the microalgae species with the highest potential for methane production from the literature reviewed are as follows: *Chlamydomonas reinhardtii*, *Spirulina maxima*, *Chlorella vulgaris*, and *Dunaliella salina*. Additional consideration was given to the species with ability to grow in fresh water environment (i.e. appropriate for microalgae integration in wastewater treatment), accessibility for the research, ability to grow easy in culture, biomass production, and literature information availability, thus *Chlorella vulgaris* was picked over the other species obtained from this review/ranking.

4.4.2 Challenges of Microalgae Digestion

Microalgae, like any other feedstock for anaerobic digestion are prone to limitations some of which have been highlighted in previous studies. These factors that can significantly impact the methane yield and production of the microalgae species include cell wall resistance, high ammonia production due to nitrogen content and sodium toxicity from marine species.

The presence of the algal cell wall which may be resistant to degradation has been observed to be a challenge in the digestion of microalgae. This was observed in an experiment by Golueke *et al.*, (1957) in which intact algal cells were observed during the digestion of microalgae as a result of resistance of the cell wall to degradation by the anaerobic bacteria. According to Mussgnug *et al.*, (2010) degradability of the cell wall is dependent on the microalgae specie as observed in an experiment where six different microalgae species were digested and results showed the specie with the highest methane yield happened to be the one with easily degradable cell walls or no cell walls at all.

Ammonia toxicity can also impact methane yield due to the high protein (nitrogen) content of some microalgae as their digestion leads to the generation of considerable amounts of ammonia which depending on the pH is inhibitory to the methanogenic Archaea. Within the UK wastewater treatment industry, an upper limit of 3000 mg/L TAN at pH of 7.4 is generally applied to ensure inhibition does not occur (Yenigün and Demirel, 2013).

Another challenge in microalgae digestion includes sodium toxicity in the case of marine species. Although sodium is a micronutrient required by the anaerobic bacteria, marine microalgae do pose a threat of sodium toxicity to the anaerobic bacteria when digested (Demirbas, 2010). Adapting the anaerobic bacteria to higher salt concentrations has been proven to minimise sodium inhibitory effects during digestion.

4.4.3 Optimizing Microalgae Digestion

To enhance the anaerobic digestion of microalgae, a number of ways exist including; pre-treatment and co-digestion with upgraded substrate (feedstock with extra Carbon to boost microalgae C:N) may be employed to achieve optimal methane production

Pre-treatment is applied in anaerobic digestion with a primary aim of enhancing hydrolysis which is the rate limiting step (Eastman and Ferguson, 1981) in anaerobic degradability. The benefits of this technique include increased digester performance, increase biogas production and a reduction in process costs by breaking the polymer chains into soluble components while employing methods such as chemical treatment using acids, bases or ozone, thermal treatment, mechanical treatment such as presses or ultrasonic lysis (Sialve *et al.*, 2009; Heerenklage *et al.*, 2010). Depending on the substrate type, the pre-treatment applied for a particular substrate may vary. For microalgae, pre-treatment aims to improve bioavailability of resistant compounds in the microalgae cell wall thus making its content available to the bacteria for degradation.

Furthermore, altering the growth conditions as well as the metabolism of the algae can cause variation in microalgae composition thus enhancing the digestive properties of microalgae. This technique increases the theoretical methane potential

of the microalgae as reported by Illman *et al.*, (2000) where nitrogen deficient conditions were used to grow microalgae. The benefits of this approach include an increase in lipid content as well as a decrease in ammonia release during anaerobic digestion (AD). On the other hand, microalgae growth rate was reduced.

Unbalanced nutrient (low C/N ratio) is an important limiting factor to anaerobic digestion of microalgae. Co-digestion on the other hand could be a measure to improve digestibility of microalgae. Co-digestion is a term used to describe the combination of several biodegradable wastes with complimentary characteristics into a singular treatment facility with the overall aim of achieving increased methane yield thus making the operation more economically feasible (Mata-Alvarez, 2003).

Several studies identifying the benefits of co-digestion have been established. These include co-digestion of sewage sludge with food waste (Mata-Alvarez, 2003), organic fraction of MSW (Kim *et al.*, 2003) to achieve enhanced methane production. Thus, it is proposed that the co-digestion of microalgae with a substrate with high carbon such as primary and secondary sludge (Samson and LeDuy, 1983; Cecchi *et al.*, 1996), oil-greases (Brune *et al.*, 2009), food waste and paper (Yen and Brune, 2007) could enhance methane production.

4.4.4 Anaerobic co-digestion of microalgae and Sewage Sludge

While co-digestion of microalgae and other biodegradable waste is gaining attention, the possible effect of co-digesting microalgae and sewage sludge is also of increasing interest. The first of this kind of study was the investigation of Golueke *et al.*, (1957) after which more recent studies have been carried out. Some of these include the work of Samson and LeDuy, (1983) who observed a 2.1 fold increase in methane yield of microalgae *Spirulina maxima* when co-digested with sewage on a 50:50 (volatile solids) basis.

Olsson *et al.*, (2013) also studied the co-digestion possibilities of microalgae harvested from Lake Malaren with sewage sludge under thermophilic and mesophilic conditions. The experiments studied the addition of microalgae to sewage sludge at four different co-digestion ratios (0:100, 12:88, 25:75 and 37:63 of algae:sludge) based on VS content. Findings from the study showed a significant increase in

methane yield in the co-digestion of microalgae and sewage sludge at 12:88 (alga:sludge) as the methane yield at this digestion ratio superseded methane yield from sludge alone by up to 12%. Co-digestion at other ratios did not have any significant effect on the achievable methane yield.

Based on the results obtained from these few investigations, there is no doubt about the possible promise the co-digestion of microalgae and sewage sludge can offer, however two things are yet to be clarified. The first is a clear understanding on how this co-digestion will affect the anaerobic digestion (AD) of waste activated sludge (WAS) and the digested product.

Another limitation in existing literature include the fact that even though very few co-digestions between microalgae and sewage sludge have been carried out, no study has looked at the possible effect of co-digesting these two substrates after pre-treatment of the microalgae. As a result of the overall aim of this research which is to utilize existing WWTP facilities to increase energy production, the possible effect of co-digesting pre-treated microalgae and sewage sludge is considered as part of the research objectives.

It is established that employing thermal hydrolysis as a form of pre-treatment can increase the biodegradation and methane yield of substrates (microalgae) up to 35%, thus this research will investigate the possibility of co-digesting pre-treated (via thermal hydrolysis) microalgae with sewage sludge to achieve enhanced energy production.

4.5 Summary of Literature review findings

- Microalgae possess great potential for nutrient removal in wastewater as well as high prospects for energy production however, there are still constraints (mainly technical and engineering) limiting the full exploitation in terms of development and sustainability.
- To overcome the existing hurdles for microalgae commercialization, more research need to be carried out to: identify potential strains of microalgae that will balance nutrient uptake and biofuel production, develop bioreactors with

higher photosynthesis efficiencies, reduce energy requirement (pumping, harvesting and extraction), and energy conversion efficiencies.

- Microalgae is a potential substrate for anaerobic digestion which requires further studies to successfully bypass its cell wall rigidity before its full exploitation may be realised.
- From the review, four microalgal species: *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Spirulina maxima*, *Tetraselmis* and *Chlorella vulgaris* were identified to have the potential to make good substrates for anaerobic digestion.
- Based on the fact that the species with the ability to grow in fresh water environment will be most appropriate for microalgae integration in wastewater treatment, accessibility for the research, ability to grow easy in culture, biomass production, and literature information availability, *Chlorella vulgaris* was picked over the other species for this research.
- This specie of microalgae are used often for commercial and research purposes and have shown to grow well in different wastewater types ranging from municipal/industrial, agricultural wastewaters to dewatered sludge. *Chlorella* has also been proven to grow heterotrophically in the presence of organic carbon sources (Sansawa and Endo, 2004).

Chapter 5 Materials and Methods

5.1 Materials

5.1.1 Algal Culture

Stock cultures of *Chlorella vulgaris* (Figure 5.1) were purchased from the Culture Collection of Algae and Protozoa (CCAP), Scottish Association for Marine Science. These were used to inoculate using 1 ml samples in duplicate, sterilized 1 litre bioreactors containing Bold's Basal Medium - BBM (Figure 5.2). These reactors were constantly supplied with air using a small aquarium pump connected via a flow meter. Mass production of the microalgae was intended to provide a viable pure stock culture.

Sub-culturing of the stock was carried out every 7 - 10 days and this was achieved by inoculating a flask containing 900ml of fresh culture medium with up to 100 ml of original stock culture.



Figure 5.1 Pure stock culture of Chlorella vulgaris

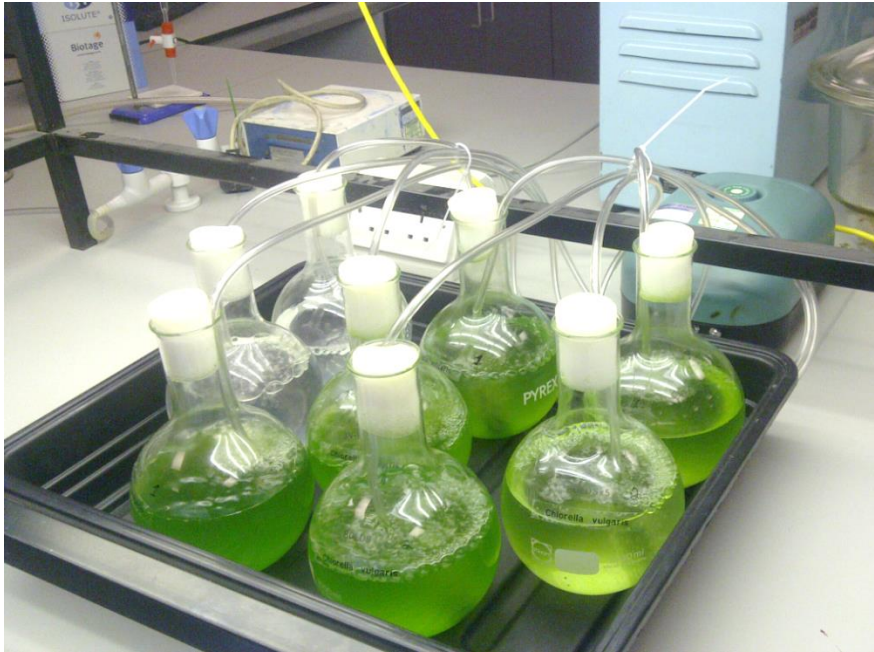


Figure 5.2 Pure Stock Culture of Chlorella vulgaris inoculated in BBM

Laboratory cultivation of *Chlorella vulgaris* (C1)

The setup for the cultivation of *Chlorella vulgaris* involved three tubular photobioreactors operated in batches. Each bioreactor had a capacity of 2.3L and individual air supply tubes (Figure 5.3).



Figure 5.3 Chlorella vulgaris culture in tubular photobioreactors

Once the stock culture was observed to be sufficiently dense with turbidity between 800 and 1200 NTU (2-3 weeks), it was used to inoculate the photobioreactors for the experiment at 10 percent (230ml) and the remaining volume of the laboratory scale photobioreactors was filled with the standard Bold's Basal (BB) media. Air was constantly supplied by a HAILEA super silent, adjustable air pump to each photobioreactor containing the culture media throughout the cultivation period to provide a source of CO₂ and also to provide agitation of the culture.

The setup was illuminated on a 16:8 light: dark photoperiod using a fluorescent lamp which was placed approximately 8cm and parallel to the bioreactors to achieve the desire level of photosynthetic photon flux (250 μ mol m⁻² s⁻¹).

Algal biomass was removed from culture media by centrifugation at 3000 rpm for 30mins to achieve 7% solid content.

5.1.2 Other *Chlorella* Sources

Two other sources of microalgae *Chlorella vulgaris* were sourced for comparison as an alternative to the laboratory cultivated *Chlorella*, as cultivation proved not to be feasible within the research period. These were purchased from Holland and Barrett, UK and Oneon, UK.

a) *Chlorella vulgaris* obtained from Holland and Barrett, UK (C2). Each tablet contained about 500mg of algae and bulking agents (magnesium stearate, steric acid, silicon dioxide, dicalcium phosphate and chlorophyllin). The tablets were crushed and ground to a fine powder (Figure 5.4).



Figure 5.4 Holland and Barrett *Chlorella* (C2)

b) *Chlorella vulgaris* obtained from Oneon, UK (C3). This was confirmed as 100% organic *Chlorella* with no additives (Figure 5.5).



Figure 5.5 Oneon *Chlorella* (C3)

5.1.3 Sewage sludge and Seed inoculum

Sewage sludge for all investigations carried out in this study was collected from Mitchell Laithes Dewsbury, UK. This is the 5th largest treatment works in Yorkshire treating mainly domestic effluents and serving a population equivalent of up to 244,000 people and treating sewage from Dewsbury, Osset, Batley and the Spenn Valley. Sewage sludge was collected directly from the blend tank feeding the mesophilic anaerobic digester (MAD). This contained a mixture of primary and

activated sludge in the ratio 50:50. This was taken into the lab and stored in fridge/freezer at 4 - 5°C.

The initial seed inoculum used in this study was digested sludge obtained from Mitchell Laithes (Dewsbury) treating sewage sludge (50:50 primary: secondary sludge). This was acclimatised by feeding the inoculum with the microalgae sources used in the research (2 grams on alternate days for 28 days) prior to the BMP experiments. This was reduced in subsequent experiments as the seed inoculum of previous experiments was added to the initial inoculum of the later experiments.

5.1.4 Nutrient Medium

This contains the essential macro- and micro- nutrients for the growth of the anaerobic bacteria. The solution used in this study was a modification of the nutrient media as described by Owen *et al.*, (1979). Preparation of the media involved dissolving the stated amount of the following reagents in 1 litre of distilled water: 0.53g NH₄Cl, 0.27g KH₂PO₄, 0.35g K₂HPO₄, 1.20g NaHCO₃, 0.075g CaCl₂.2H₂O, 0.10g MgCl₂.6H₂O, 0.02g FeCl₂.4H₂O, 0.05g MnCl₂.4H₂O, 0.05g H₃BO₄, 0.05g ZnCl₂, 0.03g CuSO₄ and 0.01g Na₂MoO₄.2H₂O.

5.2 Methods

5.2.1 Characterisation of substrates

Seasonal characterization of the digester feed (sewage sludge) used in the study was carried out in terms of TS and VS at all seasons sampled (Table 5.1). This was subsequently stored in the freezer at <10°C for no longer than 6 months to prevent any enzymatic or chemical activity. The characterization was carried out once for the microalgae *Chlorella vulgaris* used in the study as this was obtained from a consistent source and preserved throughout the study.

Table 5.1 Seasonal sampling at Mitchell Laithes Dewsbury (WWTP)

	Winter	Spring	Summer	Autumn
Dates and number of Samples	February 2012 (1) December 2012 (1)	March 2013 (1)	June 2012 (1) June 2013 (1)	October 2013 (1)
Total Samples	2	1	2	1

5.2.2 Biochemical methane potential (BMP) test

The experiments were carried out using 500ml borosilicate bottles, rubber fitted bungs with an air tight gas outlet and stop valves. Reactor contents were prepared individually with a working volume of 400ml in duplicate. Triplicate reactors of 250ml volume were also prepared (with a working volume of 150ml) basically for sample collection. The reactors were operated at a mesophilic temperature (37°C) in a mechanical shaker which provided intermittent shaking at 140 rpm (Figure 5.6).



Figure 5.6 Incubator + shaker for BMP Experiments

Reactors (150ml) were sampled on defined days (Day 0, 3, 7, 14, 21 and 28). This was more frequent in the first 7 days after which sampling was carried out weekly. During sampling, 20ml was collected, half of the sample was used for TS VS according to standard method 2540 G and pH determination while the other half was centrifuged at 4000rpm for 30mins and the supernatant used for alkalinity (APHA

2320 A), ammonia and TKN (APHA 4500 N_{org} A) analysis following methods of the American Public Health Association (APHA, 2005).

5.2.3 Gas Measurement

This was carried out daily by connecting the gas tubes on the 400 ml reactors to a liquid displacement bottle filled with 5% NaOH to remove CO₂ from the biogas produced, leaving methane to be measured. The net volume of methane produced in the reactors was corrected by subtracting the methane produced by the inoculum control from the methane produced by the other reactors, which was then corrected to STP (NL CH₄) of 273.15 K and 100 kPa. The methane yield was expressed in terms of NL CH₄/ g VS_{added} and NL CH₄/ g VS_{removed} by dividing the cumulative gas production by the amount of substrate put in the reactor and the amount of substrate destroyed respectively.

5.2.4 Analytical Methods

pH: the pH of digester effluent and feed samples were measured using a gel-type electrode and meter. This was calibrated daily using buffer solutions at pH 7 and 4 to ensure stable and reliable readings.

Alkalinity: was carried out on the supernatant of the centrifuged effluent. This was titrated against 0.02N sulphuric acid to reach a pH end point of 4.5 using an auto dispense titrator. Each titration was completed in approximately 1min.

VFA Analysis: VFA and alcohol concentrations were determined using an Agilent gas chromatograph (GC) equipped with a flame ionization detector (FID) and NORDION NB- 351 column, with a 25m length, 0.32mm internal diameter and 0.5µm film thickness. Operating conditions were: injector temperature 150°C; FID temperature 240°C; oven temperature program: 95-140°C (10°C/min), 140-200°C (40°C/min) held for 5 min. Helium was used as a carrier gas. Prior to analysis, the digester effluent samples were centrifuged in 50ml centrifuge tubes at 4000 rpm for 20min, the supernatant was then filtered through a 0.22µm filters and stored at 4°C until GC analysis.

TAN: Total ammonium nitrogen (TAN, $\text{NH}_4^+ + \text{NH}_3$) concentrations were measured using a Buchi distiller consisting of a steam generator, distiller and concentrated NaOH dispenser, which connected to an automatic titrator. The distiller is a programmable device set to perform specific distillation into boric acid absorbing solution after which the ammonium concentration of the boric acid solution is automatically titrated with a strong acid (0.05 M H_2SO_4) titrant to the pale lavender end point of methyl red-methylene blue indicator.

CNHS Analysis: To determine the elemental composition (carbon hydrogen nitrogen and sulphur), triplicated samples were analysed using a CHNS analyser Model Thermo Flash EA12 series. Prior to this, samples were oven dried at 105°C for 24 hours and then a known weight of individual samples was analysed. The results were used to determine the C:N ratio, empirical formula and stoichiometric methane potential in the case of substrate samples (Rodriguez, 2012)

SEM: Characterization of the structure and morphology of the preferred *Chlorella* source was performed using a scanning electron microscope (SEM) in order to validate its cell wall intactness. The equipment used was a ZEISS Auriga SEM with an energy dispersive X-ray spectroscopy (EDX) detector to study composition.

Biogas Analysis: Biogas composition from the CSTRs were regularly analysed for methane, hydrogen and carbon dioxide content using a gas chromatograph with a thermal conductance detector (GC/TCD). The chromatograph (Figure 5.7) makes use of a 30mm long column (Supelco Carboxen 1010 PLOT) with a 0.53mm I.D. Set conditions of the GC include: manual; injection of 200 μl , split reaction of 5:1.

The injector temperature was at 200°C and a detector temperature of 230°C was applied. The chromatograph uses argon as carrier gas and the carrier flow was at 3ml/min. Oven program was 35°C with a 7 minutes holding time. Ramp temperature: 35°C to 225°C at $24^\circ\text{C}/\text{min}$.

Biogas was extracted through the biogas outlet of the respective digesters using a 5-ml syringe. To prevent contamination with air, the syringe was purged with 10ml biogas prior to gas collection. Samples were analysed within 10 minutes following extraction.



Figure 5.7 Agilent 7890A Network Gas Chromatograph

5.2.5 Solubility Determination

The disintegration of the microbial cell wall causes release of intracellular organic compounds into the liquid phase of sludge. Solubilisation on the other hand is a well-established method suitable for delineating the extent of cell disintegration by determining increase of the chemical oxygen demand (COD) in the sludge supernatant (Nickel and Neis, 2007). COD solubilisation was measured using two approaches: the degree of solubility (%) and the spectrophotometry approach.

- **Degree of Solubility (%)**

The solubilisation degree (SD) (%) of COD was calculated according to Equation 5.1 in order to evaluate the efficiency of the pre-treatments

$$\text{Degree of Disintegration \%} = \frac{(SCOD - SCOD_o)}{(TCOD_o - SCOD)} \quad \text{Equation 5.1}$$

Where sCOD refers to soluble chemical oxygen demand, tCOD refers to total oxygen demand and the subscript “o” stands for “before pre-treatment. Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were measured according to standard methods 5220 D (APHA, 2005).

- **Spectroscopy method:**

This was determined using the Biomate 3 spectrophotometer produced by Thermo scientific. This instrument had improved bandwidth of 1.8nm, patent optical design thus providing compact high performance dual-beam systems. With a xenon lamp, the instrument produces a balanced light over the instrumental wavelength (190 – 1100nm). The supernatant from pre-treated samples were analysed to determine variation in absorbance at 750 nm, indicating the release of organic compounds such as protein, carbohydrates and lipids.

5.2.6 Determination of biodegradability and hydrolysis rate

The BMP data obtained in this study was analysed using a first order kinetic model which allows the estimation of the apparent degradability f_d [L CH₄ gVS⁻¹] and the apparent hydrolysis rate, K_{hyd} [day⁻¹]. This was achieved by fitting the data into the first order equation below (Equation 5.2) (Batstone *et al.*, 2009; Keymer *et al.*, 2013):

$$Y_{CH_4} = V_{CH_4}/VS = f_d(1 - \exp(-K_{hyd}t)) \quad \text{Equation 5.2}$$

Where Y_{CH_4} = Specific methane yield at given time [L CH₄ gVS⁻¹]

V_{CH_4} = Volume of the methane produced [L CH₄]

VS = the mass of the volatile solids present in the reactors [gVS]

t = time from start of BMP till maximum cumulative methane is reached [days]

5.2.7 Dewaterability

Tests on the digested effluent were carried out using a patent capillary suction time (CST) device (Model 304B) manufactured by Triton Electronics. The device includes a cylindrical steel funnel resting on a Whatman 17 chr filter paper between two perspex plates having electrode sensors across the top plate. The test procedure involves filling the steel funnel to the brim with a representative sample of the

digester effluent, while the filter paper generates a capillary suction pressure that sucks the water from the sludge. Water permeates through the filter paper at a rate that is dependent on the sludge condition as well as the filterability of the residue cake formed on the filter paper.

The electrode sensors across the top plate are placed at a standard interval from the steel funnel and the time it takes for the water front to pass between the electrodes is termed the capillary suction time (CST). This instrument gives a proxy parameter used to determine dewaterability of digested product by normalizing the capillary suction time by the total solid (TS) content of the digestate or sample of interest.

5.3 Experimental Setup

5.3.1 Substrate characterization

This was carried out using a series of qualitative and quantitative analytical procedures including: pH, Alkalinity, Total Solids (TS), Volatile Solids (VS), Volatile Fatty Acids (VFA) and Total Ammonia. Solutions of the solid and semi-solid substrates (1g in 100ml of water) eased the analysis

Biochemical Methane Potential (BMP) tests were carried out on all three substrates (microalgae sources) for justification. Other parameters such as hydrolysis, rate of degradation and cell wall integrity were also of significant interest for justification of the selected *Chlorella* source.

From the TS and VS obtained from the characterization of substrates and inoculum, the respective weight and volume of the substrates and inoculum required for each reactor was worked out based on a 4.2g VS/L loading rate and an inoculum: substrate of 2 : 1 (based on VS). The final volume was then subsequently made up to the respective working volume of each reactor using the nutrient media prepared.

A total of 11 reactors were set up (Table 5.2).

The reactors were set up using the quantities of substrate, inoculum and nutrient media shown in Table 5.2. The reactors were then placed on a shaker (at 140 RPM) in an incubator set at 37°C (Mesophilic temperature)

Table 5.2 Substrate composition of anaerobic reactors

	Reactor	Effective volume (ml)	Inoculum (ml)	Nutrient media (ml)	Substrate (g)
1	control inoculum	400	288.24	111.76	–
2	Control inoculum	400	288.24	111.76	–
3	C1: Cultivated <i>Chlorella 1</i>	400	288.24	86.26	25.5
4	C1: Cultivated <i>Chlorella</i> (Duplicate)	400	288.24	86.26	25.5
5	C1: Cultivated <i>Chlorella</i> Sampling	150	109.18	31.22	9.6
6	C2: H & B <i>Chlorella</i>	400	288.24	111.76	25
7	C2: H & B <i>Chlorella</i> (Duplicate)	400	288.24	111.76	25
8	C2: H & B <i>Chlorella</i> Sampling	150	109.18	31.42	9.4
9	C3:Oneon <i>Chlorella</i>	400	288.24	214.20	28.2
10	C3:Oneon <i>Chlorella</i> (Duplicate)	400	288.24	196.49	28.2
11	C3:Oneon <i>Chlorella</i> Sampling	150	109.18	30.12	10.7

5.3.2 Pre-treatment on microalgae degradability

To evaluate the effect of pre-treatment on microalgae, *Chlorella vulgaris* (Oneon) produced as detailed earlier was used to evaluate the efficiencies of these pre-treatments. Prior to the individual pre-treatments, the substrate was diluted to 7% solids using distilled water. The pre-treatment types applied include:

- **Autoclave Pre-treatment:** Autoclaving is a pre-treatment method that employs a similar technique to the thermal hydrolysis. It works basically by steam treating the content at a constant temperature and pressure, leading to a pasteurization and break down of organic matter within the feedstock. For the pre-treatment, a Touchclave lab “K” series autoclave was employed, algal sludge

samples were transferred into 500ml Duran bottles and then autoclaved at 121°C for 30 minutes. This pre-treatment was carried out on algal sludge before it was fed into the batch reactors.

Following all pre-treatments on microalgae, BMP experiments were carried out to compare effectiveness of these approaches on microalgae methane yield, VS destruction, biodegradability and hydrolysis rate. An energy balance with the adoption of these approaches was also considered.

- **Thermal pre-treatment:** a temperature of 90°C for 3 hours was adopted for thermal treatment as this has proven effective in increasing methane production of microalgae up to 220% (González-Fernández *et al.*, 2012). Thus a Gallenkamp Hotbox oven Size 2 was employed (at the set temperature) for the treatment of the microalgae in the study.
- **THP:** The thermal hydrolysis conditions were produced in the laboratory using a Parr Hydro-thermal reactor (Figure 5.8). The reactor was conditioned to 165°C at 8 bar pressure for 30 minutes prior to feeding into the digester.



Figure 5.8 Laboratory scale hydrothermal reactor

5.3.3 Co-digestion experiments

The possible co-digestion of microalgae and sewage sludge was studied in a biochemical methane potential test. Prior to the BMP experiments, *Chlorella vulgaris* was pre-treated using the thermal hydrolysis (165°C @ 8 bar pressure).

For the methane potential monitoring of this experiment, 500ml anaerobic batch reactors were used with hermetically sealed stoppers and controlled opening valves for gas removal and sampling. Each reactor contents was prepared to an effective volume of 400 ml and 100 ml of headspace. Anaerobic batch reactors were loaded at 4.2g VS/L with an inoculum: substrate ratio of 2:1, each. The final volume was then subsequently made up to the respective working volume (400ml) using a modified nutrient media as described by Owen *et al.*, (1979), this solution contain the essential macro and micro nutrients for the growth of the anaerobic bacteria.

In order to investigate the impact of co-digesting algal biomass and sewage sludge, different ratios of algae: sludge blends were tested (0:100, 25:75, 50:50, 75:25 and 100:0, based on VS content). Experimental setup and gas measurement were carried out as detailed in section 5.2.2 and 5.2.3.

5.3.4 Semi-CSTR Setup (Effect of OLR and HRT)

The experiments were conducted in a CSTR type digester, in order to evaluate the effect of increased OLR on anaerobic co-digestion of microalgae and sewage sludge, and also to identify possible operational problems. The digesters were constructed using 2.1 litres Nalgene air tight bottles equipped with ports to enable digester feed influent, effluent and biogas collection (Figure 5.9).

The reactors were heated with heater tape and kept at a constant temperature of 37±1°C and wrapped in foil insulator to maintain temperature. Respective digesters were fed simultaneously with effluent removal and biogas measurement on a daily basis. Process parameters (VFA, Alkalinity, NH₃ and pH) were measured on the effluent on daily basis. Digester feed and effluent characteristics were measured on a weekly basis for analysis of solid concentration, total carbon, TKN, TAN and VFA.



Figure 5.9 CSTR systems during the operation of co-digestion experiments

The biogas produced was estimated using the displacement method. This was done by connecting the biogas tube on the respective digester to a liquid displacement bottle (Figure 5.9). This displacement bottles were filled with water signifying biogas production to be equivalent to the amount of water displaced. Biogas characterization was carried out twice a week to determine methane and CO₂ concentration.

Microalgae *Chlorella vulgaris* (Oneon) was produced from the same source. Sewage sludge and seed inoculum samples were collected from the blend tank, combining primary and secondary sludge, and from the mesophilic anaerobic digester at Yorkshire Water's sewage treatment works in Dewsbury, UK. The cell wall of both substrates was disintegrated using thermal hydrolysis at 165°C and 8 bar pressure simulating standard conditions for hydrothermal sewage sludge pre-treatment. Substrates were then stored in tightly sealed 5-L plastic containers and placed in a freezer (-14°C) prior to use.

Digesters were loaded as detailed in Table 5.3. Frozen substrate were thawed and based on the VS content, feed batches were prepared to achieve 25:75, 50:50, and 75:25 (algae:sludge) as well as the desired solids concentrations for respective digester experiments. The prepared feed was then stored at 4°C and used within 7 days.

The feeding process involved switching off the digester mixer 1 hour prior to feeding and switching back on 1 hour after feeding. The calculated amount of feed was fed into the reactor and effluent was collected based on the HRT.

Co-digestion of pre-treated microalgae (*Chlorella vulgaris*) and sewage sludge were carried out in experimental runs 1, 2 and 3. The digesters were fed slurries consisting of 3 main proportions (25:75, 50:50 and 75:25 based on VS content) of *Chlorella vulgaris* and sewage sludge (Figure 5.10). Prior to the experiments, individual reactors were brought to a steady state characterized by process parameters including pH, alkalinity, and steady biogas production. This steady state was achieved in about 3-4 weeks.



Figure 5.10 Prepared co-digestion of feedstock for AD

In Experiment #1, 100% algae digester was used as a representative of steady state in which different OLR and HRT ranging between 2 - 5g VS/L/d and 20 – 8 days respectively were tested

Experiment #2 tested the co-digestion effects under the most optimal conditions for HRT and OLR obtained in experiment 1. This was to have a clear understanding of the achievable methane yield under the CSTR conditions and validate the BMP results obtained from co-digestion in the previous chapter.

A constant HRT of 20 days was designed to simulate a full scale plant operation while varying OLR and co-digestion ratios were studied in Experiment 3. The idea of changing the OLR at a constant HRT was basically with the aim of understanding the effect of increasing OLR on methane yield/production, digester performance, effluent characteristics (dewaterability), biogas yield, and biogas composition. Table 5.3 summarizes the experimental setup.

Table 5.3 Experimental design for anaerobic co-digestion study

Experiment	Digester Name	Algae Ratio (based on g/vs)	Sludge Ratio (based on g/vs)	OLR	HRT
1	1A1	100	0	2	20
1	1B1	100	0	2.7	15
1	1C1	100	0	4	10
1	1D1	100	0	5	8
2	2A1	0	100	2	20
2	2B1	25	75	2	20
2	2C1	50	50	2	20
2	2D1	75	25	2	20
2	2E1	100	0	2	20
3	3C1	0	100	3	20
3	3C2	25	75	3	20
3	3C3	50	50	3	20
3	3C4	75	25	3	20
3	3C5	100	0	3	20
3	3D1	0	100	4	20
3	3D2	25	75	4	20
3	3D3	50	50	4	20
3	3D4	75	25	4	20
3	3D5	100	0	4	20
3	3E1	0	100	5	20
3	3E2	25	75	5	20
3	3E3	50	50	5	20
3	3E4	75	25	5	20
3	3E5	100	0	5	20
3	3F1	0	100	6	20
3	3F2	25	75	6	20
3	3F3	50	50	6	20
3	3F4	75	25	6	20
3	3F5	100	0	6	20

5.3.5 Carboxylate Platform (anaerobic fermentation) Experiments

Similar to the BMP experiments, the batch fermentation experiments were carried out using 500 ml borosilicate bottles, rubber fitted bungs with an air tight gas outlet and stop valves. Reactor contents were prepared with a working volume of 400ml in duplicate, and triplicate reactors of 250 ml volume were also prepared (with a working volume of 150 ml) basically for sample collection.

Inoculum for the experiments was obtained from the laboratory scale anaerobic digesters digesting a mixture of different blending ratios of microalgae and sewage sludge. Prior to THE batch experiments, inoculum was heat shocked at 80°C to inactivate the methanogens. To keep the reactor in fermentation phase, iodoform (CHI₃) was used as methanogenic inhibitor. This was dosed every other day. CaCO₃ powder (1.0 g/g of substrate) was added to control the pH between 6.5 and 7.0.

From the TS and VS obtained from the characterization of substrates and inoculum, the respective weight and volume of the substrates and inoculum required for each reactors was worked out based on 7g VS/L loading rate and an inoculum: substrate of 2 : 1 (based on VS). The final volume was then subsequently made up to the respective working volume of each reactor using the nutrient media prepared.

In order to investigate the impact of co-digesting algal biomass and sewage sludge, different ratios of algae: sludge blends were tested (0:100, 25:75, 50:50, 75:25 and 100:0, based on VS content). Each sample was prepared in triplicates to CHECK reproducibility. A constant internal temperature of 37°C was achieved by incubating the reactors which were mixed at 140 rpm for a 15 minutes period twice a day.

During the experiment, sampling was carried out on defined days (Day 0, 1, 4, 8, 11, 17, 20, 24 and 30). During sampling, 10 ml was collected: 5ml for TS, VS and pH determination while the other half was centrifuged at 10,000 rpm for 5mins and the supernatant was used for alkalinity, VFA, and ammonia following standard methods of the American Public Health Association as previously mentioned (APHA, 2005).

Experiment #1 was carried out to identify optimal iodoform concentration to favour methanogenic inhibition. Two iodoform concentrations were tested, 3mg/l (Sludge

1) and 10mg/L (Sludge 2). Experiment #2 tested respective co-digestions using the optimal iodoform concentration.

5.3.6 Mass and energy balances in the optimal co-digestion

The balances were calculated using the data obtained previously in the MAD experiments. Prior to the energy balance, the mass balance was carried out on the organic matter using the VS and COD data obtained from the solid and liquid samples respectively. The methane and CO₂ concentrations in the biogas were also put into consideration at this phase.

For the energy balance, COD data of the liquid streams as well as the concentrations of methane and hydrogen in the biogas were used. To determine the energy content in the solids, elemental analysis (CNHSO) was used to calculate the theoretical heat of combustion (Q) using Dulong's equation for calculating calorific value of the fuels.

$$Q = 1/100[8080 C + 34500 (H - O/8) + 2240 S] \text{ kcal/kg} \quad \text{Equation 5.3}$$

where C, H, O, S refer to % of carbon, hydrogen, oxygen and sulphur respectively.

For the assessment of energy performance with the successful integration of microalgae into WWTP flow sheet, the methodology included: determination of the configuration of the proposed plant, selecting the plant size, assumptions in regards to the plant operation, establishing the terms necessary for inclusion into the energy balance equations and finally the calculation of the different energy flows.

The calculation of each individual term in the equation required combination of real data from the experiments in this study, data from literature, and reasonable assumption with regards to specific decision criteria. Calculations carried out in this research are not intended as accurate guide but rather to provide indicative effort for evaluation of the process. The integral evaluation of the process included the environmental benefits and economic analysis of the proposed symbiosis.

Table 5.4 below shows the various stages of this research.

Table 5.4 Summary of the different phases of this research

Phases of the research	Parameters evaluated
Characterization of substrate	<ul style="list-style-type: none"> • Characterization of substrates • Composition and seasonal variation of sewage sludge from Mitchell Laithes • BMP applied to respective substrates • Hydrolysis rates and biodegradability (AquaSim software), • Cell wall integrity
Effect of Pre-treatment	<ul style="list-style-type: none"> • Effects of several pre-treatment on solubility • BMP applied to respective pre-treated substrates • Hydrolysis rates and biodegradability (AquaSim software),
Effect of co-digestion (BMP)	<ul style="list-style-type: none"> • Effects of several co-digestion (0:100, 25:75, 50:50, 75:25 and 100:0, based on VS) • BMP applied to substrates • Hydrolysis rates and biodegradability (AquaSim software),
Effect of co-digestion (CSTR)	<ul style="list-style-type: none"> • Effect of co-digestion in a semi-CSTR • Range of OLR and HRT for the production of methane
Anaerobic Fermentation (Batch)	<ul style="list-style-type: none"> • Effect of co-digestion • Production of VFA and possible alcohols
Evaluation of the process	<ul style="list-style-type: none"> • Mass and energy balances of the two approaches (AD and Carboxylate) • Global energy balance for the microalgae symbiosis

Chapter 6 The Source of *Chlorella* source used in this study

6.1 Introduction

The first step in the research involved finding a suitable and sustainable source of microalgae biomass needed for the research/experiments. Based on a literature review *Chlorella vulgaris* was chosen over the other two microalgae (*Chlamydomonas reinhardtii* and *Dunaliella salina*) for the research on the grounds that it is a fresh water microalgae, with easy access and productivity in the laboratory.

Chlorella vulgaris was cultured in the laboratory and the achievable methane production from this substrate was determined. It soon became obvious that it would be too time consuming to cultivate the quantities needed for the digestion studies, with the equipment available in the laboratory, even with the most optimal condition for algal growth. Thus there was a need to find *Chlorella* source that would have the same specific methane yield (SMY) as the *Chlorella* cultured and cultivated in the laboratory.

In order to find this sustainable *Chlorella*, criteria were: a pure strain with no additives or anti bulking agent; a strain grown in a nutrient rich environment and with an intact cell wall since part of the experimental objectives were to observe the effect of breaking the microalgae cell wall using the existing thermal hydrolysis in the UK water industry. This step is regarded as a necessary one in order to achieve the overall aim of the research, which is to increase the methane production of the industry using the already setup facilities.

Two *Chlorella* sources were obtained. *Chlorella* powder purchased from Holland and Barrett (UK) and *Chlorella vulgaris* was purchased from One-on, UK. To justify the selected *Chlorella* source for the research, it is expected to portray similar characteristics with the laboratory cultivated *Chlorella* in terms of substrate characteristics, biodegradability, methane yield (g VS_{added/destroyed}) and process stability. Thus, at this experimental stage, a Biochemical Methane Potential (BMP) test was carried out on all the three substrates for justification.

6.2 Results

Feed stocks were analysed prior to the digestion experiments to ensure optimal conditions in terms of carbon, nitrogen, TS and VS content. Substrates used in this study include *Chlorella vulgaris* from three different sources name C1, C2 and C3. All *Chlorella* sources had VS content between 83.77 and 93.95%. Carbon content of these substrates was in the range of 39-45% TS (Table 6.1). The substrates and the inoculum used in this study contained high alkalinity and adequate VFA which project its amenability to anaerobic digestion. Based on substrate characterization and VS content, respective digestions for this study were undertaken.

Table 6.1 Summary of Substrate Characterisation

Parameters	pH	TS (%)	VS (%)	Alkalinity (mgCaCO ₃ /L)	C (%T S)	N (%T S)	H (%TS)	O (%T S)	C:N
Cultivated <i>Chlorella vulgaris</i> (C1)	7.0	7.1	92.75	380	42.8	6.9	5.7	24.2	6.2
Holland & Barrett <i>Chlorella</i> (C2)	7.0	7.16	93.95	375	39.1	8.2	5.7	29.2	4.8
Oneon Uk, <i>Chlorella</i> (C3)	7.0	7.11	83.77	436	45.1	7.4	6.6	27.3	6.1

6.2.1 Methane yield obtained in the study

The methane yield from the comparative experiment of the several *Chlorella* studied ranged between 150 and 264N mL CH₄/g VS_{added} (Table 6.2). While the lowest methane yield was observed in the Holland and Barrett *Chlorella* (C2), the cultivated *Chlorella* and the Oneon *Chlorella* demonstrated similar yield of 264 and 256N mL CH₄/g VS_{added} respectively. While this values fall within the achievable methane production from a wide range of microalgae species studied in literatures, it also agrees with values obtained from *Chlorella* when cultivated in nutrient rich environment (Sánchez Hernández and Travieso Córdoba, 1993).

Methane yield of the three *Chlorella* sources studied were compared in terms of cumulative yield, per g VS_{added} and per g VS_{destroyed}. Results showed consistency under each criterion as cultivated *Chlorella* (C1) and Oneon *Chlorella* (C3) portrayed similar/higher yield compared to the Holland & Barrett *Chlorella*.

Table 6.2 Achievable methane production from the digested substrates

	Cumulative methane yield (N ml)	Methane Yield (N mL CH ₄ /g VS _{added})	Methane Yield (N mL CH ₄ /g VS _{destroyed})	% of VS destroyed
Cultivated <i>Chlorella vulgaris</i> (C1)	443.99	264.30	331.22	45
Holland & Barrett <i>Chlorella</i> (C2)	252.28	150.17	180.53	36
Oneon UK, <i>Chlorella</i> (C3)	430.75	256.40	329.50	45

A noticeable lag phase up to three days (Figure 6.1) was observed in Holland & Barrett *Chlorella* (C2) and this was coupled with the lowest methane yield. This delay could be attributed to improper acclimatization of the feed with the inoculum, presence of resistive compounds to degradation or the presence of a thicker cell wall thus making the cell wall more recalcitrant amongst other possibilities including S:I ratio.

Results (Table 6.2) showed similarities between the cultivated *Chlorella* (C1) and the Oneon *Chlorella* (C3). C2 *Chlorella* demonstrated the least performance under all comparative criteria studied in regards to methane yield. VS destruction from the comparative study also showed similar percentage destruction of 45% for the cultivated *Chlorella* and the Oneon *Chlorella*. C2 on the other hand demonstrated a very low VS destruction up to 25% less than the cultivated and the Oneon *Chlorella*.

6.2.2 Process parameters

The process parameters below (Table 6.3) give an indication of the anaerobic processes as well as the reactor health during digestion.

Table 6.3 Mean digester parameter

	pH	VFA (mg/L)	NH ₃ (mg/L)	Alkalinity (mg CaCO ₃ /L)
Cultivated <i>Chlorella vulgaris</i> (C1)	7.4± 0.1	1479± 25	1560± 39	3312± 125
Holand & Barrett <i>Chlorella</i> (C2)	7.6± 0.1	840± 25	2740± 41	1805± 107
Oneon UK, <i>Chlorella</i> (C3)	7.6± 0.1	1436± 25	1620± 35	3295± 105

Alkalinity levels in the reactors showed concentration high enough to enhance methanogenesis. While the highest alkalinity was observed in the cultivated *Chlorella* (C1) 3295 mg CaCO₃/L, no obvious difference was observed when compared to the Oneon *Chlorella* with alkalinity of 3312 mg CaCO₃/L. C2 *Chlorella* however demonstrated the weakest alkalinity of 1805 mg CaCO₃/L. Nevertheless, all reactors demonstrated sufficient buffering capacity to maintain a stable pH which averaged between 7.4 and 7.6 for all *Chlorella* tested.

6.2.3 Comparison of biodegradability rate and methane production

The BMP data obtained in this study was analysed using a first order kinetic model which allows the estimation of the apparent degradability f_d [L CH₄ gVS⁻¹] and the apparent hydrolysis rate, K_{hyd} [day⁻¹]. This was achieved by fitting the data into the first order equation provided by Batstone *et al.*, (2009) and Keymer *et al.*, (2013).

The model was implemented in aquasim 2.1d. The objective function used was the sum of squared errors (χ^2). Average values from the triplicates were used and the uncertainty was assessed as described by Keymer *et al.*, (2013) via parameter uncertainty analysis.

Results of the BMP tests with model simulations are shown below (Figure 6.4). The error bars indicate the standard errors from triplicate tests while the model lines shown are based on the best fit of f_d and k_{hyd} with standard errors which are shown in Table 6.4.

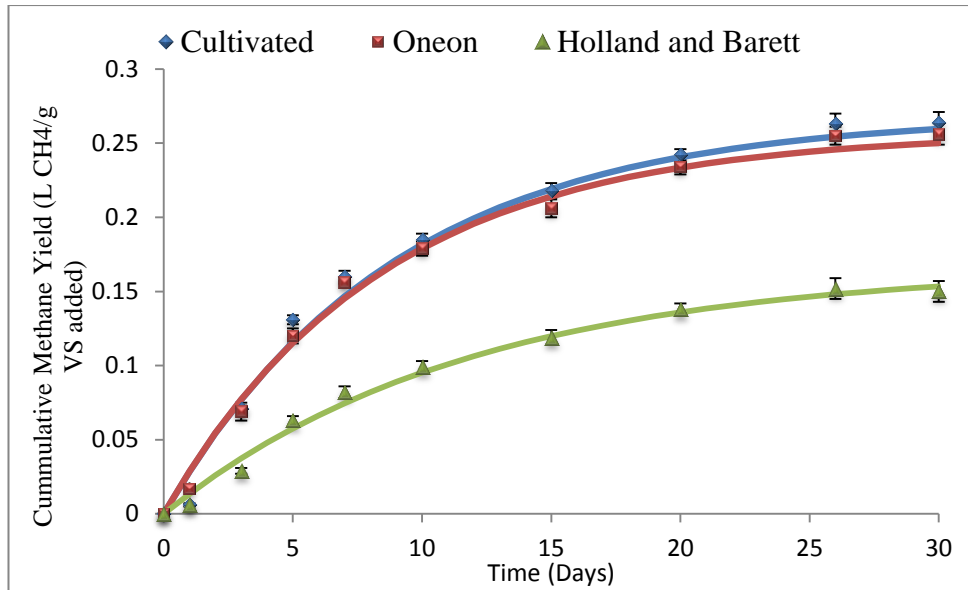


Figure 6.1 Cumulative methane yield from respective digesters. Error bars indicate standard error in triplicate tests while the lines show the predicted model trend.

Table 6.4 parameter estimation obtained for degradation (f_d) and first order hydrolysis rate (K_{hyd}) showing standard errors of predictions.

Substrate	f_d [L CH ₄ gVS ⁻¹]	K_{hyd} [day ⁻¹]	χ^2
Cultivated <i>Chlorella</i> (C1)	0.26 ± 0.01	0.11 ± 0.003	0.001
Holland & Barrett <i>Chlorella</i> (C2)	0.15 ± 0.01	0.08 ± 0.002	0.001
Oneon <i>Chlorella</i> (C3)	0.26 ± 0.01	0.11 ± 0.003	0.001

Findings once again proved the similarities between the Oneon *Chlorella* and the cultivated *Chlorella* which both demonstrated the same hydrolysis rate 0.11 day^{-1} , while the least hydrolysis rate was obtained from C2 *Chlorella* with a corresponding degradation rate of 0.08 day^{-1} . In view of this C3 *Chlorella* was picked over C2 as it portrayed the most similar characteristics in terms of methane yield and identical process stability to the cultivated *Chlorella* in the study.

6.2.4 Oneon *Chlorella* (C3) Review/Cell wall Integrity

The methane yield of the *Chlorella* adopted for the research ($0.26 \text{ L CH}_4 \text{ gVS}^{-1}$) falls within the literature values ranging between 0.1 and 0.39. This shows the *Chlorella*

adopted for the research is suitable for further experiments. Nevertheless, the potential of these substrates (microalgae) for anaerobic digestion could be improved as the theoretical methane potential suggests.

This may only be achieved with a complete degradation of the substrate, thus there is need to look into possibility of enhancing microalgae degradation bearing in mind that the cell wall of these microalgae has been identified to be resistive to bacterial attack (Golueke *et al.*, 1957; Sánchez Hernández and Travieso Córdoba, 1993). With the enhancement of the cell wall, the possible methane yield from these substrates of interest stands a chance of competing with high potential substrates, like food waste.

Having identified the C3 (Oneon *Chlorella* source) to possess the most identical properties and methane yield with that of cultivated *Chlorella*, another paramount requirement was to confirm the cell wall structure of this *Chlorella* source to be intact. This step was taken for two reasons:

- 1) It has been reported that the harvesting and processing of microalgae can cause damages to its cell wall.
- 2) Considering the fact that one of the research objectives was to see the effect of pre-treatment on cell wall destruction, solubility and overall methane yield, an already disrupted cell wall as a result of cultivation or any other reason would have nullified the use of the *Chlorella* source.

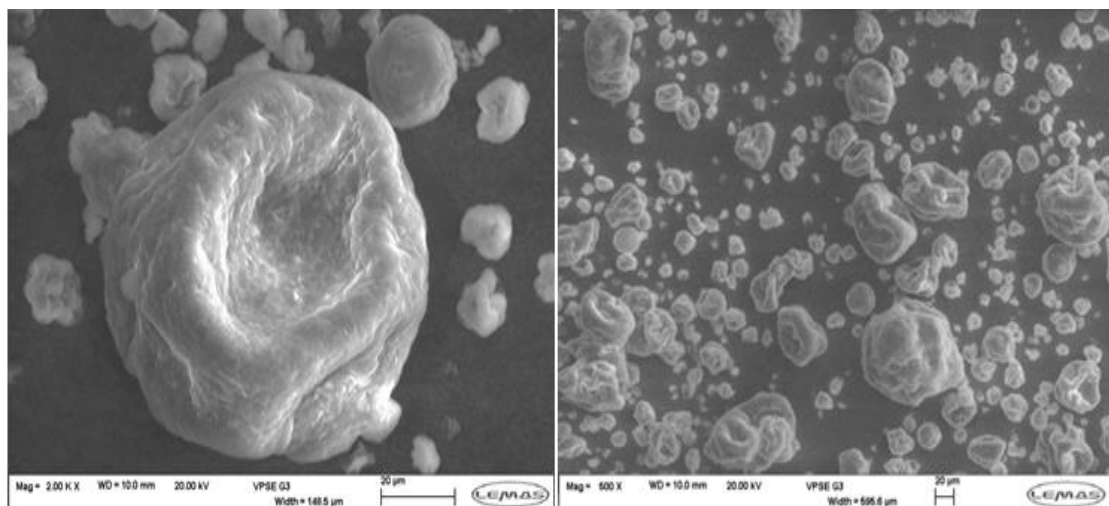


Figure 6.2 Graphical (SEM) Representation of the C3 *Chlorella vulgaris* Source

Finally, cell wall integrity of the preferred *Chlorella* source (C3) was confirmed using the SEM microscope (Figure 6.2). This confirmation seals any other doubts about the source of *Chlorella* adopted for the research. Also it gives a firm ground for evaluating the effects of pre-treatment on increasing the methane yield achievable from microalgae with intact cell wall. The SEM results visually proved the intactness of the *Chlorella* (C3) cell wall thus confirming its suitability for further experiments in the research.

6.3 Discussion

The aim at this experimental stage was to find out the most suitable *Chlorella* source that would provide the quantities needed for future work. It required properties similar to that of *Chlorella* cultivated under our rich nutrient media thus a comparative BMP experiment was carried out on the three *Chlorella* sources of interest. Parameters selected for comparison include biodegradability, elemental composition, methane yield (in terms of VS_{added} and VS_{destroyed}) and finally with the use of the SEM to confirm cell wall integrity.

The Oneon *Chlorella* (C3) showed the most similar results in terms of biodegradability, methane yield per g VS added and methane yield per g VS destroyed. The carbon and nitrogen content these two *Chlorella* were also similar in balance thus yielding approximately equal C:N of 6.2 and 6.1 for cultivated (C1) and the Oneon (C3) *Chlorella* respectively. Of the three *Chlorella* sources compared the Holland and Barrett *Chlorella* (C2) proved to have the lowest result with its C:N at 4.8. Also, C2 contained anti-bulking agents such as magnesium stearate, stearic acid, silicon dioxide, dicalcium phosphate and chlorophyllin etc. which could have been one of the reasons for the inhibited anaerobic digestion performance of this substrate.

Results obtained from this study highlight similar characteristics between the Oneon UK *Chlorella* (C3) and the cultivated *Chlorella* (C1). With regards to methane yield, similar quantities were produced 256 and 264 N mL CH₄/g VS_{added} respectively. The relationship between CH₄ yield of C1 and C3 was confirmed using a 2-tailed T test with $P = 0.4$, suggesting no statistical significant difference between the achieved methane yield of both *Chlorella* sources.

Methane yield obtained from C2 (Holland & Barrett *Chlorella*) was lowest compared to the other two sources, this is assumed to be as a result of anti-bulking agents used to preserve this *Chlorella* source. The C1 and C3 *Chlorella* showed very little lag phase suggesting a well acclimatization with the seed while the C2 *Chlorella* demonstrated a 4-5 days lag phase.

Process parameters with respect to ammonia on the other hand were similar and favourable for the anaerobic process of C1 and C3 with respective values of 1560 and 1620 mg/L, while the C2 demonstrated a relatively high ammonia concentration by day 15 (2740 mg/L). Although this does not reach the inhibitory level (Chen *et al.*, 2008), possibilities that this relatively high ammonia concentration could have slowed down the process cannot be disregarded. This high ammonium build up is as a result of its high Nitrogen content 8.2% of the C2 *Chlorella*.

Overall, the study looked at the composition of substrates, behaviour in the digester, hydrolysis rate and SEM which confirmed similarities between C3 and C1 and the possible use for other research objectives. To all intents and purposes, it may be concluded that there is no statistical difference between the Oneon *Chlorella* (C3) and the cultivated *Chlorella* (C1).

6.4 Summary of the chapter

- From the experiments carried out in this chapter, One-on *Chlorella* (C3) demonstrated its suitability for the research whilst yielding similar results to the cultivated *Chlorella* in terms of characteristics, methane yield and process stability.
- Cell wall integrity confirmed the feasibility of using the C3 *Chlorella* source to achieve the next experimental objective which is to observe the effects of pre-treatment on microalgae cell wall disintegration and enhancing methane production.
- Holland and Barrett (C2) showed no similarity with the cultivated *Chlorella* and its high nitrogen content as well as the presence of anti-bulking agent in the *Chlorella* source made it unsuitable for further experiments thus, it was discarded.

- The successful establishment from the results which shows that the adopted source of *Chlorella vulgaris* tallies with the literature values as well as values obtained from our lab grown algae allows progression into further experiments and achieve the proposed research objectives.
- To enhance the energy yield achievable from microalgae, there is the need to by-pass the recalcitrant cell wall of the microalgae which is composed of cellulose, hemicellulose and pectin, which is resistive bacteria attack.
- The SEM picture verified the unruptured cell wall of the microalgae. This confirms the feasibility of using the selected strain to verify the impact of pre-treatment on cell wall degradation and methane enhancement as intended in the next chapter.

Chapter 7 Effect of Thermal Hydrolysis on *Chlorella vulgaris* methane yield

7.1 Microalgae cell wall Inhibition

The main concern/issue addressed in this chapter relates to the cell wall, the presence which is a barrier, thus limiting the anaerobic digestibility of this material . The need to overcome the limitation that comes with microalgae cell wall is high as there is a need to tap into the intracellular content to enhance its anaerobic digestion efficiency.

Several pre-treatment methods may be employed based on classification: biological (Fdez.-Güelfo *et al.*, 2011), chemical (López Torres and Espinosa Lloréns, 2008), physical (Izumi *et al.*, 2010) and thermal (Kim *et al.*, 2003). Generally, all these approaches have increased the hydrolysis rate, disintegration of microalgae and most especially the methane production achievable from microalgae by up to 220% (González-Fernández *et al.*, 2012; Passos *et al.*, 2013).

Although the success of these approaches means a significant energy increase may be obtained from digesting microalgae, recent studies have highlighted the set back that may occur as a result of pre-treatment to increase methane yield. It is believed that the net energy expended on disintegrating the microalgae cell wall does not justify the increase in CH₄ produced.

In the UK, thermal hydrolysis operation is widely used for sludge hydrolysis prior to anaerobic digestion. This approach is however considered as the preferred pre-treatment option for this study, as this facility is well established, understood and has been demonstrated to increase the overall methane yield of sewage sludge by up to 100% (Bochmann *et al.*, 2007; Rodriguez, 2012). It is hypothesized that a successful adoption of this existing facility can offset some of the excessive energy and capital cost associated with commercial exploitation of microalgae for energy.

Thus, the objective at this experimental stage was to identify the benefits of adopting thermal hydrolysis to pre-treat microalgae and increase its possible achievable methane yield, using the hydrolysis conditions currently used at those sites where thermal hydrolysis reactors are installed.

7.2 Results and Discussion

Thermal hydrolysis conditions of 165°C @ 8 bar pressure for 30mins were produced in the laboratory to disintegrate the microalgal cell wall. To evaluate the effectiveness of thermal hydrolysis on the microalgae, organic solubility was carried out followed by the specific methane yield which was used to compare biodegradation before and after hydrolysis. Two other thermal pre-treatments (heat drying pre-treatment at 90°C for 3 hours, and autoclave 120°C for 30 minutes) were also assessed at the optimal conditions suggested in the literature for comparison with thermal hydrolysis conditions .

Feedstock analysis prior to the digestion experiments showed both the substrates and the inoculum used in this study contained high alkalinity which suggests its amenability to anaerobic digestion (Table 7.1). Nitrogen content in the substrates also varied as a result of the pre-treatment leading up to increment in the range of 52-60% with equivalent TKN increase up to 0.9g/L.

Table 7.1 Summary of Substrate Characterisation

Substrate	Total solids (%)	Volatile solids (% TS)	pH	Total Alkalinity (mg CaCO ₃ /L)	TKN (mg /L)
<i>Chlorella vulgaris</i>	7.12	71.23	6.1	225	2390
<i>Dried Chlorella vulgaris</i>	7.15	78.32	5.7	225	2354
<i>Autoclaved Chlorella vulgaris</i>	7.21	75.67	5.8	225	2504
<i>TH Chlorella vulgaris</i>	7.21	73.32	5.9	225	2443
Inoculum	2.40	59.19	7.7	436	1851

7.2.1 Solubility Determination

This determines the dissolution rate and release rate of cell intracellular material from microalgae into the liquid phase for subsequent use by anaerobic bacteria. Two approaches were employed: degree of solubility in terms of COD content (Figure 7.1), and spectrophotometry at a wavelength of 750µm (Figure 7.2).

Although the thermally hydrolysed *Chlorella* was the most effectiveness using both approaches, no correlation was observed between these two approaches with regards to the autoclaved *Chlorella* and thermally treated (dried) *Chlorella*. Under the degree of COD solubilisation, heat drying pre-treatment (90°C for 3hours) proved to be a more effective pre-treatment approach compared to autoclave pre-treatment.

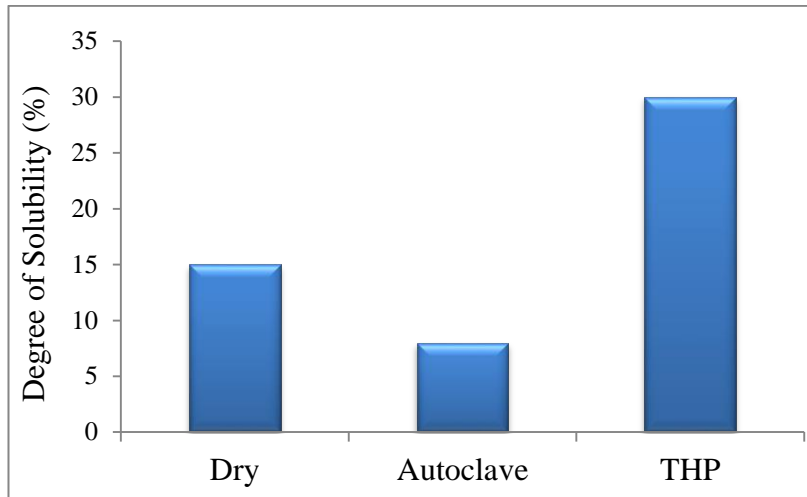


Figure 7.1 Effect of pre-treatment on degree of COD solubility

Conflicting results were obtained using the spectrophotometer (Figure 7.2), which showed more effective results with the autoclave pre-treatment compared to the heat drying pre-treatment.

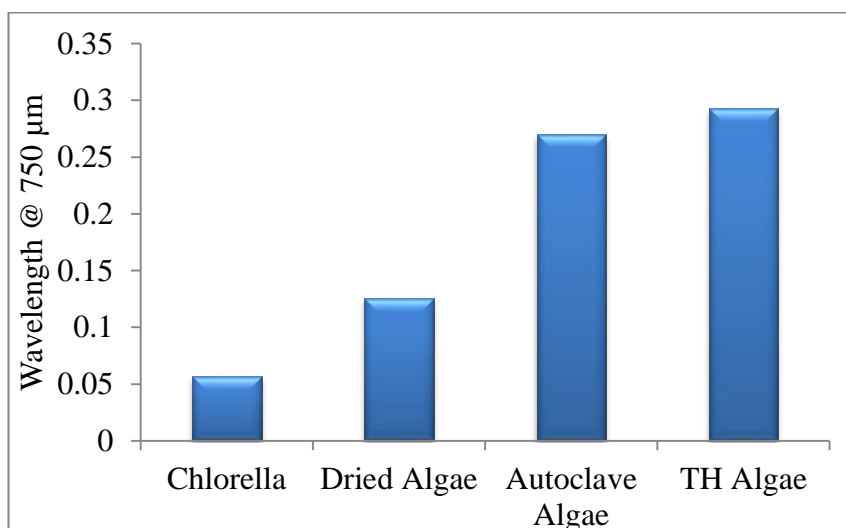


Figure 7.2 Effect of pre-treatment on solubility (spectrophotometer test)

Based on the results obtained from the degree of COD solubility, a high release of COD was found with all pre-treated *Chlorella* when tested against the control,

yielding a comparative increase of 14.5%, 8% and 30% for thermal treatment at 90°C, autoclaving and thermal hydrolysis respectively.

These results agree with the findings of Samson and LeDuy, (1983) who investigated the effects of several pre-treatment including thermal, biological and ultrasonic under various conditions. Their findings showed an increase in CODs in the range of 11 to 68%. Also, results obtained in the study agree with Alzate *et al.*, (2012), where thermal treatment was used to achieve up to a 32% increase in *Chlorella* COD. The lowest COD release was obtained from autoclaving with just an 8% increase observed for autoclaved *Chlorella*..

In addition to enhancing COD solubility, all the pre-treatments also delivered an increase in N-NH₄ concentration (Table 7.1).

7.2.2 Methane Yield

Methane yield ranged from 264 to 357 L CH₄/kg VS_{added} and the yield increased proportionately to the severity of the pre-treatment regime with the untreated *Chlorella* showing the lowest value and the THP the highest (Table 7.2).

Table 7.2 Comparative methane yield achievable from pretreatment of microalgae

	Cumulative methane yield (L CH₄)	Methane Yield / VS added (L CH₄/kg VS)	Methane Yield / VS destroyed (L CH₄/kg VS)	VS destroyed (%)
<i>Chlorella</i>	443.99	264.3	331.22	40
Dried <i>Chlorella</i>	535.16	318.55	413.65	57
Autoclaved <i>Chlorella</i>	552.45	328.84	415.58	58
TH <i>Chlorella</i>	600.33	357.34	482.65	60

The results for the yield were also mirrored for the VS destruction and the THP showed a 50% higher destruction (60%) than the untreated *Chlorella* (40%). As a result there was an additional 160 ml of CH₄/g produced from thermal hydrolysed *Chlorella*

All pre-treatments increased the microalgae methane yield with thermally hydrolysed microalgae yielding the largest increase with drying proving to be the least effective with an increase in methane yield of 20%. The percentage increase obtained for heat drying pre-treatment was less than that obtained in the study of González-Fernández *et al.*, (2012), where similar conditions were reported to achieve a 220% increase in methane yield. Similarly the percentage increase in yield obtained under the thermal hydrolysed condition (35%) was less than the findings of Keymer *et al.*, (2013) who studied the effect of thermal hydrolysis under similar experimental conditions and achieved an 81% increase in CH₄ yield.

It is possible that the different microalgal species used in these studies could explain the variation as *Chlorella vulgaris* was used for this study while *Scenedesmus* was used in the later studies. These strains differ both in cell wall size and composition which are factors that may predict effect of pre-treatment and methane yield achievable from microalgae (Torres *et al.*, 2013).

7.2.3 CH₄ yield and COD solubilisation

The relationship between the degree of solubility and the achievable CH₄ yield of the pre-treated microalgae was analysed. Apart from the thermally hydrolysed algae, no direct relationship was obtained between the solubility and achievable methane yield for the pre-treatments studied. The lowest performance was obtained from using the autoclave as a pre-treatment option where a COD solubilisation of 8% was observed leading to a proportional methane increase of 0.064 L CH₄/g VS added. Thermal treatment (drying at 90°C), demonstrated an increased solubility of 15% but a proportional methane increase of 0.054 L CH₄/g VS added, whilst thermal hydrolysis on the other hand yielded an increase in COD solubility of 30% accompanied by an increase in methane proportion of 0.093 L CH₄/g VS added.

The autoclaved *Chlorella* had the lowest COD yet a higher methane yield in terms of VS_{added} when compared to the thermally dried *Chlorella*. This finding agrees with the work of Samson and LeDuy, (1983) who saw a decrease in methane yield against the control even though solubilisation experiments highlighted a 68% increase in COD. Also, Alzate *et al.*, (2012) and González-Fernández *et al.*, (2011) confirmed that higher microalgal COD does not necessarily enhance CH₄ production.

From the results obtained, it could be inferred that the pre-treatment approaches employed for microalgae are controlled by different mechanisms thus yielding a variety of results. The thermal hydrolysis approach however provided a particle size reduction and increase in exchange area between the particles and liquid phase. This resulted in the rapid degradation of the released material thus the highest methane yield.

TKN and phosphorus analysis were also carried out at the end of the anaerobic digestion experiment to identify the percentage of nutrients released into the aqueous phase. This showed a respective average recovery of 65 and 22% for TKN and phosphate present in the raw substrate thus suggesting an exploitable avenue of these nutrients for microalgae cultivation.

7.2.4 Effect of Pre-treatment on Hydrolysis Rate

The hydrolysis rate obtained from the pre-treatment studies ranged between 0.08 to 0.142d⁻¹ with the untreated *Chlorella* showing the lowest value and the THP the highest (Table 7.3). The thermally hydrolysed algae which corresponds to the highest methane yield, produced up to a 75% higher rate of methane production than the control. Drying and autoclave studies also showed a substantial degradation of 12.5 and 25% higher than the untreated algae respectively. This shows that all the pre-treatment reasonably increased the rate of degradation of *Chlorella vulgaris*.

Results of the BMP tests with model simulations are shown below (Figure 7.3). The error bars indicate the standard errors from triplicate tests while the model lines shown are based on the best fit of f_d and k_{hyd} with standard errors (Table 7.3)

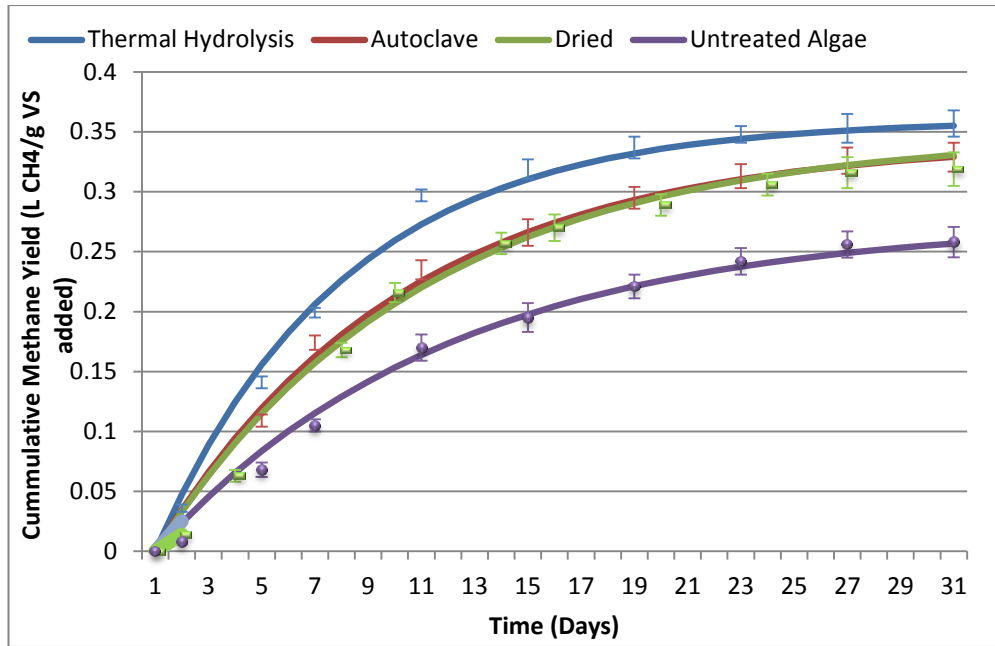


Figure 7.3 Cumulative methane yield from respective digesters. Error bars indicate standard error in triplicate tests while the lines show the predicted model trend.

Table 7.3 Parameter estimation obtained for degradation (f_d) and first order hydrolysis rate (K_{hyd}) showing standard errors of predictions.

Pre-treatment Type	f_d [L CH ₄ gVS ⁻¹]	K_{hyd} [day ⁻¹]	χ^2
Untreated	0.26 ± 0.01	0.08 ± 0.004	0.005
Dried	0.33 ± 0.02	0.09 ± 0.01	0.01
Autoclave	0.33 ± 0.01	0.10 ± 0.004	0.005
Thermal Hydrolysis	0.36 ± 0.01	0.14 ± 0.01	0.012

A visual representation of the effect of pre-treatment on the parameters of interest (methane yield [L CH₄ gVS⁻¹] and rate of hydrolysis K_{hyd} [day⁻¹]) was developed with 95% confidence envelopes. The envelopes (Figure 7.4) were calculated as described by (Batstone *et al.*, 2003) via the minimization of an objective function based on the sum of squares.

The first order rate constant (K_{hyd}) obtained in the study for substrates both before and after pre-treatment falls within the range obtained for the digestion of screenings (0.061 – 0.1773 d⁻¹) previously obtained by Rodriguez, (2012). It exceeds the hydrolysis values of grass, straw and some fruit wastes, which range between 0.016

and 0.122 d^{-1} (Tong et al., 1990; Veeken and Hamelers, 1999). Also the order of increase in the rate constant for pre-treatment aligns with the order of increase in methane yield obtained from the study.

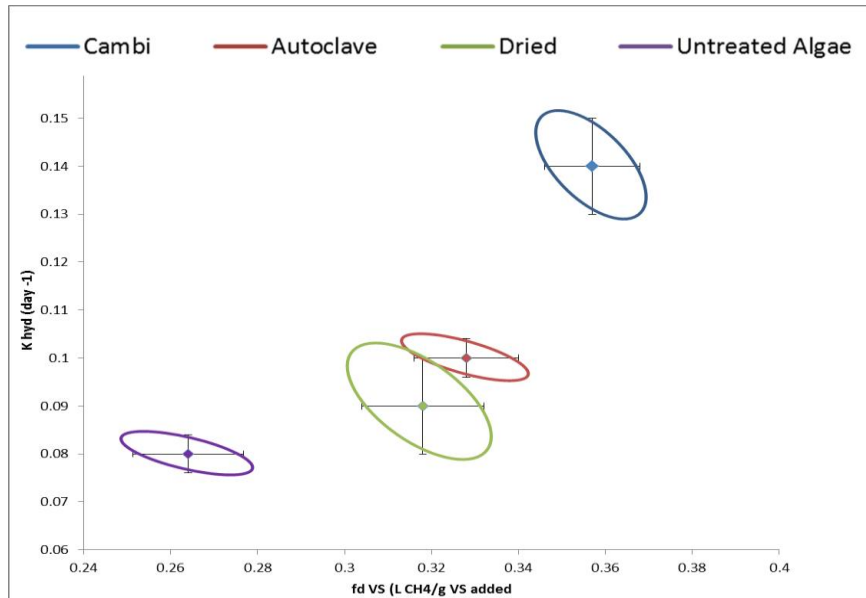


Figure 7.4 95% confidence envelopes for degradation (f_d) and first order hydrolysis rate (K_{hyd}) of the pre-treatment experiments

7.2.5 Scanning electron microscope (SEM) Analysis of *Chlorella* following thermal hydrolysis

To ascertain the effectiveness of thermal hydrolysis on *Chlorella vulgaris* SEM prior to pre-treatment was used to verify cell wall integrity. After thermal hydrolysis of the *Chlorella*, the biomass was recovered, dried and visually inspected under SEM which revealed the extent of disintegration (Figure 7.5) on the cell wall, allowing the anaerobic bacteria access to tap into the intracellular content of the microalgae cells and to enhance its anaerobic digestion efficiency.

These experiments showed that existing thermal hydrolysis of the UK water industries for sewage sludge conditioning may be adopted to pre-treat microalgae, disintegrate its cell wall, increase its solubility/hydrolysis and thus increase the methane yield of microalgae from 0.27 to 0.36 L CH₄ /g VS_{added}. This 35% increase in methane yield demonstrates the commercial potential of this option

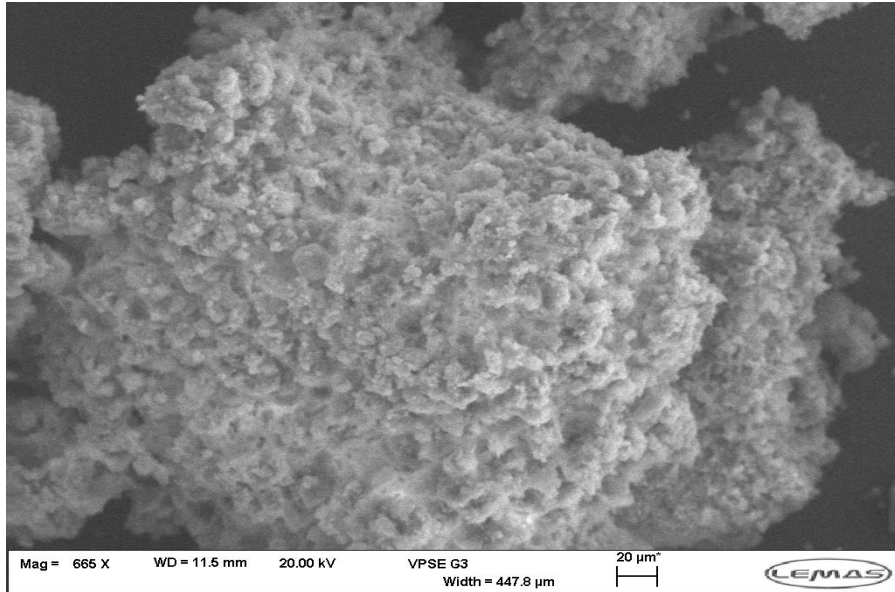


Figure 7.5 SEM image of Chlorella vulgaris after pre-treatment

7.2.6 Energy Balance

Finding a balance between energy expended on pre-treatment and energy produced is essential for the commercial feasibility of pre-treatment technologies which are aimed at enhancing anaerobic digestion performance and methane yield. The minimum requirement for feasibility will be to ensure that the increased methane yield obtained from thermal hydrolysis is enough to justify the extra energy expended on the pre-treatment.

Embodied energy will normally include energy for microalgae production, heating VS/water molecules, maintaining temperature as well as for pressure. However, at this stage, the study has only carried out the energy balance based on the VS content of substrate with further assumptions that the existing technologies employed for sludge dewaterability can be used to achieve the required concentration at minimal cost and that the excessive heat produced by the WWTP may also be used to achieve the desired solids content/pre-treatment. Subsequent chapter in the study will however attempt an extensive mass/energy balance using the available facilities within a typical UK WWTP.

Based on the experimental data obtained in this chapter, the energy balance of the thermal hydrolysis technique studied was estimated as a ratio of energy input to the energy output (E_i/E_o) (Passos *et al.*, 2013). Energy balance is attained at a value of \leq

1, while values > 1 signify an energy negative approach. E_i signifies the specific energy applied to organic biomass (Equation 7.1)

$$E_i \text{ (kJ/kg VS)} = c_p m \Delta T \quad \text{Equation 7.1}$$

Where, c_p is specific heat (kJ/kg), m is mass (kg VS) and ΔT is temperature difference of raw sample to TH temperature ($^{\circ}\text{C}$).

The energy output (E_o) was calculated using the equation 7.2 below:

$$E_o \text{ (kJ/kg VS)} = [\Delta P_{\text{CH}_4} \text{ (mL/g VS)} * \xi \text{ (kJ/m}^3\text{)}] / 1000 \quad \text{Equation 7.2}$$

Where, ΔP_{CH_4} = is the change in methane yield as a result of pre-treatment (mL/g VS)

ξ = lower heating value of methane (35,800 kJ/m³CH₄)

Table 7.4 Energy ratio of microalgae biomass under TH pre-treatment conditions

Pre-treatment Type	VS Content	Energy Input (kJ/kg VS)	Energy output (kJ/kg VS)	Energy ratio (E_i/E_o)
Thermal Hydrolysis	0.73	832	3333	0.25

The obtained E_i/E_o had a value of 0.25 showing the process is energy positive and that energy output supersedes energy input Nevertheless, a more energy positive approach will be achieved with the proposed integration as the existing THP in WWTP is equipped with facilities that may help enhance heat recovery for re-use within the system.

Recovery of this energy using the heat exchangers and as flash steam for the re-use by hydrolysis reactor suggests about 70% of the heat requirement can be returned/recycled to the system (Mills *et al.*, 2011; Nan *et al.*, 2012). A sankey diagram may however be used to describe the energy flow within the THP (Figure 7.6). Putting the recycled heat back into the reactor, a more positive E_i/E_o of 0.1 will be achieved.

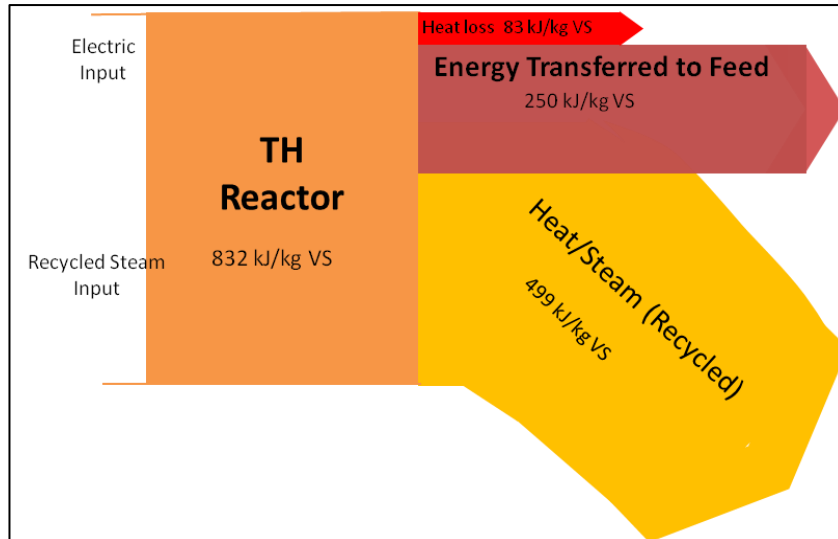


Figure 7.6 Sankey diagram representing energy utilization of the TH reactor

The finding in this study however differs from other similar studies such as work of Passos *et al.*, (2013) and González-Fernández *et al.*, (2012) where pre-treatment was evaluated with E_i/E_o ratio ranging between 18.7 and 97.3. This is mainly because biomass concentration in this studies has been assumed using the existing facilities within the UK WWTP at no significant cost.

7.3 Summary of the chapter

- All three pre-treatments studied demonstrated an improvement in the solubility and hydrolysis rate of microalgae. The most efficient and consistent result was obtained from the thermal hydrolysis treatment which increased microalgal solubility and hydrolysis rate up to 75%.
- The thermal hydrolysis currently used for sludge pre-treatment in UK WWTP may be used for enhancing microalgae cell wall and increasing the methane yield for achievable from this substrate up to 35%, increasing the methane yield of microalgae from 0.27 to 0.36 L CH₄ /g VS_{added}. This highlights a better chance for microalgae commercialization.
- Nutrients recovered in the liquid phase after anaerobic digestion was large at 65 and 22% for TKN and phosphate respectively and this may be beneficial for microalgal cultivation.
- The thermal hydrolysis process proved energy positive with E_i/E_o of at least 0.25.

Chapter 8 Co-digestion of Pre-treated Microalgae and Sewage sludge

8.1 Introduction/Background

To date, limited literature exist on the co-digestion of microalgae and sewage sludge, and it is common agreement that the cell wall of microalgae is a set back as it is resistant to digestion thus negatively impacting achievable methane yield (Samson and LeDuy, 1983; Heimel, 2010; Olsson *et al.*, 2013). In addition, no research has documented the effect of co-digesting alga and sewage sludge after pre-treatment.

The previous chapter suggests that the use of thermal hydrolysis, for instance the Cambi process, can achieve up to a 35% increase in methane yield. This new yield suggests a better performance of microalgae as a substrate for anaerobic digestion thus a mutualistic relationship is expected from the co-digestion of this upgraded feedstock and sewage sludge. This chapter investigates the possible benefits that may be reaped from co-digesting microalgae and sewage sludge after pre-treatment.

8.2 Results and Discussion

8.2.1 Substrate Characterization

Feedstock analysis prior to the digestion experiments demonstrated the potential in terms of elemental composition, of the microalgae to act as a suitable co-digestate. *Chlorella vulgaris* had VS of 73.3% and a carbon content of 44.9% while the sewage sludge used for this experiment had VS of 77.3% with a carbon content of 37.6% (Table 8.1).

Table 8.1 Characteristics of feedstock used for BMP

Parameters	pH	TS	VS	Alkalinity	C	N	H	O	C:N
		(%)	(%)	(mgCaCO ₃ /L)	(%TS)	(%TS)	(%TS)	(%TS)	
<i>Chlorella vulgaris</i>	5.9	8.6	73.32	2250	44.9	7.4	6.6	27.3	6.1
Sewage Sludge	5.4	4.5	77.29	1310	37.6	6.9	5.7	24.2	5.4

8.2.2 Process parameters

This higher carbon content suggests that the microalgae should perform better than sewage sludge. Both the substrates and the inoculum used in this study contained a high alkalinity which shows its amenability to anaerobic digestion. Throughout BMP the experiment, alkalinity increased and remained fairly constant (Figure 8.1) with values predominantly between 2,536 and 5,520mg/L (Table 8.2). This sufficient buffering capacity helped to provide a stable pH which ranged between 7.3 and 7.5 as observed for the co-digestions studied. This pH falls within the pH range predicted for optimum digester performance without the need for chemical dosing (Heo *et al.*, 2004).

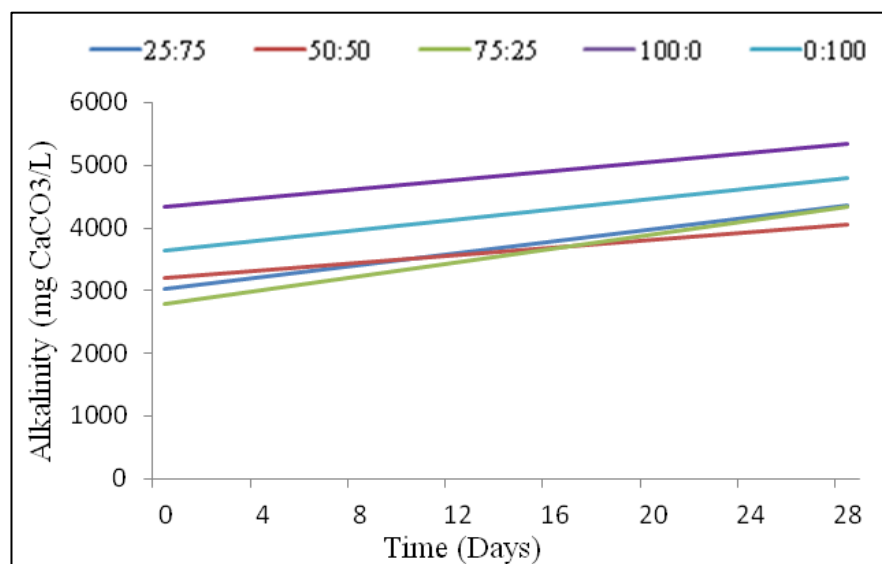


Figure 8.1 Alkalinity profiles of co-digestion experiments

The concentration of VFA is equally important for monitoring the performance of a digester. Quantitative and qualitative information on the VFAs formed may be used to delineate and report the dominating species of acidogens within a reactor. In this study, major intermediate products of anaerobic co-digestion, were acetic (HAc), propionate (HPr), n-butyrate (n-HBu), isobutyrate (i-HBu), isovalerate (i-HVa) and n-valerate (nHVa) acids in this order.

Table 8.2 Mean value for each digester parameter during the BMP experiments

Reactor	pH	Alkalinity (mg CaCO ₃ /L)	VFA (mg/L)	TAN (mg/L)
0% Algae	7.5 ±0.1	4707 ±138	813±108	1526±113
25:75	7.4±0.1	3605±135	781±65	1002±107
50:50	7.4±0.1	3569 ±125	781±76	1113±115
75:25	7.5±0.1	3466 ±125	907±68	959±76
100% P\T Algae	7.5±0.1	5192±135	1094±95	1532±129

±Figures are one standard deviation

The VFA profile of the co-digestion reactor (Figure 8.2) showed significant occurrence of acidogenesis in the first 3 days with the 75:25 co digestion experiments having the most VFA accumulation, which coincided with a decrease in alkalinity. All reactor VFA concentration declined between days 3 and 13, which was expected as methane production was significant.

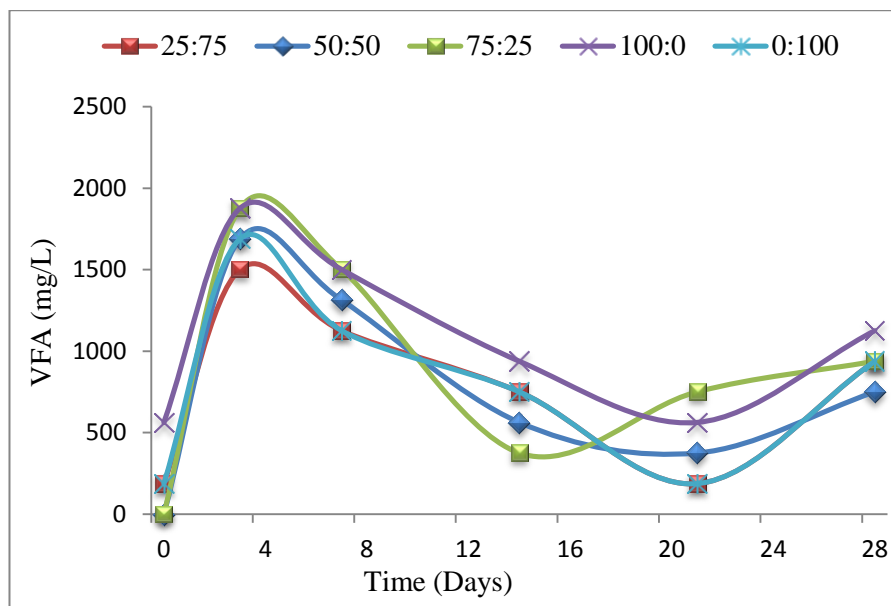


Figure 8.2 VFA profiles of co-digestion experiments

Throughout the study, VFA concentration in all the reactors ranged between 187 and 1877 mg/l, all of which fall within the safe limit of concentration. At the end of the experiment, the VFA concentration in the co-digestion reactors dropped with the least value obtained in the 50:50 reactor with a corresponding VFA of 750.625 mg/L.

The digester stability was also monitored using VFA:Alkalinity which is a “substrate dependent” parameter which measures the relative proportion of compounds that may act to reduce pH and buffering capacity needed to maintain it. The start-up days

demonstrated a relatively high VFA:Alkalinity as a result of rapid break down of carbohydrates into acids. After day 6, a steep decline in VFA:Alkalinity ratio was observed, corresponding to the rapid conversion of VFA to methane after which the VFA:Alkalinity in all reactors remained below 0.3, indicating a stable digestion process (Figure 8.3). It can be inferred that 100% algae reached stability after 8 days and all other reactors were stable in 12 days which is good as UK digesters are always operated at retention times greater than 12 days, thus suggesting the co-digestions studied can fit into the current operating conditions in the UK WWTPs.

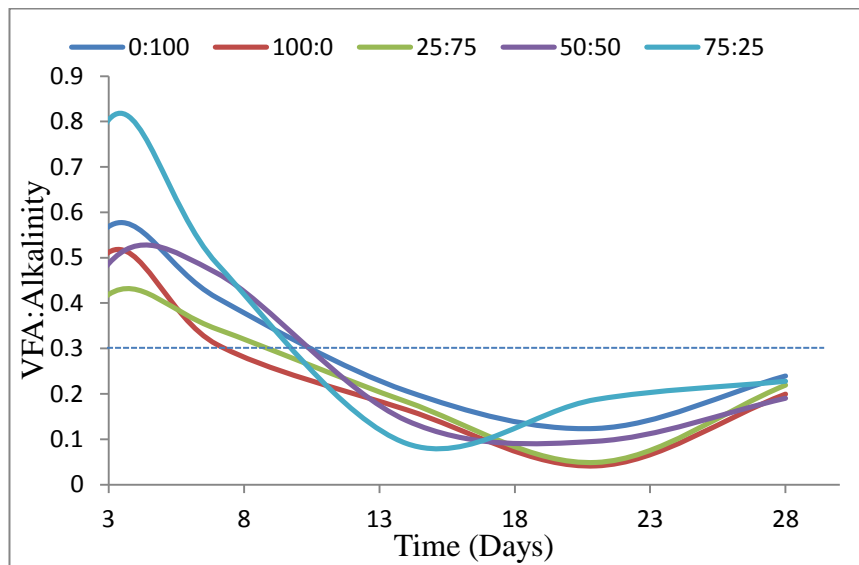


Figure 8.3 VFA:Alkalinity profile of co-digestion experiments

8.2.3 Methane yield obtained from respective digesters

The accumulated gas volume obtained in the respective reactors (Table 8.3) ranged between 451.3 and 620.3 N ml CH₄, with 100% sludge showing the lowest yield and 75:25 co-digestate having the highest yield. This shows the feasibility and a stable co-digestion relationship between the pre-treated *Chlorella vulgaris* and sewage sludge.

A correlation plot between alga/sludge ratio vs. methane yield in terms of VS_{added} and VS_{destroyed} (Figure 8.4) showed a linear relationship; $R^2 = 0.76$ and 0.87 respectively. The obtained methane yield from the 100% sludge digester (batch) was 0.360 L/g VS_{destroyed}. On the other hand, methane yield obtained from the 75:25 (batch) and 100% algae under the same condition was similar with respective values of 0.48 and 0.48 L

$\text{CH}_4/\text{g VS}_{\text{destroyed}}$. Methane yield at these two co-digestion ratios were significantly higher by 34% than the obtained methane yield from digesting 100% sludge thus highlighting the linear relationship co-digesting algae with sludge can have. This was confirmed by the ANOVA which revealed significant statistical differences ($P < 0.00002$) between the co-digestion ratios with regard to CH_4 yield. Although 75:25 proved the highest yield in the study, it may also be suggested that very little additional benefits was obtained over the 50:50 (algae:sludge) study just about 6 percent increase.

Most literature reported values for anaerobic digestion of microalgae are reported in terms of VS added ranging between 0.14 – 0.45 $\text{L CH}_4/\text{g VS}_{\text{added}}$. Comparing the literature values to the obtained methane yield from the 100% algae in the experiment in terms of VS added (0.36 $\text{L CH}_4/\text{g VS}_{\text{added}}$), it falls within the expected range.

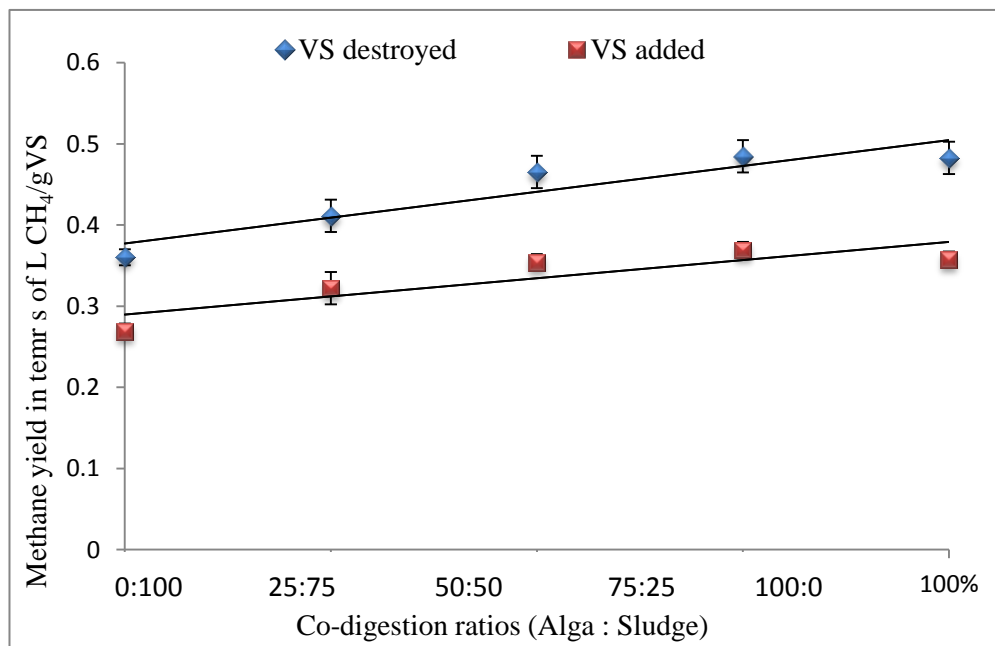


Figure 8.4 Methane yield from respective co-digestion

A linear relationship $R^2=0.92$ (Figure 8.5). was also observed between algae/sludge ratio and VS destruction. The VS destruction obtained from the 100% sludge digester was 50%: while the VS destruction obtained from 100% pre-treated algae digester (batch) was more substantial with a 60% VS destruction. Co-digestion 75:25 had the next highest destruction at 59% VS destruction.

The methane content of biogas may be used to delineate the efficiency and health of a digester. High values imply steady and stable digester performance while low values imply inhibition which limits the methanogenic activity within the microbial consortium (Park and Li, 2012). Co-digestion experiments in this study had a significant effect on methane content for all ranges studied. The methane content in all reactors ranged between 56% and 60% with 100% sludge and 100% algae having the lowest and highest methane content respectively.

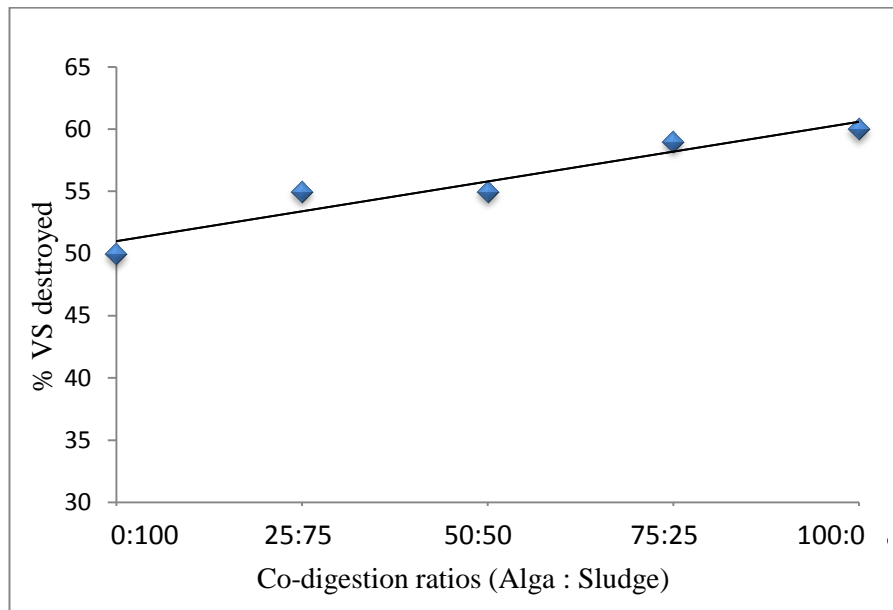
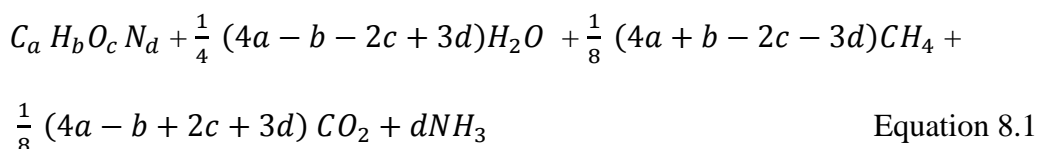


Figure 8.5 %VS destroyed from respective co-digestions

8.2.4 Relationship between specific methane yield (SMY) and Theoretical Methane potential (TMP)

Using the Buswell's equation (Equation 8.1), the theoretical methane potentials were estimated (Symons and Buswell, 1933) and compared to the respective specific methane yield obtained from the study (Table 8.3).



The TMP varied over the range 0.52 to 0.65 L CH₄ /g VS with the highest yield estimated for the 100% algae and the least for the sewage sludge. Results showed that the SMY obtained in the study (Table 8.3) at all digestion ratios were in the

range of 64 and 77% of the calculated methane potential of algae. These ranged between 79 and 93% of the theoretical methane potential of sewage sludge.

Table 8.3 Performance data of co-digestion experiments during a 30 days BMP test

Reactor (Alga: Sludge)	Cumulative mL CH ₄ /	BMP L CH ₄ /g VS added	BMP L CH ₄ /g VS destroyed	VS destroyed %	Methane Content %	TMP of Algae (L CH ₄ /g VS)	TMP of Sewage Sludge (L CH ₄ /g VS)
0:100	451 ±12	0.27 ±0.01	0.36 ±0.01	50	56		
25:75	541 ±29	0.32 ±0.01	0.41 ±0.02	55	59		
50:50	580 ±19	0.35 ±0.01	0.46 ±0.02	54	56	0.64	0.52
75:25	620 ±21	0.37 ±0.01	0.48 ±0.02	59	59		
100:0	600 ±20	0.36 ±0.01	0.48 ±0.02	60	60		

±Figures are one standard deviation

Findings from this study differ from earlier studies for instance Samson and LeDuy, (1983), who used sewage sludge to increase the C:N content of *Spirulina* from 4.2 to 6 and achieved an improved performance of AD of microalgae. Furthermore, Heimel, (2010) observed that co-digestion of sewage sludge and algal sludge gave no significant synergistic benefits.

This present study shows a positive relationship between the co-digestion of algal sludge (*Chlorella vulgaris*) and sewage sludge as the proportion of pre-treated algal sludge added to the sewage sludge led to a direct proportional increase in overall methane yield. There was a substantial increase up to a co-digestion ratio of 50:50 after which little additional benefits was obtained at 75:25 (algae:sludge). The improved results are due to the cell wall status of the algal sludge used in this study which was disintegrated (prior to anaerobic digestion) using thermal hydrolysis under conditions typically used within the UK WWTPs, thus enabling more biodegradability of the substrate. Moreover, the C:N ratio of *Chlorella vulgaris* used in this experiment (6.1) is greater than that of the *Spirulina* (4.2) studied by Samson and LeDuy, (1983).

Comparatively, the 75:25 and the 100% algae reactors yielded similar methane production of 0.48 and 0.48 L CH₄/g VS_{destroyed} respectively, this highlights a linear relationship with the addition of pre-treated algae up to this co-digestion ratio (75:25) thus favouring the methanogenic activities. Methane yield obtained at this ratio surpassed the yield obtainable from sludge digestion (0.36 L CH₄/g VS_{destroyed}) by 34% and is recommended for further studies.

8.2.5 Co-digestion effects on biodegradability rate and methane production

From the results obtained (Table 8.4), it is obvious that the 100% algae had improved degradation rate 57% higher than the rate of methane production in the 100% sludge. This is presumed to be as a result of the thermal hydrolysis pre-treatment effect on the algae cell wall releasing the cell content and making it available for the anaerobic bacteria to degrade. Also, a direct relationship ($R^2=0.94$) exists between the three co-digestion studies highlighting the direct benefits of addition of algal sludge to sewage sludge to increase methane yield and the rate of degradation.

Results of the BMP tests with model simulations are shown below (Figure 8.6). The error bars indicate the standard errors from triplicate tests while the model lines shown are based on the best fit of f_d and k_{hyd} with standard errors (Table 8.4).

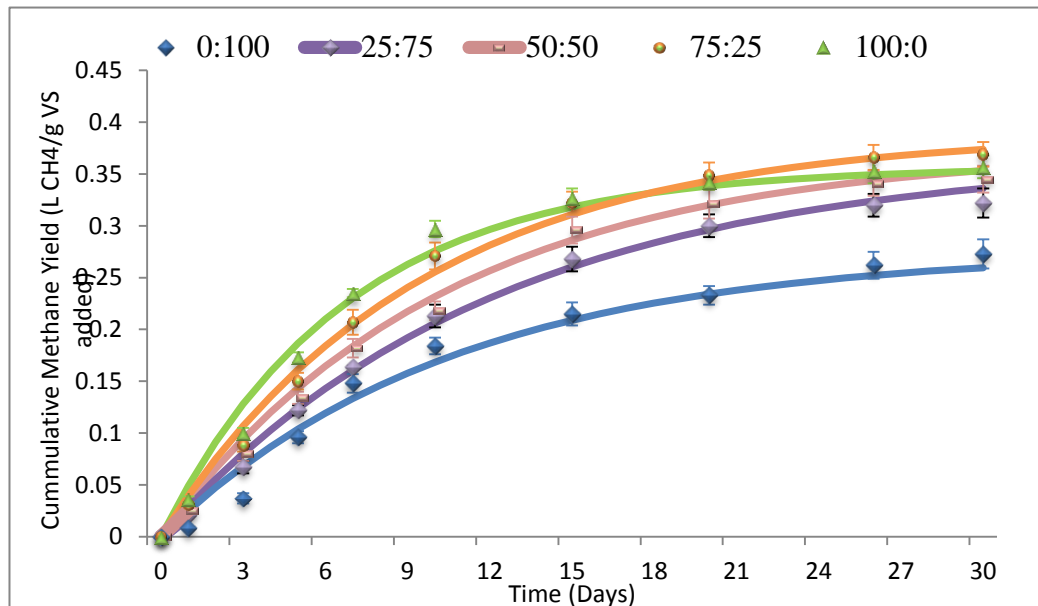


Figure 8.6 Cumulative methane yield from respective co-digesters (algae : sludge). Error bars indicate standard error in triplicate tests while the lines show the predicted model trend.

Table 8.4 parameter estimation obtained for degradation (f_d) and first order hydrolysis rate (K_{hyd}) showing standard errors of predictions.

Algae:sludge	f_d [L CH ₄ gVS ⁻¹]	K_{hyd} [day ⁻¹]	χ^2
0:100	0.27 ± 0.01	0.090 ± 0.01	0.012
25:75	0.32 ± 0.01	0.088 ± 0.003	0.003
50:50	0.35 ± 0.01	0.094 ± 0.002	0.001
75:25	0.37 ± 0.01	0.108 ± 0.005	0.006
100:0	0.36 ± 0.01	0.142 ± 0.01	0.010

Nevertheless, it was established that while the 100% algae co-digestion had the highest degradation rate, it did not produce the highest methane yield as the produced methane was similar to that obtained from co-digestion 75:25 with a corresponding K_{hyd} of 0.108.

Overall, the first order rate constant (K_{hyd}) obtained in the study for both substrates and co-digestions ranged between 0.09 and 0.142 d⁻¹. These hydrolysis rates are however similar and competitive with other biodegradable wastes (Tong *et al.*, 1990; Veeken and Hamelers, 1999). Also, the order of increase in the rate constant for the co-digestions align with the order of increase in methane yield obtained from the study.

8.3 Reflections

To date, no research has documented the effect of co-digesting alga and sewage sludge after pre-treatment using the existing thermal hydrolysis conditions thus the importance of this work, which highlights the feasibility of integrating microalgae whilst exploring the existing assets in the water industries and the unutilized digester headroom.

Overall, the integration of algae into the UK WWTP seems like a feasible option from the results obtained in this study. Co-digesting sewage sludge with microalgae proved to be a beneficial approach for increasing energy produced in the UK water

industry while utilizing the digester headspace and using the pre-treated algae to increase degradability and overall methane production.

This study suggests a feasible approach to enhance sludge methane yield by co-digesting sewage sludge with thermally hydrolysed alga sludge. The favourable co-digestion result obtained suggests added benefits of available carbon and extra nutrients in the microalgae. Also, the disintegrated cell wall enhanced digestion and methane production.

The thermal hydrolysis is an existing facility in the UK water facility and requires no alteration to conditions neither will it add significantly to production cost; moreover this algal biomass will be readily available at no extra cost in the WWTP as against using other alternatives such as food waste and MSW.

Nevertheless, to justify the feasibility of this integration, extensive energy balances with regard to commercial applications for the most optimal scenario tested will have to be examined in the next study to see how viable the processes are.

8.4 Summary of the chapter

- The study satisfied the hypothesis that microalgae can be a compatible substrate to co-digest with sewage sludge with capability of utilizing the existing/unutilized digester capacity in the UK WWTP.
- Addition of pre-treated alga to sludge has a directly proportional relationship to methane yield with best co-digestion (75:25) yielding up to 34% increase in L CH₄/ g VS compared to digesting sludge alone. This ratio suggests a balance between substrates thus favoring methanogenic activities.
- It is essential to generate sufficient knowledge as well as understanding of the key operational conditions (optimum HRT and OLR) to achieve a successful and optimal mesophilic co-digestion process in a continuously stirred-tank reactor (CSTR) for large scale and commercial explorations.
- To justify the feasibility of this integration, energy balances with regard to commercial applications for the most optimal scenario tested (in a laboratory scale semi CSTR) will have to be examined to see how viable the processes are.

Chapter 9 Effects Of Operational Parameters On Anaerobic Digestion Of *Chlorella vulgaris* And Sewage Sludge In A Semi Continuous CSTR

9.1 Introduction

The previous chapter compared BMP potential of the substrates used in this research as well as the ultimate methane potential achievable under different co-digestion ratios between the microalgae *Chlorella vulgaris* and sewage sludge. This established the potential benefits of co-digesting microalgae and sewage sludge, and highlighted the need to generate sufficient knowledge and an understanding of the key operational conditions necessary to achieve a successful and optimal mesophilic digestion process.

Process parameters such as pH value offer a guideline to identify the type of fermentation occurring in the reactor. Whilst an optimal pH between 6.8 and 7.2 will favour CH₄ production, a low pH value between 5 and 6.5 will lead to the production of H₂ and highly reduced volatile acids and alcohols as the electron flow is diverted from CH₄. Likewise, the role of organic loading rate (OLR) and hydraulic retention time (HRT) determine the rate of production of intermediary metabolites and also determine the efficiency and economy of the process e.g. a high organic loading rate (OLR) in the digester can reduce the HRT and capital cost generated by digester size.

While benefits such as increased removal of organic matter and volumetric CH₄ production rates are associated with increasing the organic loading rate of the digester, there is a need to not exceed the critical OLR of a digester, as this can lead to a washout of the microbial population therefore leading to a digestate composition that contains a considerable amount of undigested organic matter, which simultaneously leads to digester instability and eventual failure (Fdez.-Güelfo *et al.*, 2011).

Few studies have been carried out on the optimal OLR and HRT for anaerobic digestion of microalgae, and of these an OLR of 2 - 4g VS/L/d and 20 days HRT have been identified as optimal (Mairet *et al.*, 2011; Passos *et al.*, 2014). No study has investigated the effects of HRT and OLR on the co-digestion of the substrates

studied in this research (sewage sludge and pre-treated algae). Thus, the objective at this experimental stage was to identify the most optimal condition in terms of OLR and HRT for maximum methane yield/productivity in a continuous stirred tank reactor (CSTR).

9.2 Results

The results obtained from the algae co-digestion experiments are divided into six sections:

9.2.1 Start-up of the CSTR process

The start-up of the process began with acclimatizing individual reactors with their respective feed at 1 g VS/l/d and this was achieved in a span of 3-4 weeks. Steady states of the reactors were characterized by stable biogas production (Figure 9.1), relatively low levels of ammonia and moderate VFA in the digesters (Table 9.1).

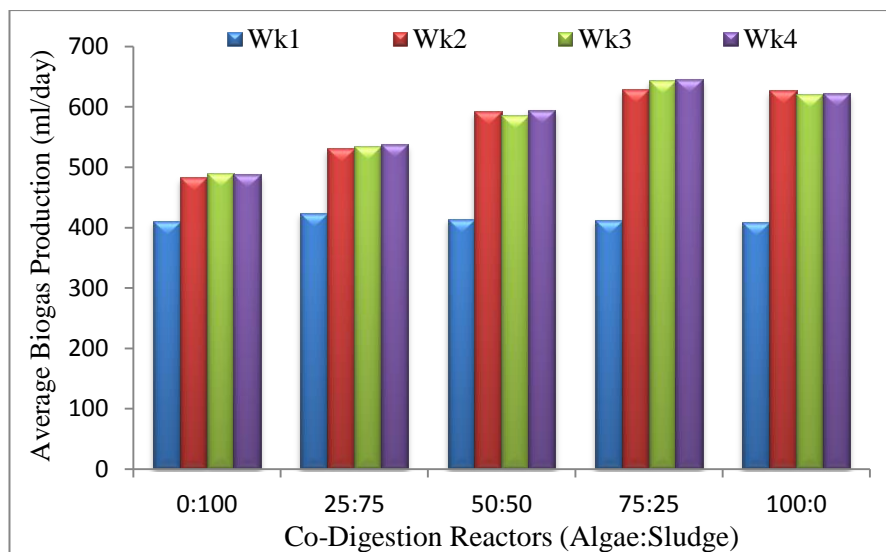


Figure 9.1 Biogas production during start-up of semi-continuous CSTR

According to Ripley *et al.*, (1986), the VFA:alkalinity ratio is of more importance than the actual level of individual parameters. The obtained VFA:alkalinity in this study was in line with Behling *et al.*, (1997) who suggested that a VFA:alkalinity below 0.4 implies that the digester is in a steady state with a tendency to withstand

minimal fluctuations without any major change in pH. For the digesters operated, a VFA: alkalinity ratio ranging between 0.2 and 0.25 was obtained.

Table 9.1 Average process parameters during reactor steady state

Digester (Algae:Sludge)	pH	Alkalinity (mg CaCO ₃ /L)	VFA (mg/L)	Ammonia (mg/L)	Biogas Yield (L Biogas/kg VS)	Methane Content (%)
0:100	7.6	5210	1126	785	489.5	60
25:75	7.9	3500	1126	697	535.4	63
50:50	7.5	3450	1314	715	586.8	64
75:25	7.8	3780	1314	790	643.5	63
100:0	7.7	4650	1314	798	621.5	63

During the steady state operation of the digesters, a stable pH and alkalinity ranging between 7.6-7.9 and 3,450 – 5,210 mg CaCO₃ respectively were observed which falls between the optimal range for a steady methanogenic reactor (Heo *et al.*, 2004). This showed that microalgae and sewage sludge possess good buffering capacity at all co-digestion ratios without need for external pH correction during digestion. Ammonia levels also fell within recommended limits.

During the steady state operation of the digesters, methane content in biogas ranged between 60 and 64%. This falls within the stable expected range depicting stable conditions and it is anticipated to vary with higher OLR or a shorter HRT (Mata-Alvarez, 2003).

9.2.2 Impact of OLR and HRT on CSTR performance

Using the 100% algae digester as a representative of steady state, a HRT between 20 and 8 days and OLRs between 2 and 5g VS/L/day were studied. Time constraints meant that it was not possible to carry this out on all co-digestion ratios. The performance of the mesophilic anaerobic digestion (MAD) for each operational condition carried out was compared using methane yield and production (Table 9.2).

Table 9.2 Effect of operational conditions on the performance of semi continuously fed MAD process

HRT (Days)	20	15	10	8
OLR (g VS/L/day)	2	2.7	4	5
Parameters				
Specific Biogas L/g VS	0.63± 0.09	0.56± 0.07	0.50± 0.09	0.45± 0.09
Methane Content (%)	65	62	59	47
Methane Yield (L/g VS)	0.41	0.35	0.29	0.21
Methane Production CH ₄ /L/d	0.45	0.53	0.47	0.58
Alkalinity (mg CaCO ₃ /L)	7475± 128	5303± 125	4148± 125	3302± 125
Ammonia (mg/L)	1930± 23	2100± 19	2400± 22	3124± 74
VFA (mg/L)	1501± 145	1750± 97	1950± 125	2100± 115
VFA/Alkalinity	0.2± 0.03	0.33± 0.04	0.47± 0.06	0.58± 0.06
pH	7.8± 0.2	7.6 ± 0.2	7.2 ± 0.3	6.5± 0.2
% VS destroyed	66	60	49	40

±Figures are one standard deviation

During the experiment, the effect of increasing organic loading rate was observed on methane content which decreased simultaneously with the HRT until the methane content reached its lowest value of 47% at an HRT of 8 days and corresponding organic loading rate of 5g VS/L/day.

In terms of specific biogas and methane production, a significant difference was observed between the HRTs tested. This was confirmed by the ANOVA which revealed significant statistical differences with regard to CH₄ yield ($P < 2E-07$). At an HRT of 20 days and corresponding OLR of 2 VS/L/day, highest methane yield of 0.41 m³ CH₄/Kg VS_{added} was obtained (Figure 9.2). This was followed by the 15 days HRT with corresponding yield of 0.35L CH₄ /g VS_{added}, while the least yield was obtained at HRT of 8 days and OLR of 5 VS/L/day.

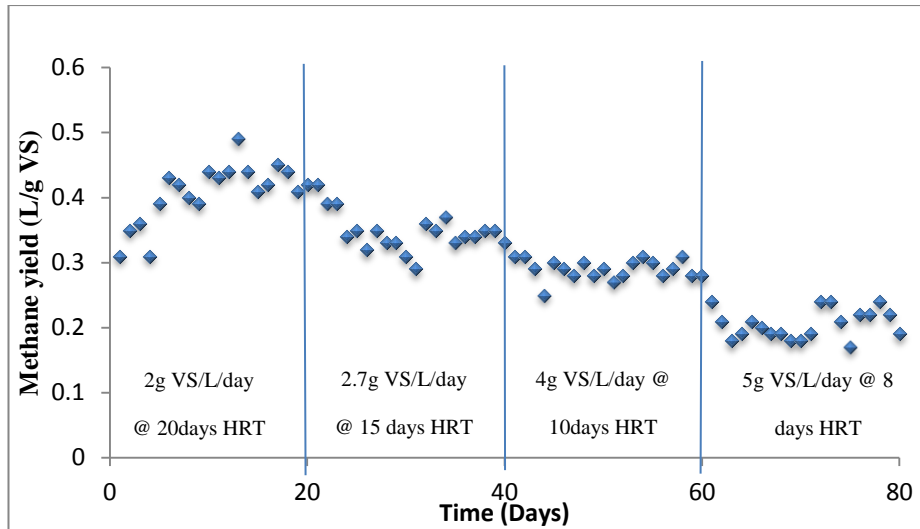


Figure 9.2 Methane yield showing CSTR performance.

An obvious reduction in methane yield of $0.29 \text{ L CH}_4 / \text{g VS}_{\text{added}}$ was obtained with a 10 days HRT, falling well below the achieved value from the BMP experiments. The least performance was observed at 8 days HRT and a corresponding OLR of 5 g VS/L/day, where the reactor condition shifted from methanogenic to acidogenic as a result of the high OLR thus causing a large fall in methane production and a drop in operating pH. Also at this HRT, some operational challenges were encountered as the digester experienced foaming while the NH_3 concentration increased substantially from 2.4 g/L to 3.2 g/L.

Findings at this stage however agree with Mairet *et al.*, (2011) who tested the effect of HRT on the anaerobic digestion of microalgae and reached the conclusions that 20 days HRT being the most optimal for the AD of microalgae in terms of methane yield and digester stability. Therefore, for the co-digestion experiments under the CSTR conditions, an HRT of 20 days was used.

9.2.3 Co-digestion of microalgae and sewage sludge under optimal HRT and OLR in CSTR

At an OLR of 2g VS/L/d and HRT of 20 days, the effect of co-digestion ratios in a semi continuous digester revealed a number of clear relationships between methane yield, methane production, VS destruction and methane content (Table 9.3).

Table 9.3 Effect of operational conditions @2g VS/L and 20 days HRT

Parameter	Digesters (Algae:sludge)				
	0:100	25:75	50:50	75:25	100:0
Specific Biogas L/g VS	0.51± 0.09	0.55± 0.012	0.59± 0.08	0.65± 0.08	0.63± 0.05
Methane Content (%)	65	65	65	64	65
Methane Yield (L/g VS)	0.31	0.36	0.39	0.41	0.41
Alkalinity (mg/L)	7397± 250	7475± 250	6713± 125	7200± 205	7475± 185
Ammonia (mg/L)	1432± 35	1202± 20	1432± 87	1659± 50	1930± 47
VFA (mg/L)	1367± 20	1287± 20	1367± 20	1475± 25	1501± 35
VFA/Alkalinity	0.18± 0.04	0.17± 0.03	0.2± 0.04	0.2± 0.03	0.2± 0.03
pH	7.7± 0.1	7.9± 0.1	7.8± 0.1	7.7± 0.1	7.8± 0.1
% VS destroyed	55	59	60	63	66

±Figures are one standard deviation

The methane yield ranged between 0.31 and 0.41 L CH₄ /g VS_{added} with 100% sewage sludge (0:100) having the least methane yield and the reactor with 75% algae (75:25) having the highest methane yield. 100% pre-treated algae (100:0) also proved to be more significant than sewage sludge and the other two co-digestion ratios in terms of methane yield, with a value of 0.41 L/g VS which falls within the range of methane yield reported in studies on AD of microalgae.

Co-digestion at this stage did not seem to have any effect on the methane content of the respective digesters however, a linear relationship was observed between the addition of microalgae to sewage sludge in terms of methane yield and methane production with $R^2 = 0.871$ and 0.8717 respectively (Figure 9.3). A similar relationship was also observed between algae/sludge ratio and VS destruction (Figure 9.4).

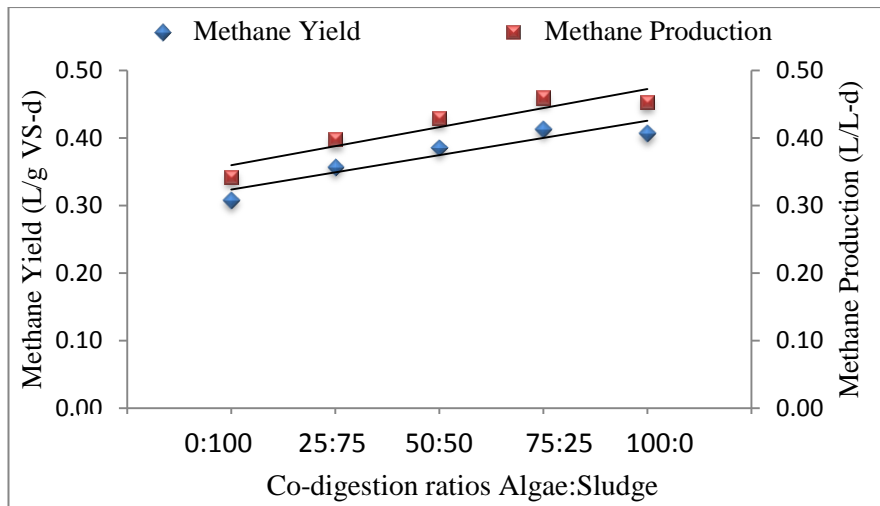


Figure 9.3 Effect of algae/sludge ratio on methane production rate and yield (@ 2g OLR and 20days HRT)

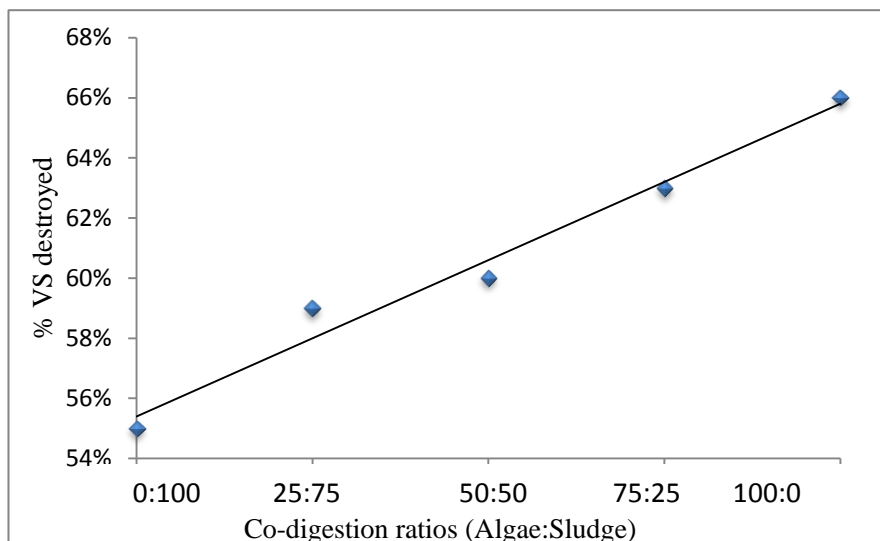


Figure 9.4 %VS destroyed from respective co-digestion

The VS destruction obtained from the 100% sludge digester was 55%: while the VS destruction obtained from 100% pre-treated algae digester was most substantial with a 66% VS destruction. Co-digestion 75:25 had the next highest destruction at 63% VS destruction (Figure 9.4).

The methane yield achievable under this condition was compared with the SMY obtained from the BMP experiments carried out in the previous chapter. Although the CSTR conditions experimented agree with the order of methane yield increase obtained in the BMP experiments, the obtained methane yield superseded that of the BMP experiment up to 14% increase (Table 9.4).

Table 9.4 Comparative Methane yield obtained from CSTR vs BMP

Algae: Sludge	0:100	25:75	50:50	75:25	100:0
BMP results (Previous chapter)					
L CH ⁴ /g VS _{added}	0.29	0.32	0.35	0.37	0.36
CSTR (2g VS/L/d and HRT of 20days)					
L CH ⁴ /g VS _{added}	0.31	0.36	0.39	0.41	0.41

This increased finding in the CSTR experiments agree with the finding of Lesteur *et al.*, (2010) and Rodriguez, (2012) who also observed an improvement in methane yield obtainable during continuous operation of anaerobic digestion. Also the VS destruction and methane content of the biogas obtained in the CSTR study comparatively exceeded that obtained in the BMP experiments.

9.2.4 Study of increasing OLR on optimal/fixed HRT

The impact of increasing OLR at a fixed HRT of 20 days can have on methane yield, methane production and volatile solid destruction was observed for the co-digestion carried out in this experiment. Each co-digestion digester was initially loaded at 2g VS/L^{-d} and were operated to a maximum loading at 6g VS/L^{-d} (Table 9.5).

Table 9.5 Effect of operational conditions on anaerobic co-digestion of microalgae and sewage sludge at 20days HRT and varying OLR (g VS/L)

OLR	Parameter	Digesters (Algae:sludge)				
		0:100	25:75	50:50	75:25	100:0
2	CH ₄ Production	0.34	0.40	0.41	0.46	0.45
	CH ₄ Yield (L/g VS)	0.31	0.36	0.39	0.41	0.41
	VFA/Alkalinity	0.18± 0.06	0.17± 0.03	0.2± 0.03	0.2± 0.06	0.2± 0.05
	% VS destroyed	55	59	60	63	66%
	pH	7.7± 0.1	7.9± 0.1	7.8± 0.1	7.7± 0.1	7.8± 0.1
	NH ₃	1432± 21	1202± 24	1432± 20	1658± 39	1930± 37
3	CH ₄ Production	0.53	0.61	0.66	0.71	0.70
	CH ₄ Yield (L/g VS)	0.32	0.37	0.39	0.43	0.42
	VFA/Alkalinity	0.2± 0.06	0.22± 0.03	0.23± 0.05	0.24± 0.06	0.24± 0.09
	% VS destroyed	55	60	61	63	66
	pH	7.8± 0.1	7.9± 0.2	7.7± 0.1	7.8± 0.2	7.9± 0.1
	NH ₃	1502± 45	1366± 20	1505± 20	1929± 22	2188± 39
4g	CH ₄ Production	0.72	0.82	0.89	0.96	0.94
	CH ₄ Yield (L/g VS)	0.33	0.37	0.40	0.43	0.42
	VFA/Alkalinity	0.22± 0.04	0.22± 0.03	0.21± 0.04	0.22± 0.04	0.22± 0.03
	% VS destroyed	55	59	60	62	64
	pH	7.6± 0.1	7.9± 0.1	7.7± 0.1	7.7± 0.1	7.9± 0.1

	NH ₃	1710± 56	1502± 20	1710± 25	2579± 37	2602± 41
5g	CH ₄ Production	0.87	0.94	0.83	0.96	0.85
	CH ₄ Yield (L/g VS)	0.31	0.34	0.30	0.30	0.31
	VFA/Alkalinity	0.25± 0.04	0.27± 0.04	0.27± 0.06	0.3± 0.06	0.3± 0.04
	% VS destroyed	50	55	56	55	56
	pH	7.2± 0.1	7.1± 0.1	7.3± 0.1	7.0± 0.1	7.0± 0.2
	NH ₃	2580± 35	2086± 70	2210± 48	3317± 35	3177± 28
6g	CH ₄ Production	0.75	0.71	0.78	0.81	0.77
	CH ₄ Yield (L/g VS)	0.23	0.21	0.24	0.24	0.23
	VFA/Alkalinity	0.36± 0.04	0.33± 0.05	0.38± 0.03	0.42± 0.04	0.39± 0.04
	% VS destroyed	42	44	43	38	40
	pH	7.1 ± 0.1	7.1± 0.2	7.1± 0.1	7.0± 0.1	6.9± 0.1
	NH ₃	3214± 52	3026± 35	3372± 45	3778± 40	3716± 55

±Figures are one standard deviation

No significant increase in methane yield was observed by increasing the OLR of all co-digestion ratios studied from 2 gVS/L^d to 4 gVS L⁻¹d⁻¹, however, at 5 gVS L⁻¹d⁻¹ OLR all digesters showed a reduction in methane yield (Figure 9.5).. This was however not so prominent with the 100% and 25:75 digesters but when the digesters were loaded at 6 g VS L⁻¹d⁻¹, an obvious reduction in the methane yield of co-digestion reactors was observed at 30-45% compared to the optimal OLR of 4 gVS L⁻¹d⁻¹ (Figure 9.5). This loading rate suggests the possibility of the newly added feed exceeding the methanogenic growth rate and potentially causing digester instability.

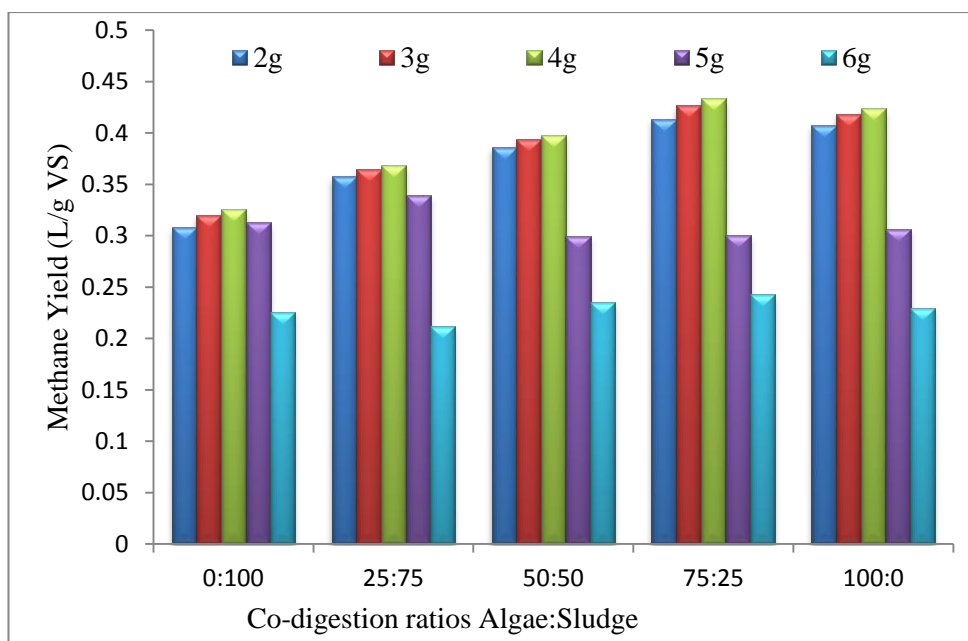


Figure 9.5 Effect of OLR on methane yield of respective co-digestions operated at an HRT of 20 days

Increasing organic loading rate (OLR) up to 4 gVS L⁻¹d⁻¹ had a positive effect on methane production at all co-digestion ratios studied (Figure 9.6). At 5 gVS L⁻¹d⁻¹ a slight increase in methane production was observed in 0:100 and 25:75 (algae:sludge), whilst no increase and in some cases a significant decrease was observed in methane production at the other three (50:50, 75:25 and 100:0) co-digestions studied. At 6 gVS L⁻¹d⁻¹, a more obvious reduction in methane production was demonstrated in all digesters.

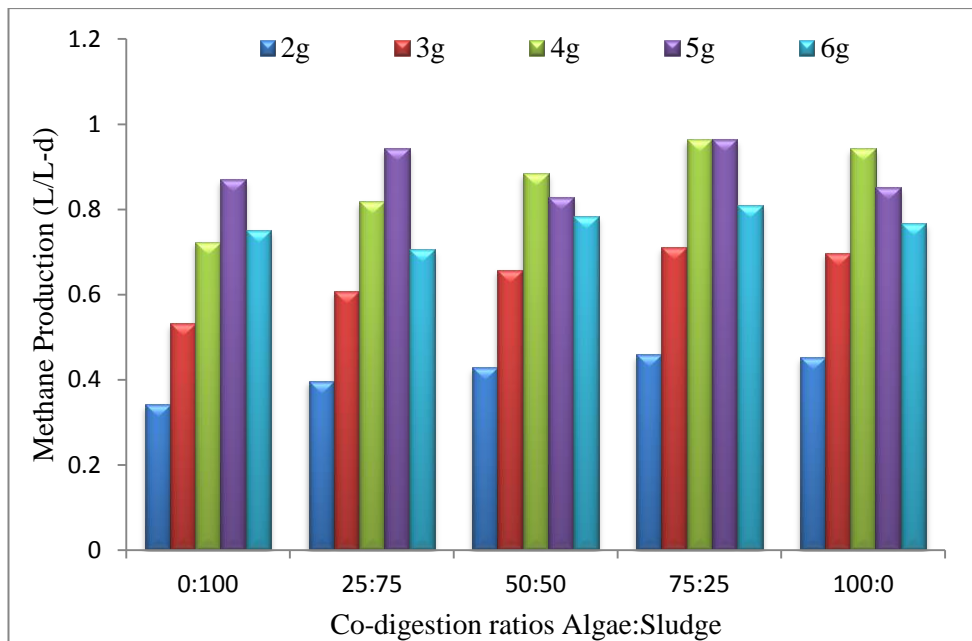


Figure 9.6 Effect of OLR on methane production of respective co-digestions operated at an HRT of 20days

The study showed all digester conditions to be optimal at a loading at 4g VS/L. Nevertheless, loading the digesters at 5 gVS L⁻¹d⁻¹ was more favourable with the 100% sludge and 25:75 (alga:sludge) digesters than the other digesters at this experimental phase. At this loading rate, methane yield of the 100% sludge and 25:75 (alga:sludge) digesters surpassed that of other co-digesters and the methane production rate was competitive with 75:25 (alga:sludge).

Increasing OLR beyond reactor capacity is normally expected to cause a decrease in volatile solids destruction (Menardo *et al.*, 2011) however, no significant difference was observed in VS destruction up to 4 gVS L⁻¹d⁻¹. At 5 gVS L⁻¹d⁻¹, all

reactors demonstrated peak loading with reduced efficiency and reduced VS destruction up to 17%. This reduction was more severe when the digesters were loaded at 6 gVS L⁻¹d⁻¹. (Figure 9.7).

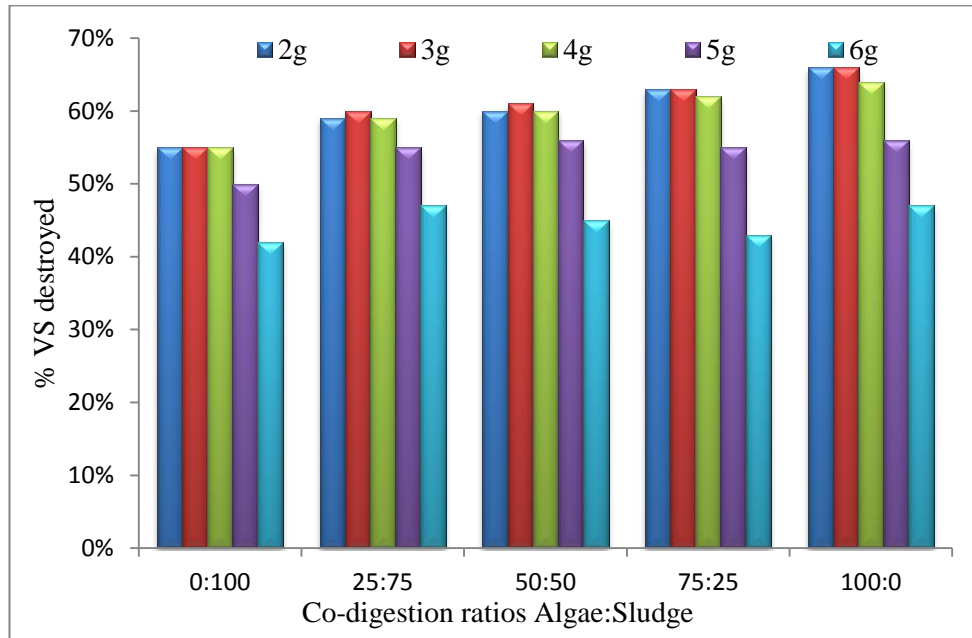


Figure 9.7 Effect of OLR on % VS destroyed of respective co-digestions operated at an HRT of 20days

9.2.5 Digester Stability

Digester stability was monitored using the specific energy loading rate (SELR) (Figure 9.8) which expresses the stability of a digester in terms of the energy conversion rate and the mass of microorganisms and the VFA:Alkalinity which measures the relative proportion of compounds that may act to reduce pH and buffering capacity needed to maintain it.

All the digesters were in the safe zone (< 0.4 for VFA:Alkalinity and < 0.5 for SELR) for all digesters loaded between 2g and 4 gVS L⁻¹d⁻¹. At a loading of 5 gVS L⁻¹d⁻¹ and above, the SELR of digesters exceeded 0.5. This signifies the microbial consortium exceeding its maximum capacity for digestion and thus the rapid reduction in digester performance even though the VFA:Alkalinity was still maintained within the recommended limit.

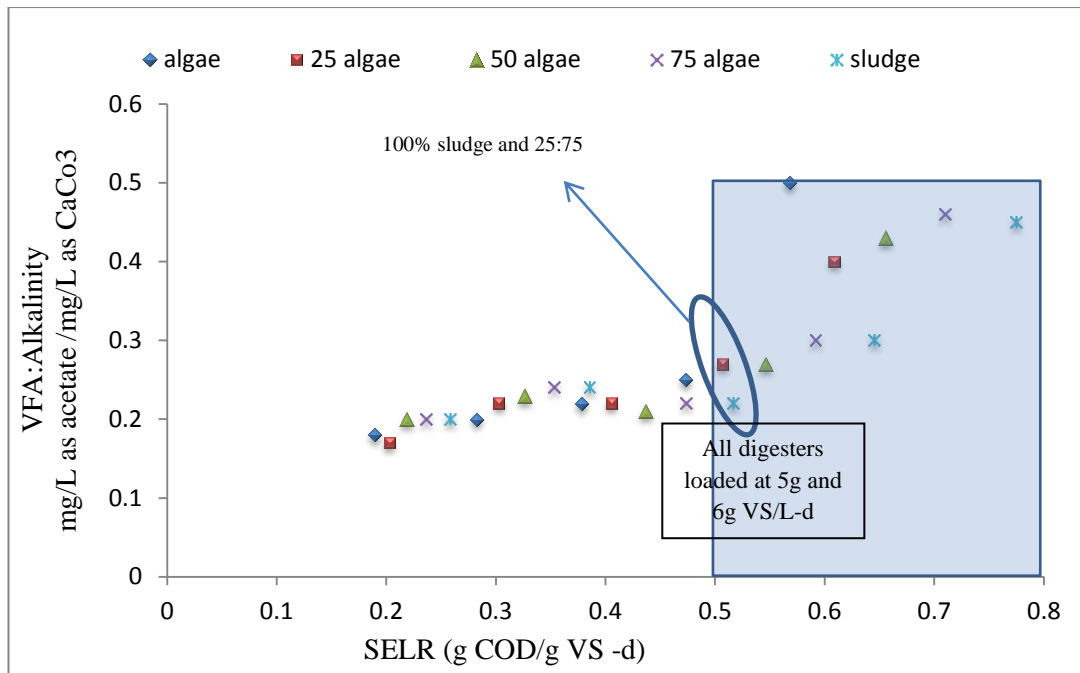


Figure 9.8 SELR and stability of respective co-digestions operated at an HRT of 20days

At 5g VS loading, the 100% sludge and the 25:75 digesters demonstrated a safe VFA: alkalinity of 0.25 and 0.27 respectively but the SELR was just on the boundary with respective values of 0.47 and 0.51. This explains the reason why the reduction in methanogenic activities in these digesters was not severe as the other digesters. At a loading of 6 gVS L⁻¹d⁻¹, all the digesters demonstrated a SELR of up 0.57 to 0.78 with a rapid reduction in SMY, methane production and VS destruction.

9.2.6 Digestate Quality

9.2.6.1 Dewaterability characteristics of microalgae and WAS digestate

The need for dewatering after anaerobic digestion is an inevitable process, thus the necessity to investigate the effect co-digestion of microalgae with sewage sludge can have on the digestate produced.

Comparing the two controls (Figure 9.9), 100% algae showed more effective dewatering up to 2.6 times that of 100% sludge. This improved dewaterability of algae is suggested to be as a result of the thermal hydrolysis pre-treatment carried out on the microalgae used in the study.

This finding was expected as it has been established that thermal hydrolysis aids dewatering of digested substrates (Kepp et al., 2000). The results of dewaterability demonstrated a linear increase in the rate of dewatering in response to increases in the fraction of pre-treated algae, in co-digestion with sewage sludge up to an optimum ratio of 75% algae. This suggests a favourable co-existence between the anaerobic digestion of microalgae and sewage sludge with possible benefits for dewatering of digested products therefore a possible re-use for microalgae cultivation.

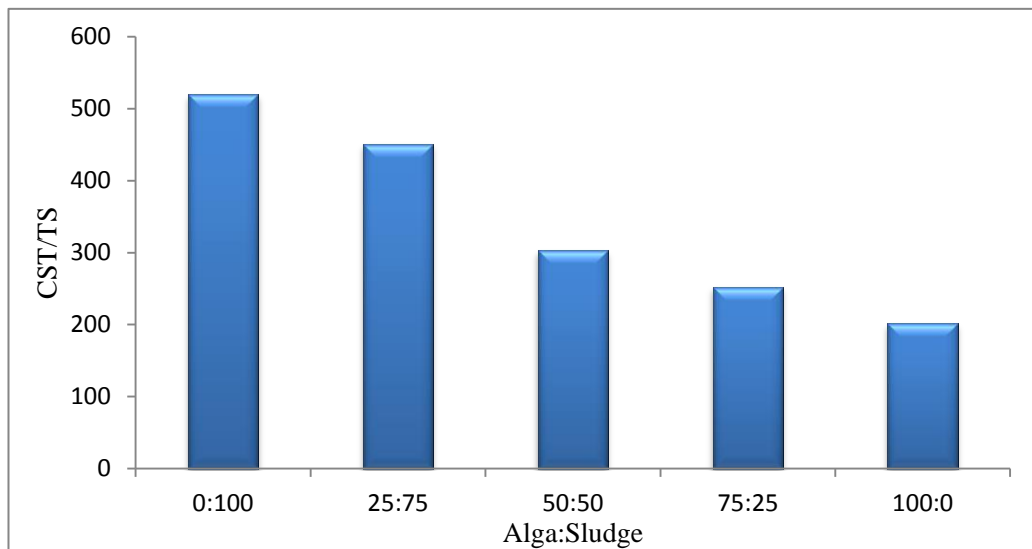


Figure 9.9 Dewaterability at optimal HRT (20days) and OLR (4 g VS/L-d)

9.3 Summary of the chapter

- With a fixed HRT of 20 days, an increase in OLR was favorable for the co-digestion ratios studied up to $4 \text{ gVS L}^{-1}\text{d}^{-1}$, after which increased loading can lead to digester instability. Nevertheless, loading the digesters at 5 g VS was feasible with the 100% sludge and 25:75 (alga:sludge) digesters compared to the other ratios studied.
- Co-digesting at 75:25 (algae: sludge) based on VS content proved to be most optimal for all OLR studied, with the most optimal performance at to $4 \text{ gVS L}^{-1}\text{d}^{-1}$ having a corresponding CH_4 yield of $0.43 \text{ L/g VS}^{-\text{d}}$ and productivity of $0.96 \text{ L/L}^{-\text{d}}$.

- Although, co-digestion results obtained in a conventional semi CSTR agreed with the BMP co-digestion results, the advantage of a continuous digester to yield improved methane yield compared to BMP was identified with an increased yield up to 14% increase for all co-digestion ratios studied.
- The digestate quality of the respective digesters showed that the addition of microalgae to sewage sludge was favorable with a linear increase in dewaterability up to an optimum ratio of 75% algae.

Chapter 10 The carboxylate approach

10.1 Introduction/Background

Anaerobic digestion of sewage sludge was originally used to stabilise sludge prior to recycling to agricultural land, and thus prevent offensive odours and in this role it has been in place for over a century. But more recently its focus has shifted to the generation of renewable energy whilst still preserving its original role (DEFRA, 2012).

Anaerobic digestion achieves conversion of the organic fraction of biomass to biogas through the action of anaerobic bacteria. Furthermore, the use of AD in the water industry is a very simple process using a mixed culture of bacteria to convert the waste into biogas; the process generally requires no energy input for sterilization and is stable, robust and inexpensive. The energy produced in the form of biogas is of benefit considering its easy separation from the liquid slurry thus making the process more cost efficient (Tchobanoglous *et al.*, 1991).

Although producing biogas/methane for the water industry is a relatively beneficial approach, there is currently a shift of focus into how economical biomass conversion could be if other liquid biofuels such as alcohols, could be produced instead of simply biogas (Agler *et al.*, 2011). There are many metabolic pathways able to produce stable sources of energy, biofuels and other alternative compounds with higher values from conversion of organic wastes (Agler *et al.*, 2011), as compared to methane production which is a relatively low-value compound (Kleerebezem and van Loosdrecht, 2007; Holtzapple and Granda, 2009).

This alternative approach, known as the carboxylate platform, is more attractive, since for example, the market value of biofuels obtained from the alternative pathway may worth up to 4 times more that methane production and this yield may be obtained in less than 7 days as opposed the biomethanation which takes up to 15 - 20days (Mairet *et al.*, 2011).

Furthermore, the carboxylate platform is now becoming mainstream in the UK with the “House of Lords” report suggesting that the bioeconomy is going to play a major part in delivering economic and environmental opportunities for the UK (HOUSE

OF LORDS, 2014). Nevertheless, to realise the full potential and associated opportunities of this approach, the government, industry and academia are encouraged to increase focus on the approach.

The objective at this experimental stage was to identify and evaluate the possible benefits of co-digestion of microalgae and sewage sludge under the carboxylate approach might offer. This is with the ultimate intention to produce results which can compare with the anaerobic co-digestion process for methane production. Findings from this study will help understand and open for further studies, the possible benefits associated with producing more valuable biofuels (using the co-digested substrates) in a shorter time.

10.2 Results

The results obtained from the co-fermentation of microalgae and sewage sludge study are divided into three sections (10.2.1, 10.2.2 and 10.2.3):

10.2.1 Optimal Iodoform determination

From a 31 days experiment the results obtained in the batch fermentation study were compared in terms of yield, productivity and VFA composition (Table 10.1). A comparison of the achievable VFA yield under the two iodoform concentrations (3 mg/L and 10 mg/L) revealed a clear disparity (Figure 10.1)

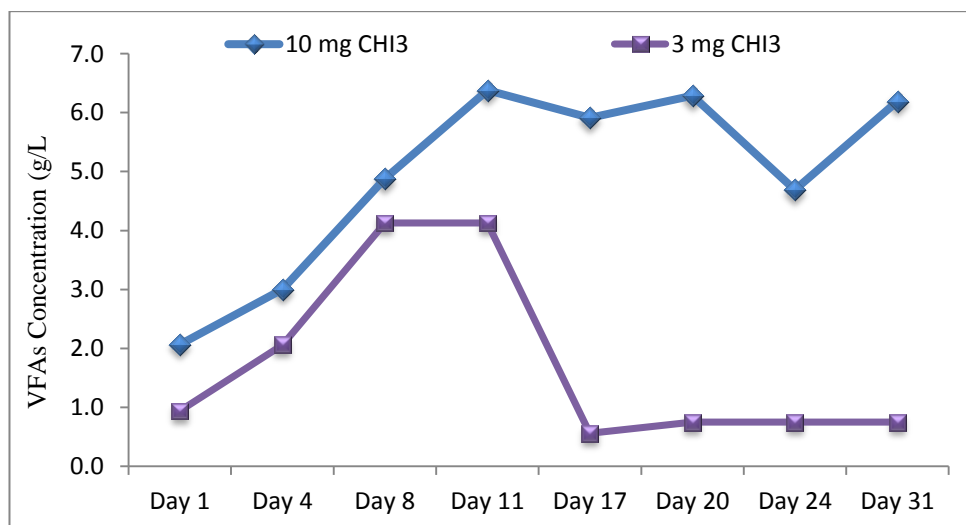


Figure 10.1 VFA concentration for WAS at the tested iodoform concentrations (3mg/L and 10mg/L)

Table 10.1 Average result for batch anaerobic fermentation study for sludge 1 and sludge 2 (3 mg/L and 10 mg/L CHI₃ respectively)

	Day 1		Day 4		Day 8		Day 11		Day 17		Day 20		Day 24		Day 28	
	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2	Sludge 1	Sludge 2
Acetic acid (mg/L)	0.43	0.87	1.27	2.05	2.73	3.78	2.85	4.94	0	4.75	0.43	4.95	0.43	3.16	0.34	4.14
Propionic acid (mg/L)	0.26	0.62	0.4	0.51	0.72	0.62	0.75	0.81	0	0.5	0.07	0.48	0.07	0.24	0	0.3
Isobutyric acid (mg/L)	0.03	0.072495	0.11	0.1	0.2	0.12	0.21	0.16	0	0.11	0.25	0.12	0.25	0.09	0.41	0.12
Butyric acid (mg/L)	0.12	0.26	0.09	0.14	0.1	0.17	0.03	0.224	0	0.28	0	0.43	0	0.86	0	1.2
Isovaleric acid (mg/L)	0.04	0.09	0.14	0.11	0.29	0.12	0.28	0.15	0.55	0.13	0.00	0.14	0.00	0.11	0.00	0.16
Valeric acid (mg/L)	0.06	0.14	0.06	0.10	0.08	0.08	0.02	0.10	0.01	0.14	0.00	0.17	0.00	0.23	0.00	0.29
Other compounds (mg/L)	0.08	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.00
Total acid concentration (g/L)	1.02±0.26	2.06±0.39	2.06±0.39	3.0±0.39	4.13±0.38	4.88±0.38	4.13±0.26	6.38±0.26	0.56±0.20	5.91±0.39	0.61±0.20	6.29±0.39	0.76±0.20	4.69±0.27	0.75±0.20	6.19±0.39
Total acid productivity (g/L·d)	1.02	2.06	0.52	0.75	0.52	0.61	0.38	0.58	0.03	0.35	0.03	0.31	0.03	0.20	0.02	0.20
Yield (g total acid/g VS fed)	0.15	0.29	0.29	0.43	0.59	0.70	0.59	0.91	0.08	0.84	0.09	0.90	0.11	0.67	0.11	0.88

While the highest VFA concentration at 3 mg iodoform concentration was 4.12 g/L, it was subsequently reached within the first 8 days of the experimental run. After day 11, VFA concentration reduced to about 0.7 g/L which remained fairly constant until the end of the experiment (Figure 10.1).

This VFA reduction suggests the conversion of the product into other forms which in this case was biogas. To accurately delineate the process conversion, gas analysis was carried out on the fermenters and biogas composition showed a relatively high concentration of methane (65%) in the biogas (Figure 10.2). This suggests the presence and activities of methanogenic bacteria at an iodoform concentration of 3mg/L and thus this is not inhibitory.

At an increased iodoform concentration of 10mg/L, a higher VFA yield of 6.4g/L was obtained. This yield was an increase of 54% over the yield obtained at the lower iodoform concentration. Unlike the 3mg/L iodoform concentration, the highest VFA yield was achieved on day11 after which it stayed relatively stable till the end of the experiment. This suggested inhibition of the methanogens thus preventing VFA conversion.

As a result of the limited literature available on the carboxylate approach, a comparison with the results in the study existing was limited. The closest research work with WAS under this platform was the work of Rughoonundun *et al.*, (2010) who observed the effect of lime pre-treatment times (0-240mins) on VFA productivity with obtained concentrations ranging between 5.45 - 10.72 g/L. The experimental conditions vary as alkaline pre-treatment was used in their research and the fermentation experiments were carried out under thermophilic conditions unlike our study where mesophilic conditions were applied with thermal hydrolysis pre-treatment. Also a higher loading rate of 50 g/L dry solid content was performed in their study as against the 7g VS/L employed in our study.

Biogas analysis was carried in the study to confirm the inhibition of methane production. Biogas composition of the initial study (3 mg/L iodoform) was basically CO₂ and CH₄ while the higher iodoform (10 mg/L iodoform) influenced biogas composition which was in this case mainly hydrogen and CO₂ (Figure 10.2).

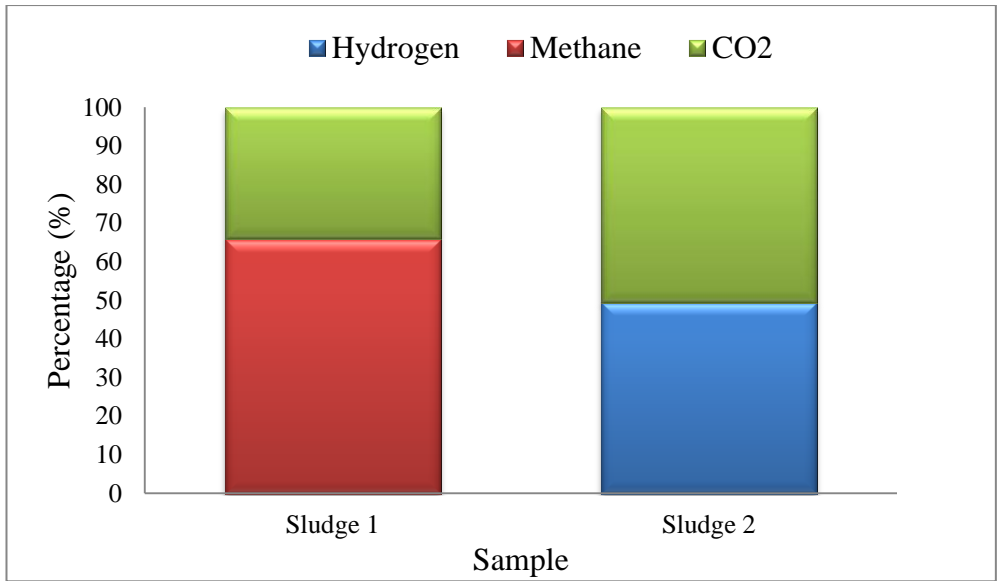


Figure 10.2 Average Biogas analysis for WAS acid fermentation

10.2.1.1 Effect of iodoform concentration on VFA composition

The VFA composition of the two concentrations studies were also compared and contrasted. From the 3 mg/L iodoform experiment, a range of acids were recovered with varying concentrations (Figures 10.3).

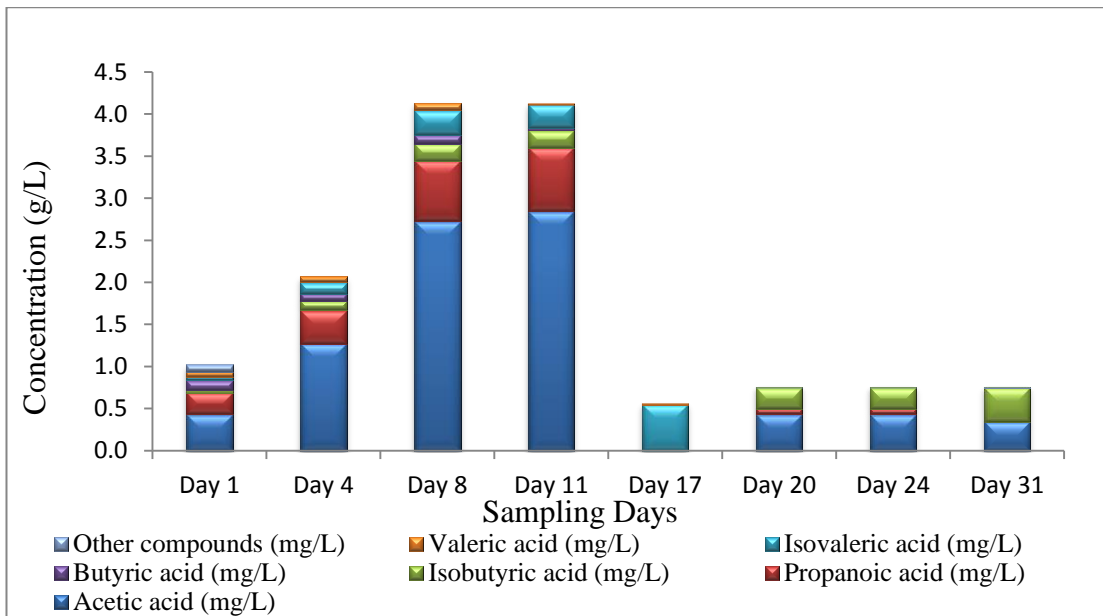


Figure 10.3 VFA composition of batch fermenter for sludge 1 (3mg/L CHI3)

Of these acids, acetic acid proved to be dominant reaching its highest concentration at days 8 and 11 with respective concentrations of 2.73g/L and 2.83g/L. Throughout the experiments, acetic acid dominated with its percentage concentration ranging between 45 and 69%. Nevertheless, the absence of butyric, isovaleric and valeric acids were noticed on days 24 and 31. Day 17 also showed no acetic, propionic, isobutyric and butyric acids present.

Analysis of the VFA produced at 10mg/L iodoform concentration was also investigated (Figure 10.4). The proportion of the range of acids obtained in the study in which acetic, propionic and butyric acids dominated in that order. All through the experiments, acetic acid maintained dominance with its percentage concentration ranging between 41 and 81% which aligns with the findings from the lower iodoform experiment.

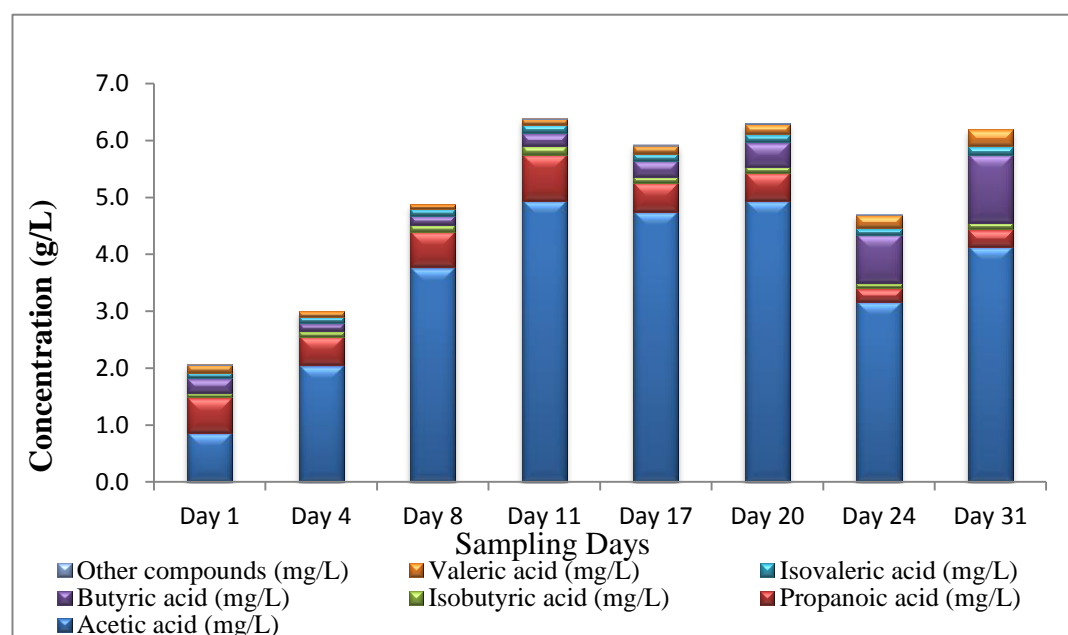


Figure 10.4 VFA composition of fermenter for sludge 2 (10mg/L CHI_3)

The highest acetic acid concentration was produced at day 11 with a concentration of 5.11g/L amounting to 80% of the total produced acids. This is similar to literature values (Rughoonundun *et al.*, 2010; Forrest *et al.*, 2012; Golub *et al.*, 2013). From the VFA analysis, it can be seen that propionic acid concentration increased gradually up to day 11 after which it began to decline. Butyric acid on the other hand gradually increased until the end with a final percentage concentration of 19.5%.

10.2.2 Co-digestion experiments using optimal iodoform concentration

Having identified an optimal iodoform concentration capable of inhibiting methanogens and simultaneously favour the VFA production of sewage sludge in our study, this optimal concentration was adopted for studying a range of co-digestions between microalgae and sewage sludge (Table 10.3). The co-digestion ratios studied were similar to the ratios carried out for the BMP and continuous MAD (0:100, 25:75, 50:50, 75:25, 100:0) of algae:sludge based on the VS content.

Table 10.2 Performance data of anaerobic fermentation during a 31 days batch test

	Algae:Sludge					
	Control	Sludge	25:75	50:50	75:25	100:0
Average pH	7.1± 0.2	6.9± 0.2	6.8± 0.2	7.0± 0.2	7.3± 0.3	6.9± 0.2
Alkalinity	6974±132	6015±1	6509±	6605±	8785±	9050±
Total acid concentration (g/L)	4.13± 0.39	6.38±	6.66±	6.94±	6.01±	6.57±
Total acid productivity (g/L•d)	0.243	0.13	0.39	0.39	0.13	0.26
Time of maximum acid concentration (d)	0.243	0.580	0.392	0.631	0.300	0.328
Yield (g total acid/g VS fed)	17	11	17	11	20	20
Acid concentration (g in the fermenter)	0.590	0.911	0.952	0.992	0.858	0.938
VS digested (g/d)	1.651	2.552	2.665	2.777	2.402	2.627
Selectivity (g total acid/g VS digested)	-	1.82	1.86	1.78	1.81	1.84
Conversion (g VS digested/g VS fed)	-	1.401	1.433	1.560	1.327	1.428
	-	0.650	0.664	0.636	0.646	0.657

±Figures are one standard deviation

10.2.2.1 Effect of co-digestion on VFA concentration

It is evident that the control experiment reached its peak concentration of 4.12g by Day 17 after which it remained relatively constant till the last day of the experiment with a final value of 3.37g. The 50:50 co-digestion experiment produced the highest VFA concentration amongst all co-digestions studied of 6.94g by day 11, peak VFA concentration was also observed for 100% sludge at day 11.

All other experiments reached their respective peak concentrations at Day 20, except for 25:75 which demonstrated peak concentration at Day 17. This showed that co-

digestion could impact the residence time under this platform in no particular order, as all the studied ratios reached peak at different days (Figure 10.5).

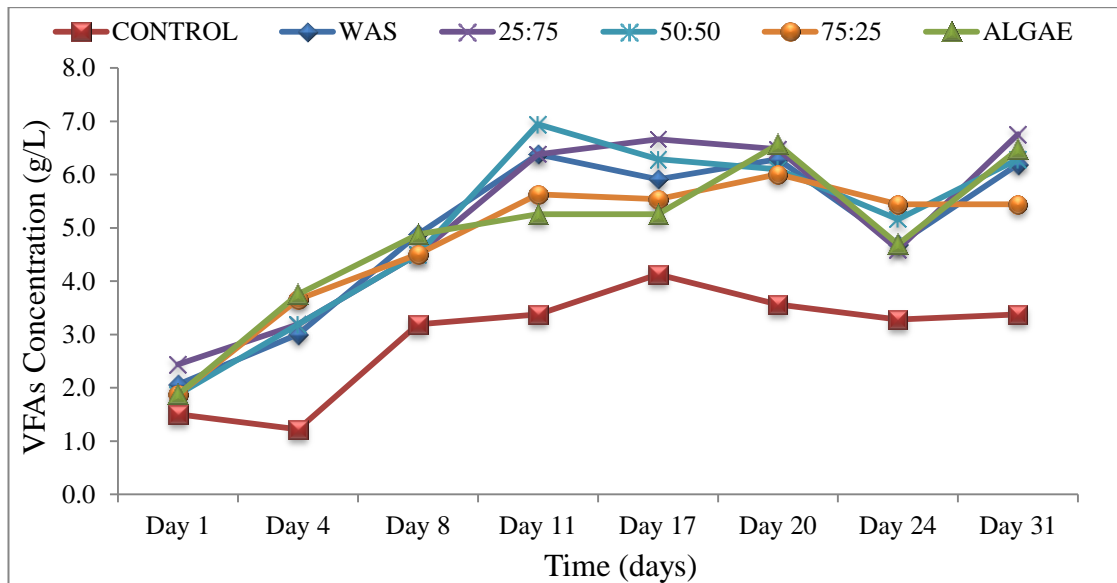


Figure 10.5 Average VFA concentrations for the respective co-digestions studied

Biogas analysis was carried out in which findings showed complete inhibition of the methanogens as no methane was detected in the biogas produced.

10.2.2.2 Effect of co-digestion on VFA composition

From the co-digestion experiments, a similar trend of VFA composition was recorded over the experiment time for all co-digestion ratios. All fermenters demonstrated a range of acids including valeric, isovaleric, butyric, isobutyric, propionic and acetic acids all of which with varying concentrations (Figure 10.6).

Of these acids, acetic acid proved to be the most dominant reaching its highest concentrations on day 11. At this day, acetic acid concentrations in all reactors ranged between 4.2 and 5.12 g/L with the highest obtained in the 50:50 fermenter while the 100% algae fermenter demonstrated the lowest percentage acetic acid concentration. All through the experiments, acetic acid was dominant with a concentration ranging between 45 and 82%.

Propionic acid in all fermenters also showed a similar trend with a relative increase up to day 11 after which percentage composition began to decline till the end of the

batch experiments. In contrast to propionic trend, the percentage composition of butyric acid in all fermenters increased gradually till the last sampling day.

Overall, co-digestions studied showed a similar trend of acids composition, suggesting that the operating conditions play a major influence on the VFA composition, indeed more than the feedstock itself. The pattern of VFA percentage/composition in this study was also similar to that observed in the available literature of Forrest *et al.*, (2010), where similar operating conditions were applied.

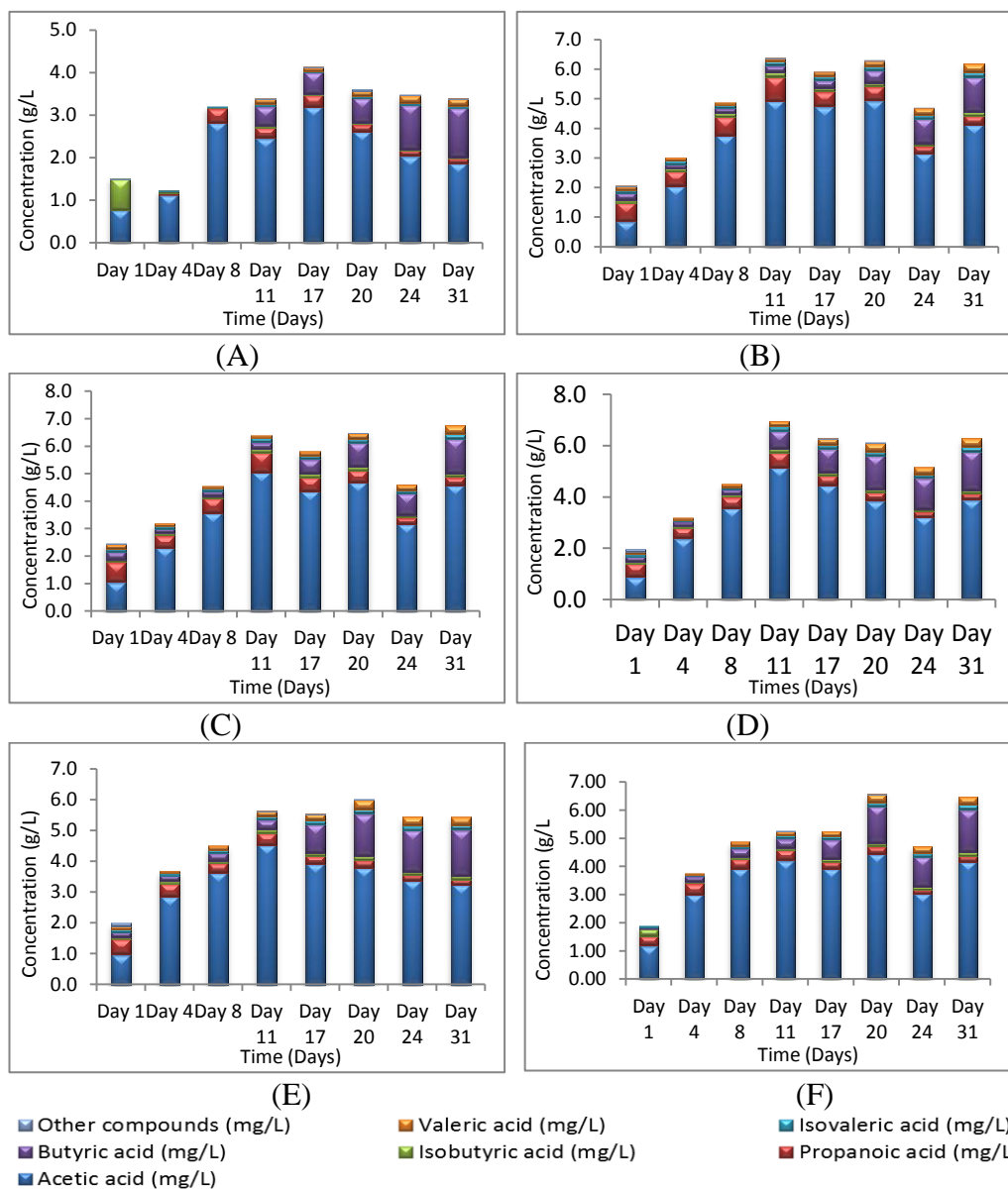
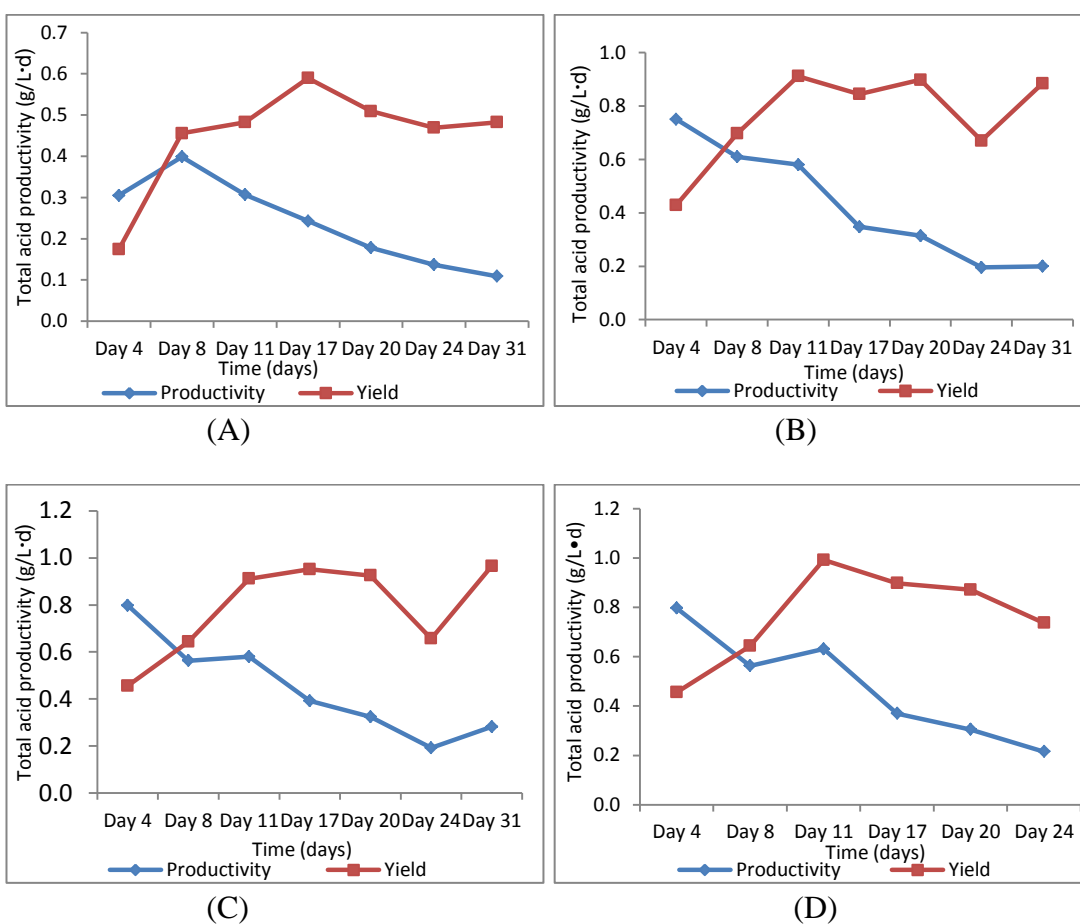


Figure 10.6 VFA composition of respective fermenters (A: control, B: WAS, C:25:75, D:50:50, E: 75:25 and F: algae)

10.2.2.3 Effect of co-digestion on correlation between productivity and yield

The correlation between total acid production against time as well as the VFA yield in respect to time for all studied co-digestions was used to analyse the performance of the batch fermenters in this study (Figure 10.7).

All fermenters demonstrated a reduction in VFA production with respect to time demonstrating the utilization of the degradable portion of the fed substrates. Nevertheless, while the VFA production declined, VFA yield (acids/g VS added) increased in all fermentation experiments. This inverse relationship between productivity and the yield aligns with other studies (Chan and Holtzapple, 2003).



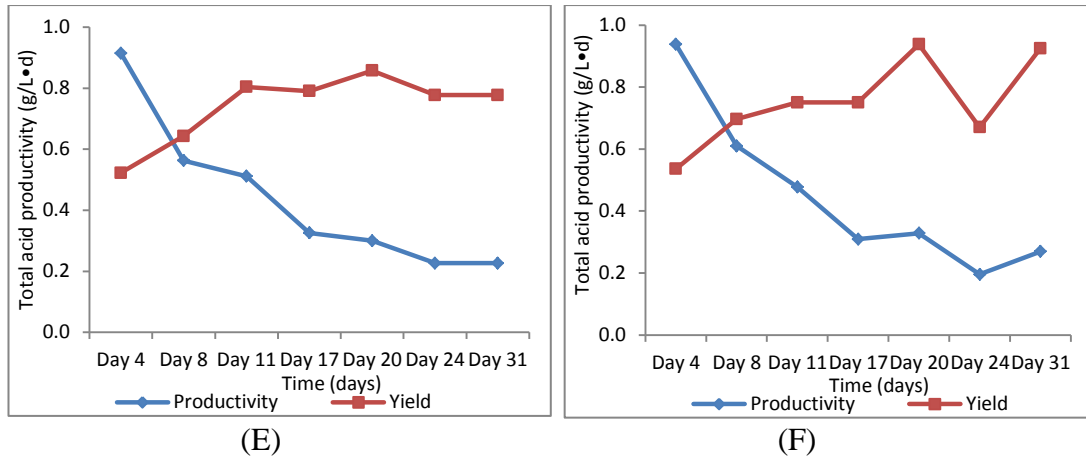


Figure 10.7 Correlation of Total acid productivity Vs Yield (A: control, B: WAS, C:25:75, D:50:50, E: 75:25 and F: ALGAE)

The VFA yield for all the co-digestions ranged between 0.86 and 0.99 g acid/g VS with the highest yield obtained in the 50:50 co-digestion and the lowest obtained from the 75:25 experiment. Since no similar literature is available in the category studied (i.e. microalgae and sewage sludge), the yield obtained in this literature was compared to the initial review (Table 3.3).

The yields in the review ranged between 0.04 and 0.55 g acid/g VS and when comparing this to the yield obtained in this study, which ranged between 0.86 – 0.99 g total acid/g VS, it is clear that the feedstock and the method of pre-treatment adopted in this study have made a large improvement.

10.2.3 Comparison between BMP and Fermentation Experiments

This section compares the results from the carboxylate experiments with those obtained from the AD experiments.

10.2.3.1 VFA yield comparison

Comparing the VFA yield obtained from the initial BMP experiments to the peak VFA yield in the carboxylate study, an improvement in VFA yield of the latter was observed at all co-digestion ratios. They demonstrated an increase between 92 and 166% with the lowest increase reported on the 72:25 while 25:75 showed the highest comparative increase (Figure 10.8).

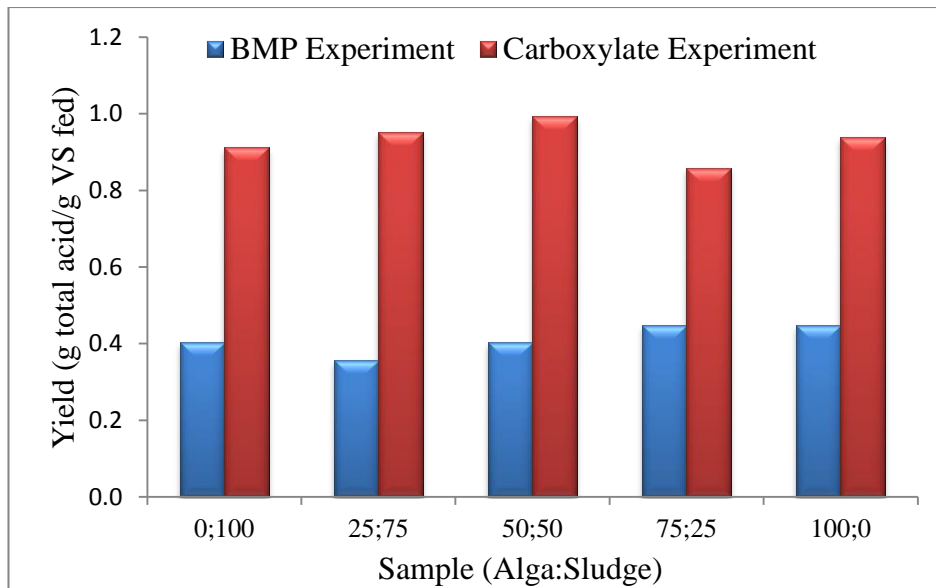


Figure 10.8 Comparison of VFA yield (BMP Experiment vs Carboxylate Study)

It was also observed that the highest VFA yield obtained in the BMP experiments was obtained at 75:25 with a corresponding yield of 0.45 g total acid/g VS fed. This ratio yielded the lowest VFA of 0.86 g total acid/g VS fed under the carboxylate platform. This suggests the possible effect operational parameters can have on bio product yield.

10.2.3.2 Digestate Quality

The fermentation broth from the experiments were analysed for dewaterability using the CST test.

Comparing the dewaterability (Figure 10.9) from the carboxylate experiment which ranged between 163 and 464 CST/TS, to that obtained in the BMP experiment (201 and 520 CST/TS), it is clear that the carboxylate had a better dewaterability tendency between 10 and 19% less the BMP dewaterability values. This could be as a result of the increased amount of volatile solids tested in the carboxylate experiment.

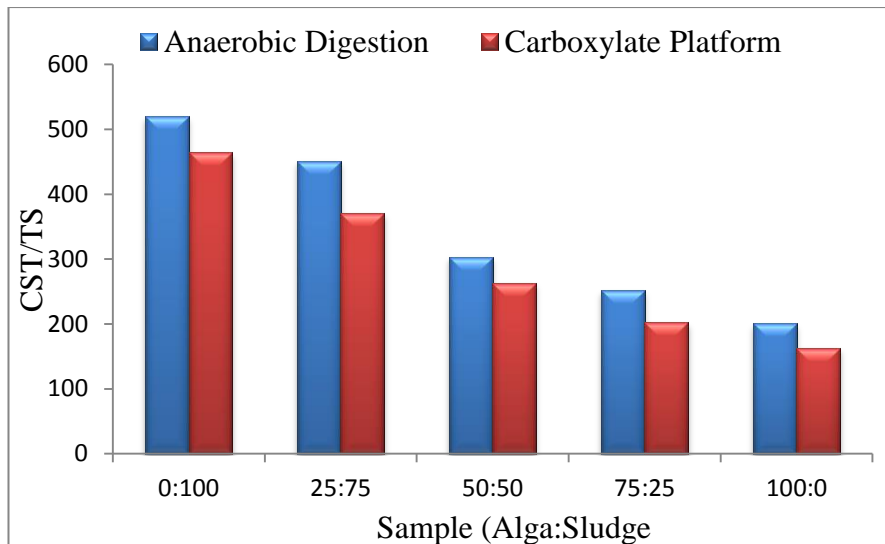


Figure 10.9 Dewaterability of the anaerobic fermentation experiments

10.3 Summary of the chapter

- Two iodoform concentration (3 and 10 mg/L) were studied for effective methanogenic inhibition. Findings suggest the higher iodoform concentration as effective while the lower concentration proved insufficient.
- Under the co-digestion experiment, peak VFA concentrations ranged between 6.01 and 6.94 g/L. The highest VFA concentration was produced in the 50:50 (alga:sludge) fermenter with a corresponding yield of 0.99 g total acid/g VS fed, this was seconded by 25:75 (alga:sludge) with a yield of 0.95 g total acid/g VS fed and least performance was obtained in 75:25 with a corresponding yield of 0.86 g total acid/g VS fed.
- All reactors reached peak production at different days, with 25:75 having the lowest retention time of 11 days and 75:25 having a retention of 20 days. This suggests that the liquid retention time for VFA production of codigestion is expected to vary between 11 and 20 days in a continuous stirred tank reactor (CSTR).
- All co-digestions studied showed a similar pattern of acids composition, suggesting that the operating conditions play a major influence on the VFA composition, indeed more than the feedstock itself.

- Comparing the carboxylate study and the AD route, increase in VFA yield was obtained from the fermentation experiments with an increase between 92 and 166% for all tested co-digestion ratio.
- Anaerobic fermentation of WAS and microalgae at several co-digestion ratios to produce VFAs may be an alternate option to methane production.

Chapter 11 Integral analysis of energy recovery with MAS

11.1 Introduction

Considering the advantages of microalgae which include: ability to utilize nutrients from wastewater; carbon sequestration potential; ability to utilize waste heat; biomass production and bioenergy/biogas potential, there is no doubt that the integration of microalgae into the existing wastewater treatment flow sheet should offer a beneficial approach with the prospect of improving both the economic and environmental sustainability within the industry (Graham *et al.*, 2009; Johnson and Wen, 2010).

But for the proposed integration of microalgae into wastewater treatment, it is essential to identify and clarify possible concerns and benefits with regards to the sustainability of the intended approach over the conventional route, as this is a prerequisite to achieving a sustainable solution via the integrated approach. Some of the possible hypothetical designs of microalgae integration into the existing flow sheet for the water industry have been extensively documented (Sahu *et al.*, 2013). A combination of these scenarios has however been adopted for the proposed integration in this study thus the mass and energy balance of the approach will be essential for a cost benefit analysis.

In anaerobic digestion systems for WWTP, energy is generally chosen as the unit to analyse the respective roles as it is the most significant and prominent product of the digestion process, coupled with the fact that it allows other flows related to the AD benefits. This net energy is however dependent on the specific configuration, which in turn is determined by site-specific conditions as described by Pereira, (2009).

The study has considered the direct and indirect benefits that may be associated with the systems. Some of the direct benefits include the emission savings when fossil fuels use are replaced, while the indirect benefits which may be the most important (Börjesson and Berglund, 2007) include handling of organic by-products. Other indirect benefits include the possible use of digestate produced as soil conditioner,

which directly benefits plants and can lead to further recycling (Berglund and Börjesson, (2006).

This section aims to demonstrate some of the possible benefits of integrating microalgae into the existing WWTP to benefit from the existing infrastructure and to contribute to sustainability in terms of renewable energy production, reduction of CO₂ footprint and nutrient re-use using a proposed scenario (Figure 1.1). This will review the feasibility of the hypothetical design of microalgae integration system well as provide some energy balance and economic analysis.

11.2 Proposed integration and assumptions of the co-digestion of microalgae and sewage sludge

Although this study focuses on the use of the existing facilities in a typical UK WWTP it is accepted that complementary units may be required particularly for microalgae cultivation using photobioreactors. Thus, these additions to the existing configuration/flow need to be considered (Figure 1.1).

Since the intended co-substrate (microalgae) will be produced within the WWTP, the boundary conditions in this case are limited to the environmental regulations. Although all the materials (apart from the microalgae) used in the study were obtained from Mitchell Laithes Dewsbury, UK, serving a population up to 244,000 people however, a different WWTP (Esholt WWTP, UK) was used as a reference model for this analysis.

This was on the grounds of size, available data and existing infrastructure including the thermal hydrolysis reactors, which may support some of the assumptions to be made for the proposed energy balance. The initial assumptions in this study include:

- The WWTP was assumed to be the size of ESHOLT, UK serving a population of approximately 700,000 and currently treating approximately 80 tonnes dry sludge per day (Yorkshire Water, 2014). Assuming the unutilized digester headspace is estimated to about 20% which is the average in the UK (WMW, 2012) it signifies the possible addition of 20 tonnes (dry solids) of feedstock per day.

- The co-digestion of microalgae and sewage sludge will take place within the existing treatment works (Figure 11.1 A & B).

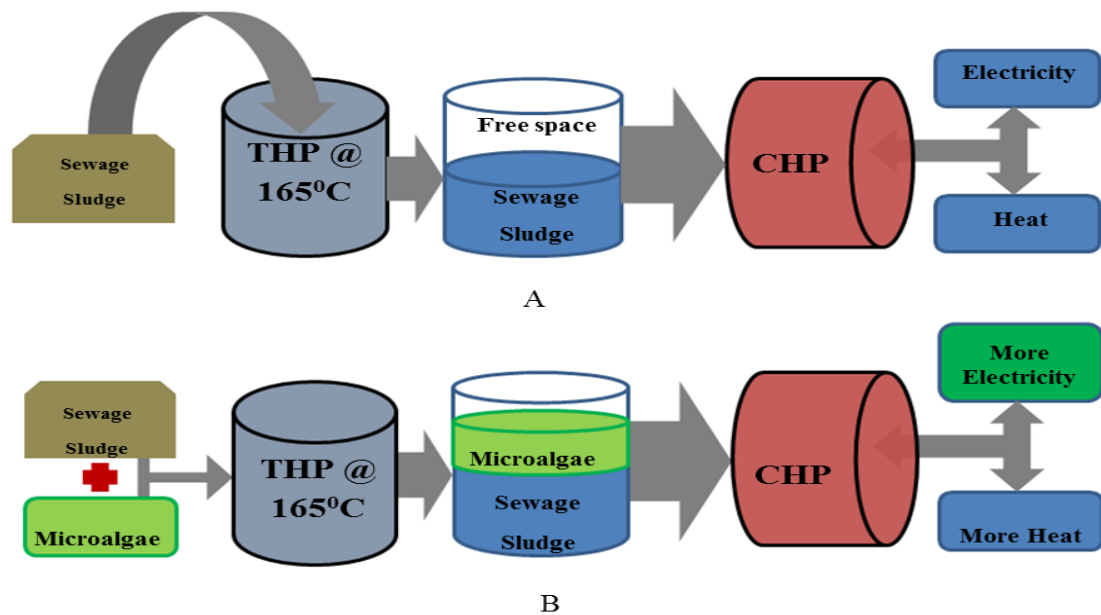


Figure 11.1 (A) Existing flow sheet for energy production; (B) integration of MAS

- Several conditions may influence the ratio of microalgae and sewage sludge produced in an integrated system, some of these include: configuration, available nutrients or CO₂ availability (Sahu et al., 2013). Assuming the possible achievable ratio for the integration will be a factor of the available space in the digester, moisture content of the algal sludge, and most importantly algal biomass yield achievable within the integrated system. Therefore the most minimal co-digestion ratio 25:75 based on VS was assumed to avoid possible overestimations of energy output.

Pre-treatment (THP): Pre-treatment of substrates entering the digester is a necessary process which enhances the substrate solubilisation, degradation and overall methane yield. This facility is usually established in the UK water industry, thus it was assumed to be used for microalgae cell wall disintegration and enhancement of methane yield as observed in chapter 7.

Anaerobic Digester/ Biogas Upgrading: Pre-treated substrates are fed into the digester which operates at a mesophilic temperature and an HRT of 20 days. It is anticipated that the extra digester space from this will be utilized by microalgae from

the integrated process. Biogas upgrade using the existing CHP will also be used to process the additional biogas produced from the AD of microalgae.

Nutrient Recovery: Digestate produced from the AD is a rich nutrient source containing nitrogen and phosphorus. Potential use of this includes struvite formation which may be used as a fertiliser, however, the mode of recovery assumed for this integration was for the re-use by microalgae to produce biomass which will then be digested. Therefore, the nutrients are recycled within the system. Alternatively, the excess N & P produced in the system may be processed for struvite formation.

Microalgae cultivation: This is anticipated to be the biggest challenge. For example, assuming biomass production is 25gVS/m^2 per day (90 tonnes ha⁻¹yr), to produce a substantial amount of microalgae to fit the suggested integration, land requirement and achievable yield of microalgae must be accurately quantified. Nevertheless, it is anticipated that existing facilities in the UK WWTP may offset some of the cost for microalgae cultivation as the basic growth requirements include CO₂, N and P and light (for photosynthesis) and are readily available within the WWTP. Therefore, infrastructure will have to be considered for the integration which includes photobioreactor installation and maintenance. Other associated cost/energy associated with cultivation will include harvesting. Thickening/concentration may also be carried out using the existing techniques for sludge dewatering.

11.3 Mass and Energy Balance of the Proposed Integration

The mass balance based on the organic loading to the digesters was analysed to determine the efficiency of the process in converting the organic waste into the final products.

Organic matter loaded into the methanogenic reactor based on VS content showed a percentage of organic matter conversion into biogas while the remaining organic matter passed out as effluent (Figure 11.2).

The mass balance for the digester loaded with 100% algae assumes the conversion of 64% of the substrate into biogas with the remaining 36% passing out in the nutrient rich effluent. The reason for the high conversion of substrates into biogas is however as a result of the thermal hydrolysis treatment on the microalgae used in the study.

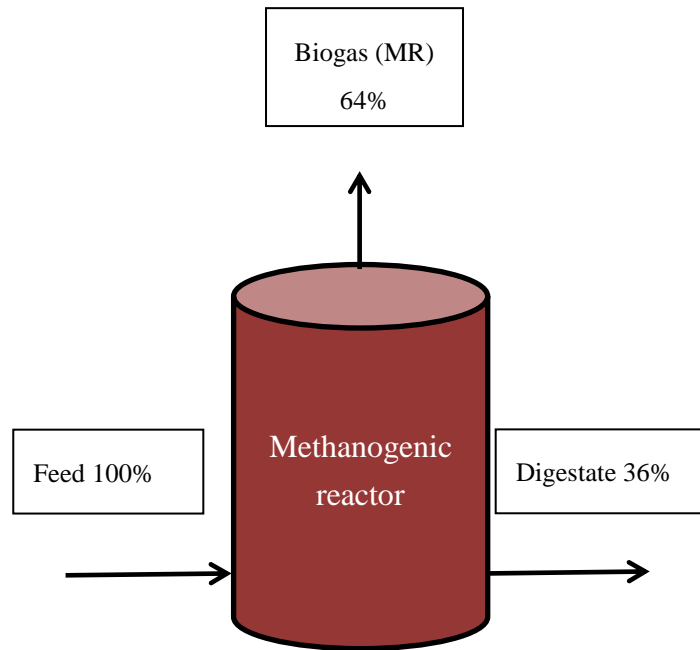


Figure 11.2 Organic matter balance for the anaerobic digestion of the 100% algae digester

Likewise, the mass balance of other co-digestions studied were observed (Table 11.1). Results obtained suggested that the 100% algae had the highest substrate conversion 64% into biogas while sewage sludge had the least organic transfer to biogas with 55% conversion. The addition of pre-treated microalgae biomass to sewage sludge let to a proportional increase of substrate conversion to biogas up to an optimum ratio of 75:25 (alga:sludge).

Table 11.1 Organic matter balance for the anaerobic digestion of the co-digestion ratios

		Algae:Sludge		0:100		22:75		50:50		75:25		100:0	
		g/vs	%	g/vs	%	g/vs	%	g/vs	%	g/vs	%		
Input	Substrate	144	100	144	100	144	100	144	100	144	100	144	100
Output	Biogas from MAD	79	55	85	59	86	60	90	62	92	64		
	Digestate from MAD	65	45	59	41	58	40	54	38	52	36		

11.4 Possible global energy balance for a WWTP with MAS (Microalgae systems)

Finding a balance between energy expended on the proposed integration of microalgae into wastewater treatment and energy produced from the overall process is essential for evaluating the commercial feasibility of the applied

technique/integration which is aimed at enhancing sustainability via anaerobic digestion performance and methane yield.

The main requirement for feasibility in adopting the proposed integration will be to ensure that the energy output (E_o) outweighs the energy input (E_i) using (Equation 11.1). The main energy output in this context is in the form of methane (E_m) while additional output energy may be recovered in the form of nutrients (E_n). In respect to the energy input E_i , this will include: substrate pre-treatment (E_{sp}), digester operation (E_{do}), biogas post treatment (E_{bp}), digestate post treatment (E_{dp}), and additional energy input associated with development of photobioreactors and maintaining different operational units (E_{dm}).

$$E_{AD} \left(\frac{MJ}{Kg} \right) = (E_m + E_n) - (E_i + E_{sp} + E_{do} + E_{bp} + E_{dp} + E_{dm}) \quad \text{Equation 11.1}$$

11.4.1 Energy Outputs

Methane energy yield (E_m): The achievable methane yield may be calculated from BMP experiments using the total yield/g VS of substrate added. The actual methane production is a fraction of the ultimate yield which is dependent on the process efficiency. Process efficiency may however range between 45 and 85% in a CSTR, but from this study, efficiency averaged 60%. Therefore, total energy from methane production is described by equation 11.2.

$$E_m \left(\frac{MJ}{Kg} \right) = VS \left(\frac{Kg VS}{Kg} \right) * BMP \left(\frac{m^3 CH_4}{Kg VS} \right) * Eff(\% \text{ BMP}) * 35 \left(\frac{MJ}{m^3 CH_4} \right) \quad \text{Equation 11.2}$$

Energy in nutrient (E_n): The re-use of nutrients produced in AD facilities in agriculture is the current norm for recycling/re-use. The energy gained via this approach is estimated between 2 and 8% of the energy content in the biogas produced (Berglund and Börjesson, 2006). Depending on the technique adopted for re-using the nutrients, there may be variation in the E_n . Specified energy content for nutrients (N&P) is equivalent to 45MJ/kg and 29 MJ/kg for N and P fertilizer production respectively according to Hofman *et al.*, (2011)

11.4.2 Energy Inputs

Energy for substrate pre-treatment (E_{sp}): This accounts for the energy expended on the thermal hydrolysis for substrate conditioning. Since similar TS (<10%) is reached for microalgae as well as sewage sludge, it is assumed that same energy will be utilized thus, additional pre-treatment of microalgae will require additional electricity input corresponding to 33MJ per tonne of raw material (Berglund and Börjesson, 2006). Surplus energy required for drying also need to be included thus it will be fair to assume double the pre-treatment input (66 MJ per tonne) for drying. Total energy input for substrate pre-treatment will be 99MJ per tonne of raw material.

Energy for digester operation (E_{do}): Energy expended on digester operation is dependent on the plant type and the substrate treated within the facility. Nevertheless, the main energy consuming aspect will include pumping, mixing and heating. Equation 11.3 below accounts for the energy required for the main energy consuming aspects (Climenhaga and Kapoor, 2009). The electricity demand for the pump is a factor of pump type/power P [kW] and the time dedicated to this labour, t_p [h/day], Energy expended on the mixer is a factor of the digester type and volume V [m^3] and is depicted E_{mx} [W/m^3] which ranges between 2.5 and 6.5 in a typical CSTR. Heating of a digester is a factor of the digester thickness, insulating material used, influent flow and temperature, temperature difference between the air and the ground with respect to the digester and finally the cross sectional areas within them (Rodriguez, 2012)

$$E_{do} = \left(P[kW] * t_p \left[\frac{h}{d} \right] * 3.6 \left[\frac{MJ}{kWh} \right] \right) + \left(V[m^3] * 5 \left[\frac{W}{m^3} \right] * 0.864 \left[\frac{MJ}{Wd} \right] \right) + \left(\left(0.265 \left[\frac{j}{m^3 s^{\circ}C} \right] * A_{air}[m^2] * \Delta T_{air} [^{\circ}C] + 0.235 \left[\frac{j}{m^3 s^{\circ}C} \right] * A_{ground}[m^2] * \Delta T_{ground}[^{\circ}C] \right) * 0.0864 \left[\frac{MJs}{j d} \right] + (C * Q * \bar{O} * \Delta T_{influent}) \right) \quad \text{Equation 11.3}$$

In the above equation, 3.6 MJ/kWh and 0.0864 MJ/W/day are conversion factors, the co-efficient of heat transfer [$J/s.m^2.^{\circ}C$] are 0.265 and 0.235, the area of the digester in contact with air or ground is represented as A in m^2 , while ΔT [$^{\circ}C$] represents the temperature change between the air, the influent and the temperature needed to be

maintained in the digester. The specific heat of influent C in a CSTR is equal to the specific heat of water 4.187×10^{-3} MJ/kg/°C, Q [m³/day] is the influent flow and ρ [kg/m³] is the density of the influent. In a typical mesophilic CSTR digestion of degradable wastes values for energy demand as a function of energy yield for pumping, heating and mixing are 0.03%, 1.1% and 6.6% respectively (Climenthaga and Kapoor, 2009). According to Berglund and Börjesson, (2006), heating and electricity demand for a large scale biogas plant is 110MJ/tonne and 66MJ/tonne respectively.

Biogas post treatment (E_{bp}): This is employed for upgrading (removal of CO₂, H₂S and H₂O) of the produced biogas and the desired quality is dependent on the intended usage. In a typical WWTP, the produced biogas is burnt in a CHP unit to produce heat and electricity. These units operate at an efficiency of 85-90% i.e. 35% electricity and 50% heat (Haefke, 2009). According to Berglund and Börjesson, (2006) the primary energy input in large-scale upgrading plants is assumed to correspond to 11% of the energy content in the biogas produced, furthermore, the methane loss via upgrading is usually neglected as this is considered small and not able to influence the net energy output from the biogas systems.

Digestate post treatment (E_{dp}): Handling, disposal and possible use of the digestate depends on several factors, these include: characteristics of the substrate, quality of the digestate produced (TS, VS, nutrients and toxicants) and the intended use.

Regardless of the disposal route, dewatering is a necessary step accounting for energy uptake of up to 10MJ/tonne digestate (Berglund and Börjesson, 2006). Subsequently, the solid digestate may be used as soil conditioner or biocompost while the liquid portion is sent back to the beginning of the treatment. This however encompasses a huge amount of nutrient which adds to the amount of energy expended on wastewater treatment however it is proposed in the study that this liquid digestate will be employed for microalgae production thus offsetting some of the energy expended in the process. Excess N and P from the process may also be used for other processes for struvite formation.

Energy input into logistics (E_l): this category includes the amount of energy spent on transport and storage. Considering that microalgae is produced within the

WWTP, transport energy may be neglected nevertheless, some of energy will still be expended on substrate handling and storage. Some of the literature energy consumptions for truck transport range between 0.5 and 4.5 MJ/tonne/km (Berglund and Börjesson, 2006; Pereira, 2009).

Energy in photobioreactor development and maintenance (Ed_m): This is meant to vary depending on the intended plant and technology to be adopted. Using an Austrian AD plant and an amortizing period of 25 years as a reference, a total energy of 100 MJ/tonne may be expended on construction and maintenance (CROPGEN, 2006; Rodriguez, 2012).

For the integration of microalgae system (MAS) into the WWTP flow sheet, the construction of photobioreactors would be required thus, a significant impact on energy required for construction and maintenance is expected. Some of the anticipated energy uses from the integration are highlighted (Table 11.2). Based on this it seemed reasonable to assume that only 30% of the total energy demand of a full plant will be channelled in this direction.

Table 11.2 Possible energy demand with microalgae integrated systems

Use	Energy	Reference
Mixing photobioreactor contents and anaerobic digestion	250kWh/d	(Sturm and Lamer, 2011)
Pumping/ bubbling CO ₂ into the system	1350kWh/d	
Harvesting using chemicals for coagulation and flocculation or Dissolved air floatation (DAF)	84 - 3150kWh/d	
Dewatering using belt filter press	340kWh/d.	
Total	2024 - 5090kWh/d	

11.5 Energy performance in the proposed AD facility

A summary of the inputs for energy applied and gained with the integration of microalgae to benefit from the existing infrastructures in the WWTP is analysed (Table 11.3). This highlights the possible values and main variables influencing the calculations however, it is essential to note that although the values are reasonably justified, they are only indicative since the review is not extensive.

Subtracting the total input from the obtainable energy in the table above gives the possible energy balance as expressed below:

$$E_{AD} = 3495 - 1569 = 1926 \left(\frac{MJ}{tonne} \right) \quad \text{Equation 11.4}$$

Based on the assumption/scenario proposed in this study, it could be inferred that a positive energy balance may be achieved with the integration of microalgae to benefit from the existing infrastructure as well as the unutilized digester headspace (Equation 11.4). From the study, each tonne of microalgae digested in a WWTP would potentially lead to an energy gain of 1926 MJ (535 kWh).

Table 11.3 Energy outputs and inputs for a WWTP with microalgae systems (MAS)

	Value (MJ/tonne) ^a	Variable/method for calculation
ENERGY OUTPUT		
Energy from Methane	3108	CSTR Experiment, reactor efficiency (obtained from this study)
Energy from nutrients	387	Nutrient content (N&P), nutrient demand
ENERGY INPUT		
Substrate pre-treatment (E _{sp})	99	Assuming equal energy for sludge treatment (Berglund and Börjesson, 2006) + energy spent on drying.
Digester operation (E _{do})	311	Using similar energy for pumping, mixing and heating (Rodriguez, 2012)
Biogas post treatment (E _{bp})	231	Assuming 11% of the energy content in the biogas produced (Berglund and Börjesson, 2006; Rodriguez, 2012)
Digestate post treatment (E _{dp})	10	Assuming similar energy is expended on dewatering of the solid digestate (Berglund and Börjesson, 2006) and the liquid effluent is used for microalgae cultivation.
Logistics	77	Assuming 22 km of transportation (2 km within microalgae cultivation site and 20 km to the point of digestate re-use) (Rodriguez, 2012)
Photobioreactor development and maintenance (E _{dm})	841	Assuming 50% of new infrastructure (Rodriguez, 2012) + energy for pumping, mixing and heating

^a values expressed per tonne of raw microalgae

The obtained net energy ratio (E_o/E_i) under the scenario will be 2.2 and this falls slightly short of literature range obtainable for the digestion of organic wastes which ranges between 2.5 and 5 (Berglund and Börjesson, 2006) and is as a result of the energy expended on microalgae cultivation and harvesting. Also the majority of the energy output is from the methane produced accounting for 89% while the energy in nutrients was 11%. The most energy intensive aspect of the process was the photobioreactor development and maintenance followed by the digestion operation and the biogas pre-treatment. These processes are however not negligible. Nevertheless for the intended integration, it is essential that this analysis be validated using data referring to the specific local conditions.

Asides from the above analysed scenario, other possible scenarios were analysed to identify the possible obtainable energy ratio. The scenarios assumed different proportions of microalgae and sewage sludge (50:50 and 75:25 respectively) produced with the integrated process. All energy inputs were not altered however, the influence of respective co-digestions on methane output and digester efficiency was put into consideration (Table 11.4).

Table 11.4 Energy ratio obtained from alternative scenarios

Scenarios	Energy ratio
Co-digestion ratio of 50:50 (alga:sludge)	2.4
Co-digestion ratio of 75:25 (alga:sludge)	2.5

This confirms that for any co-digestion ratio explored for sewage sludge with microalgae integration systems, a positive energy balance may be achieved.

11.6 Energy performance in the proposed AD facility for carboxylate production

For the carboxylate experiments, the intended product was alcohols however, despite the trace presence of alcohols including ethanol and butanol, acetic acid dominated the reactors. The presence of alcohol showed the possible conversion of these acids to alcohols with the provision of favourable conditions.

The conversion of this acids generally requires a high hydrogen partial pressure, however, laboratory regulation restricted the use of this gas. Also, it is expected that

with the right configuration, hydrogen may be produced in the reactor (Zhang *et al.*, 2006) thus making the anaerobic fermentation self-sufficient.

Nevertheless, the energy balanced based on the acetic acid concentration was determined using similar assumptions made in Table 11.3. Slight assumption was made to the energy output by replacing the methane yield the acetic yield, also same amount of energy was assumed for VFA separation from fermentation broth as energy expended on biogas post treatment. This gave an energy gain value of 3536 MJ/tonne (982 kWh) per ton of microalgae.

Furthermore, assuming a 70% conversion of the produced acetic acid to ethanol, and that same energy expended on biogas pre-treatment is substantial for the carboxylate separation from the fermentation broth (Table 11.3); each tonne of microalgae digested in a WWTP would potentially lead to an energy gain of 6654 MJ (1848 kWh).

Under this approach, improved NER will be obtained for the production of alternative biofuel using a modified AD system with values ranging between 6.9 and 7.8, therefore highlighting a significant improvement over the traditional AD route of the baseline scenario studied.

The possible energy gain obtained per tonne of microalgae in this study ranged between 1926 and 6654 MJ/t dry algae depending on the final product targeted. This was then compared to the study of Aresta *et al.*, (2005) who studied the integration of macroalgae to generate biomass and biofuel from wastewater effluent. Their net energy was calculated using an LCA approach with the best scenario demonstrating a net energy yield of 11,000 MJ/t dry algae.

Comparatively, the energy gain obtained in our study falls a bit short however, the lower gain in this study is mainly as a result of the energy conversion technology which in their case was biodiesel production using supercritical CO₂ as solvent for extraction. Other possible reasons is suggested to be a result of the intensive energy expended on microalgae cultivation and harvesting. Also, it is worthy to mention that macro algae were used in their study as against this study that explored the use of microalgae *Chlorella vulgaris*.

11.7 Environmental Impact

The environmental impact of a particular digestion process is a function of the conditions governing the substrate, digester type, downstream processing of the biogas/digestate and the possible reference system replaced (Rodriguez, 2012)

It is known that direct benefits are associated with the AD process, which include: safe and ecological waste management, monetary value from the output from the process, reduction of waste to landfill thus reducing environmental costs amongst many other benefits (Edelman 2005).

Nevertheless, in this particular case, some of the direct benefits obtainable from the digestion of microalgae (per/tonne) in a typical WWTP include:

- Additional methane production of 370m³ per tonne microalgae with an energy value of 3108 MJ (863 kWh), or alternative production of acetic acid and ethanol with an energy value of 4718 MJ (1310 kWh) and 7836 MJ (2184 kWh) respectively.
- Therefore an existing plant with the assumed baseline scenario would gain 1926 MJ (535 kWh) of net energy. The anaerobic fermentation route to produce acetic acid and ethanol will however lead to a profit of 3536 MJ/tonne (982 kWh) and 6654 MJ (1848 kWh) respectively per ton of microalgae.
- Indirect energy credits may also be associated with the proposed integration. For example, recycling N and P in wastewater produces energy gains because of the avoidance of N fertiliser which is usually energy intensive (45kJ/g N) and (29kJ/ g P) (Hofman et al., 2011)

11.8 Economic analysis

Using the baseline scenario adopted for the research coupled with the obtained data from the energy balance, the cost benefit analysis may be developed (Table 11.5).

From the results, it could be inferred that with the integration of microalgae into the a WWTP with capacity similar to Esholt benefiting from the existing facilities,

which include thermal hydrolysis, nutrients in wastewater and the unutilized digester capacity, the water industry would generate additional revenue from renewable energy representing £15,622 per annum (per tonne microalgae produced). Alternatively, the production of acetic acid and ethanol using the modified AD approach may yield to revenue in the range of £28,703 and £53,990 respectively.

Table 11.5 Cost-benefit analysis for anaerobic digestion with MAS

	Anaerobic Digestion (Methane Production)	Anaerobic Fermentation (Acetic acid Production)	Anaerobic Fermentation (Ethanol Production)
Costs (£/year)	12,702 ^a	12,702 ^a	12,702 ^a
Revenue (£/year)	28,324 ^a	41,405 ^a	66,692 ^a
Balance (£/year)	15,622	28,703	53,990

^a Calculated using the conversion 8p/kWh electricity

This analysis is made for each tonne of microalgae produced and digested within the existing WWTP, thus for the industry as a whole enormous financial benefits could be reaped.

Other indirect economic benefits associated with the integral system include the possible re-use of nutrients (N&P) in the liquid phase of the digester (which is usually added to the influent load) thus offsetting some cost expended on wastewater treatment. Moreover, other incentives exist in which the integrated system stand to benefit from, this include: renewable obligation certificates (ROCs), feed-in tariffs (FITs) and renewable heat incentives (RHIs). Therefore it is suggested that the integration and co-digestion of microalgae using the existing WWTP infrastructure may present a feasible and cost effective approach for adoption in the UK WWTP (Johnson and Wen, 2010).

11.9 Opportunities and restriction for microalgae integration systems

Having identified the feasibility in terms of energy balance and cost benefit analysis, it is essential to highlight some of the possible opportunities and challenges that may be faced in the UK as a result of the proposed integration.

Opportunities

- One of the simplest and principle opportunities from this approach includes the fact that most of the infrastructure necessary for microalgae growth and conversion to energy already exist and are well developed thus, an algal production unit is the only retrofit unit needed to be put in place and will require no elaborate time for installation (Knud-Hansen, 1998).
- The integrated system can play a significant role in carbon recycling thus helping to meet the UK GHG emission targets.
- The co-digestion of pre-treated microalgae with sewage sludge suggests a beneficial approach which demonstrates a linear increase in methane yield up to an co-digestion ratio of 75% algae.
- Utilization of the digester's extra capacity can increase the energy and renewable heat production. The heat produced from the MAS may be used to warm up the digesters and photo bioreactors to optimal temperature while benefiting from associated RHI.

Restrictions:

- The amount of microalgae produced should be adequately estimated as this is a main determinant of the feasibility. Relatively low production may suggest other alternative approaches, such as additional co-digestions with screenings which is estimated at about 10 tonnes per day for the size of the baseline scenario used in the study.
- The integration of MAS requires a great deal of technical expertise for design and maintenance of the process as it is essential to identify the optimal configuration which will put into account challenges that may occur in managing the growth conditions and culture compositions.
- During microalgae cultivation, harvesting techniques prove energy intensive in addition to its efficiencies of 88% signifying a considerable amount of biomass lost. This will require more research and will positively influence the energy balance.

- Some challenges exist with the carboxylate approach; some of these include improving the final concentration, productivity and yield. Also complete inhibition of the methanogens at large scale will require in-depth study, as well as separation and purification cost of the final products.

Chapter 12 General Discussion

The global water industry has attracted attention as a result of the energy expended in treatment with its associated carbon footprint. As a result many regulators including those in the European Union have set targets aimed at reducing these. Whilst increasing renewable energy efficiency measures within the sector has the potential to make a contribution, at present only about 8.5% of its total energy use is produced from renewable sources, mainly through combustion and anaerobic digestion (EA, 2009). It is generally accepted that wastewater treatment companies must look to more innovative options if they are to increase their renewable energy generation and achieve 25% of total energy use from renewable source (STW, 2010).

The water industry has been operating for over a century and so new technologies that outdate existing facilities, which have also not reached the end of their life span are unlikely to be readily embraced. But the need for a proactive change to increase the energy production achievable using the existing facilities is a priority. It is therefore proposed that enhancing existing technologies as well as embracing innovation is one of the keys for achieving EU target on Carbon emission and overall sustainability intended for the water industries (EEWWT, 2008).

Thus the primary aim of the research was to investigate the possibility of enhancing energy production without making significant modifications to the existing configurations, which include thermal hydrolysis employed for sludge treatment and anaerobic digesters which have been operated for many years and are well understood.

A potential opportunity was identified in the form of digester over capacity, which highlights an opportunity for increasing energy production within the wastewater treatment works through the use of additional feed substrate, which is readily biodegradable and has a high carbon content to support co-digestion with sewage sludge.

Various substrates including food waste and farm yard manure meet these criteria amongst many others (Luostarinen *et al.*, 2009; Zuhaib *et al.*, 2011; Neczaj *et al.*, 2012), however they fall under a different regulatory framework and require a Waste

Management Licence and must meet “end of waste criteria” before recycling to land, compared to the regulatory regime based around the Safe Sludge Matrix that is followed by the water industry.

The prospects of using photosynthetic microalgae highlight a mutualistic input as this microorganism show possible integral benefits into the wastewater treatment flow sheet such as its advantage of taking up nutrients from wastewater, utilizing available CO₂ source and waste heat produced for biomass production which may be further utilised with the digester headspace to increase substrate intake of the digesters and the overall methane production. Moreover, microalgae are effectively energy crops (not waste) and so are not limited by the existing legislative framework.

The methane yield from the *Chlorella* studied was 0.26 L CH₄/g VS_{added}. A comparison of this obtained yield to that obtained from the sewage sludge used in this study 0.273L CH₄/g VS_{added} suggests that raw microalgae can at least produce the same amount of methane as sewage sludge thus highlighting its potential for anaerobic digestion.

Although the methane yield of the *Chlorella* in this study is similar to that obtained from Ras *et al.*, (2011) who demonstrated the optimal methane yield of *Chlorella vulgaris* to be 0.24 L CH₄/g VS_{added} at a 28 days HRT. Our yield also falls within the literature range 0.09 – 0.341 L CH₄/g VS_{added} (Milledge and Heaven, 2014). The low VS destruction 44% suggests possible inhibitions of the process as a result of cell wall presence. Similarly, Tran *et al.*, (2014) studied the achievable methane yield of a microalgal mixed culture in a semi continuous digestion at OLR between 2 and 3.5 to achieve CH₄ yield between 0.13 and 0.141 and a low VS destruction of 30%. This poor performance is however attributed to the recalcitrant nature of the microalgae biomass studied. Other similar studies confirming the impact microalgal cell wall can have on anaerobic digestion process have also been documented (Ras *et al.*, 2011; González-Fernández *et al.*, 2012)

Thus for a full exploitation of the energy potential of microalgae by-passing the resistive tri-laminar cell wall has been proposed (Golueke *et al.*, 1957). Although pre-treatment can help by-pass the cell wall, there are concerns regarding the

unjustifiable amount of energy expended in pre-treating microalgae to increase methane yield (Sialve *et al.*, 2009; Mussnug *et al.*, 2010). Thus, it was intended to investigate the effect of the thermal hydrolysis process currently used for sewage sludge conditioning prior to digestion. This was envisaged to help enhance the possible biodegradation of the substrate of interest as well as minimally affecting its energy balance or cost as the facilities are already in use at UK WWTPs.

Overall, thermal hydrolysis pre-treatment studied demonstrated an increase in SMY by 35% compared to untreated *Chlorella* with an overall increase in the methane yield of microalgae from 0.265 to 0.357L CH₄ /g VS_{added} suggesting the new yield to be more attractive and competitive with other organic wastes such as FYM, food waste etc. (Chynoweth *et al.*, 1993; Gunaseelan, 2004).

The energy balance suggests that the amount of energy expended on pre-treatment (thermal hydrolysis) was justifiable as the input was less the output with a range of energy ratio obtained (E_i/E_o) of 0.25. The finding in this study proved energy positive nevertheless, it is essential to note that the energy analysis carried out in this study only assumed E_i based on the VS content with assumptions that the existing facilities used for sludge dewatering will be employed for microalgae concentration at minimal cost, also the excessive heat produced by WWTP may be used to achieve the solid contents needed for microalgae concentration/pre-treatment

Having identified the possibility of adopting the existing thermal hydrolysis to enhance microalgae hydrolysis and energy yield of alga biomass, utilizing digester head room and increasing methane production was the next priority. Thus co-digestion of microalgae and sewage sludge was considered with the primary objective of identifying the best co-digestion ratio between pre-treated algae and sewage sludge.

To date, only a few studies have shown the possibility of co-digesting microalgae and sewage sludge. While some have suggested no positive benefits/influence with the addition of microalgae (Wang, 2013), some have identified an optimal ratio 12 - 15% algae for optimal performance to achieve improvement in methane yield, volatile solid destruction and sludge dewatering (Yuan *et al.*, 2010; Olsson *et al.*, 2013). Furthermore, no research has investigated the possible benefits achievable

from co-digesting pre-treated microalgae with sewage sludge. This is expected to increase substantially as pre-treatment brings the microalgae to a competitive standard in terms of methane yield. Thus, further experiment was carried out in a Biochemical Methane Potential Test (BMP) to investigate the optimal co-digestion ratio between microalgae and sewage sludge.

Findings showed a positive relationship with the co-digestion of algal sludge (*Chlorella vulgaris*) and sewage sludge as the proportion of pre-treated algal sludge added to the sewage sludge led to a direct proportional increase in overall methane yield ($R^2 = 0.87$). Methane yield achieved with the co-digestions studied surpassed the yield obtainable from sludge digestion ($0.360 \text{ L CH}_4/\text{g VS}_{\text{destroyed}}$) up to 34% with 75:25 (alga:sludge) having the highest yield of $0.484 \text{ L CH}_4/\text{g VS}_{\text{destroyed}}$. This benefit is as a result of the disintegrated cell wall of the pre-treated microalgae.

Having obtained the optimal co-digestion ratio between microalgae and sewage sludge, it is also essential to scale this up using a lab scale CSTR to appreciate what operational conditions can affect the performance of these substrates. The targeted operational conditions to be evaluated were the HRT and the OLR. Based on a literature review of similar wastes, most studies ranged between 2 and $6 \text{ kg VS}/\text{m}^3\text{d}$ for the OLR, while the HRT ranged between 8 and 25 days (Kim *et al.*, 2006; Mairet *et al.*, 2011; Silvestre *et al.*, 2011). This study proved most effective with 20 days HRT and $2 \text{ kg VS}/\text{m}^3\text{d}$ OLR with a methane maximum yield of $0.407 \text{ m}^3 \text{ CH}_4/\text{kg VS}_{\text{added}}$. The HRT obtained from this study aligns with that from Mairet *et al.*, (2011) and Passos *et al.*, (2014) who observed during the anaerobic digestion of microalgae that a lower HRT <15 days was detrimental while 20days HRT was optimal leading to a high productivity with minimal impact on digester efficiencies.

Using HRT of 20 days and OLR ($2 \text{ g VS L}^{-1}\text{d}^{-1}$) from the initial study, respective co-digestions were studied. The obtained methane yield ranged between 0.308 and $0.414 \text{ L CH}_4/\text{g VS}_{\text{added}}$ with 100% sewage sludge (0:100) having the least methane yield and the reactor with 75% algae (75:25) having the highest methane yield. 100% pre-treated algae (100:0) also proved to be more productive than sewage sludge and the other two co-digestion ratios in terms of methane yield, with a value of 0.407 L/g VS . This similar trend was observed in the BMP experiments carried out for the co-

digestions however the obtained CH₄ yield in the semi-CSTR showed superiority to the BMP up to 14%. This finding showed the possible improvement achievable from substrates during continuous operation of an anaerobic digesters (Lesteur *et al.*, 2010; Rodriguez, 2012). Furthermore, the semi continuous CSTR showed more effectiveness in terms of VS destruction and the methane content of the biogas when compared to the BMP co-digestion` results.

Although the co-digestion results obtained from the CSTR experiments validates the BMP study, it contradicts the studies of Samson and Leduy, (1985), who used sewage sludge to increase the C:N content of *Spirulina* from 4.2 to 6 and achieved an improved performance of AD of microalgae. These contradicting results are mainly a result of the substrate condition used for the experiments, as the C:N ratio of microalgae in our study was 6.1 which supersedes the C:N of sludge (5.4) used in this study. Also the pre-treatment of the microalgae cell wall of this study is an expected advantage favouring the biodegradability of the substrate.

Finally, under the semi-CSTR, the optimal HRT of 20 days was kept constant while the OLR was varied to identify the maximum OLR achievable for enhance AD. The tested OLRs include 2,3,4,5 and 6 g VS L⁻¹d⁻¹. Results for the co-digestions studied showed that increasing the OLR directly impacted the digestion process up to 4g VS L⁻¹d⁻¹ after which further increased caused upset to the digesters. A co-digestion of 75:25 (algae: sludge) based on VS content still proved to be most optimal for all co-digestion studied. In terms of VS destruction and the methane yield, no obvious variation was observed with increasing the OLR from 2 to 4 g VS L⁻¹d⁻¹. When the OLR of digesters were increased to 5 g VS L⁻¹d⁻¹, a decrease in digester performance (VS destroyed, methane yield, methane production etc.) performance was observed.

This performance reduction further increased when the OLR of the digesters were pushed into 6 g VS L⁻¹d⁻¹ and may be explained with the obtained SELR of the respective digesters which went up as high 0.78. Overall, 75:25 (alga:sludge) produced the highest methane yield/productivity and also demonstrated the most stable operational parameter at a loading rate of 4g/ VS L⁻¹d⁻¹ and an optimum HRT of 20days. Due to lack of information on the possible co-digestion of TH microalgae and sewage sludge or the impact of OLR in a semi/continuous digester comparison

of the obtained result was limited thus highlighting the need for more research in this field.

Interestingly, in the UK waste is now seen as a major contributor to the economy of the country to the extent that a recent “House of Lords” report documents its significant potential thus raising questions about what the best uses of wastes are (HOUSE OF LORDS, 2014). Although wastes may be sent for anaerobic digestion to recover methane and digestate, these may be termed as low value products, perhaps we are wasting an opportunity because putting carbon to methane is not necessarily the best route and consequently research is being carried out to look at other opportunities for processing carbon which is known as the bioeconomy.

The UK water industry has a lot of infrastructures which are capital intensive. As a result, individual sewage works are unlikely to be economically viable so what we need is an integrated approach currently called the bio-refinery. In the UK WWTP, we can handle sludge and we have all the massive assets but all these is to put carbon into a low value bio products while opportunities exist to upgrade this output for example with the use of the carboxylate platform,

Although these are capital intensive, a way to make them more valuable is to increase feedstock coming in. We cannot produce more sludge, but we can utilize the end product (digestate) to produce 3rd generation energy crop (microalgae) which will give us more output that will pay for the capital cost, to lead to more jobs in the country and most importantly can lead to export via the bioeconomy. Nevertheless, to realise the full potential and associated opportunities of this approach, the government, industry and academia are encouraged to increase focus on the approach. Thus the carboxylate approach was explored using the same co-digestion experimented in the anaerobic digestion experiments

For a successful carboxylate study, it is essential to eliminate the methanogens using inhibitors including iodoform or bromoform (Domke *et al.*, 2004). Furthermore, it is essential to not exceed the optimal iodoform concentration which may also vary depending on substrate of interest with literature studies demonstrating optimal range between 1.6 and 30mg/l (Datta, 1981; Rughoonundun *et al.*, 2010; Forrest *et al.*, 2012; Pham *et al.*, 2012; Golub *et al.*, 2013). For our studies, optimal iodoform

concentration of 10mg/L was implemented which showed superiority amongst other concentration tested in terms of conversion, selectivity, VFA yield, VFA production.

Respective co-digestion was evaluated at the optimal CHI_3 in this study with peak VFA concentrations ranging between 6.01 and 6.94 g/L. Whilst the optimal co-digestion under this platform was 50:50 (alga:sludge), peak VFA production of 6.94 g/L was obtained at a corresponding 11 days period, this day was similarly observed for the peak acid production of 100% WAS. Other fermenters including 25:75 reached its peak production at day 17 while 75:25 and 100% algae reached peak production at Day 20. All co-digestions fell within a residence time within 11 and 20 days which is recommended for further studies in a continuous experiment.

The VFA yields obtained under co-digestion were within the range of 0.86 and 0.99 g total acid/g VS digested for 50:50 and 75:25 (alga:sludge) respectively. This was compared to the available literatures as no similar work on this substrate was available. From the literature review, VFA yield obtained for most substrates (corn Stover, paper, glycerol, cattle manure, macro algae, sugarcane bagasse) ranged between 0.04 and 0.55 (Datta, 1981; Rughoonundun *et al.*, 2010; Nachiappan *et al.*, 2011; Smith and Holtzapple, 2011; Forrest *et al.*, 2012; Pham *et al.*, 2012; Golub *et al.*, 2013).

This research showed an enormous increase/improvement in VFA yield over the literature values by up to 80%. The reasons for this improvement are not conclusive as several factors may have been responsible, including:

- 1) The substrate used in the literatures differ from that used in this current study,
- 2) The fermentation condition (temperature) used in the literature studies varied between 25 and 55°C while this research used a temperature of 37°C,
- 3) Most pre-treatments carried out in the literature studies were with the use of lime while this current study employed the use of the thermal hydrolysis (165°C at 8 bar pressure).

Conclusively, the research highlighted different possible optimal ratio for the co-digestion of microalgae and sewage sludge as a result of the biofuel route intended. For the anaerobic digestion route, a co-digestion ratio of 75:25 (algae: sludge)

proved to be most optimal at a HRT of 20 days and an organic loading rate OLR of 4 g VS/L^{-d} with a corresponding methane yield and methane production of 0.43 L CH₄ VS_{added} and 0.964 L/L^{-d} respectively which is competitive compared with the likes of MSW and food waste. Likewise the carboxylate approach showed the optimum condition to be 50:50 (alga:sludge). At this co-digestion ratio, the VFA yield was 0.965 (g total acid/ g VS fed) which is about 80% more than the values for substrates studied in literature including: paper, macro algae, corn stover and MSW (Datta, 1981; Rughoonundun *et al.*, 2010; Nachiappan *et al.*, 2011; Smith and Holtzapple, 2011; Forrest *et al.*, 2012; Pham *et al.*, 2012; Golub *et al.*, 2013).

The hypothetical design for MAS were analysed to provide an energy balance and economic analysis using a simple cost benefit analysis approach. Under both biofuel platforms studied, it is evident that the co-digestion ratios of microalgae and sewage sludge had an influence on the flux of organic matter and energy with the most favourable co-digestion ratio of 75:25 (alga:sludge) for methane production and 50:50 (alga:sludge) for carboxylate production.

Several assumptions were made for the proposed integration into a typical UK WWTP based on Esholt WWTP, serving a population of about 700, 000 and digesting about 80 tonnes dry sludge a day. Assuming the current digesters in the UK have an average unutilized space of 20% (WMW, 2012), there is an avenue for additional substrate in the existing WWTP digester assets. Other assumptions were made with regards to the energy input required for the integration process, while the energy output obtainable from the integration was calculated using the yield obtained within the experiments.

The study identified a positive energy balance with MAS integration, calculations from the energy balance showed that every possible tonne of microalgae digested for methane production would lead to a potential energy gain of 1926 MJ (535 kWh). The anaerobic fermentation route to produce acetic acid and ethanol will however lead to a gain of 3536 MJ/tonne (982 kWh) and 6654 MJ (1848 kWh). Overall, energy yield of microalgae in this study ranged between 1926 and 6654 MJ/t dry algae depending on the final product targeted. This may be compared to the studies of Aresta *et al.*, (2005) who studied the integration of macroalgae to generate

biomass and biofuel from wastewater effluent. Their net energy was calculated using an LCA approach with the best scenario demonstrating a net energy yield of 11,000 MJ/t dry algae. Reason for the margin between both studies is mainly a result of the energy conversion technology route which was basically biodiesel production using supercritical CO₂ as solvent for extraction in their study. Other possible reasons include the energy intensive processes for microalgae cultivation and harvesting. Also, it is worthy to mention that macro algae were used in their study as against this study that explored the use of microalgae *Chlorella vulgaris*.

Economic analysis per tonne of microalgae from the study showed that the final product of the anaerobic process can influence total achievable revenue and suggest the water industry would generate additional revenue from renewable energy representing £15,622 per annum for CH₄ production. Alternatively, the production of acetic acid and ethanol using the modified AD approach may yield to revenue in the range of £28,703 and £53,990 respectively.

Some of the indirect benefits economic benefits associated with this integration include the added advantage of microalgae to re-use the nutrients (N and P) in wastewater/digestate thus offsetting some of the cost expended on wastewater treatment via reduction of nutrient load being treated. Also, incentives such as: renewable obligation certificates (ROCs), feed-in tariffs (FITs) and renewable heat incentives (RHIs) exist in which the integration of this system will benefit from. Therefore it is beyond doubt that the integration and co-digestion of microalgae using the existing WWTP infrastructure represents a feasible and cost effective approach for adoption in the UK WWTP.

The research highlighted the possible energy gain from microalgae to benefit from the existing facilities as well as a chance of producing alternative biofuels with more economic importance. Nevertheless, for a complete embracing of the alternative approach, a considerable amount of research and downstream solutions need to be implemented. Some of these include:

1. The use of the produced VFA within the UK WWTP,

2. Separation of the VFA from the fermentation broth as this has been identified as a main challenge for the effective recovery of carboxylates
3. Improving the final concentration, productivity and yield. Also complete inhibition of the methanogens at large scale will require in-depth study, as well as separation and purification cost of the final products.

Chapter 13 Conclusions recommendations and future work

The study investigated the potential for integrating microalgae into the existing configuration of a typical UK WWTP to benefit from the existing infrastructure, in particular the THP for sludge pre-treatment and any unutilised headspace within the existing anaerobic digestion process. This was with the overall aim of increasing energy production in the UK WWTP and possibly offset some of the excessive energy and capital cost associated with commercial exploitation of microalgae for energy. The key Conclusions from the experimental studies are that:

The BMP test showed it is possible to increase the methane yield of microalgae from 0.265 to 0.357L CH₄ /g VS_{added} using thermal hydrolysis operated under temperatures and pressures typically used in full-scale facilities. This 35% increase is competitive with other organic wastes such as MSW, paper, textile and leather which have been considered as codigestates.

THP proved effective by disintegrating the algal cell wall, thus releasing the cell content and making it available for the anaerobic organisms to metabolise. The rate of methane production could be described using first order kinetics with a hydrolysis constant for the treated microalgae of 0.14 day⁻¹, a 75% increase over the K_{hyd} of untreated microalgae.

When thermally treated algae were used as a codigestate with sewage sludge a linear increase in both the volatile solids destruction and the specific methane yield was obtained as the fraction of treated algae was increased up to a co-digestion ratio of 75% algae. This ratio yielded a 34% increase in methane yield (L CH₄/ g VS) compared to digesting sewage sludge alone. Furthermore, addition of treated microalgae to sewage sludge also increased the hydrolysis rate of the co-digested feed up to 75% algae.

Operating the digesters at 20 days HRT and OLR of 4g VS L⁻¹d⁻¹ suggests the best performance after which further increase in organic load can lead to digester instability.

The digestate quality of the respective digesters showed that the addition of microalgae to sewage sludge enhanced dewaterability up to an optimum ratio of 75% algae.

In addition to methane, a mixed culture of thermally treated algae and sewage sludge could also be used to produce alternative bio products through the carboxylate platform. Complete inhibition of methanogenesis was achieved using an iodoform concentration of 10 mg/l which also increased the VFA yield up to 54%. This resulted in peak VFA concentrations between 6.01 and 6.94 g/L. The highest VFA concentration was found with a 50:50 (alga:sludge) fermenter with a corresponding yield of 0.992 g total acid/g VS fed and a retention time of 11 days. This was followed by 25:75 (alga:sludge) with a yield of 0.952 g total acid/g VS fed at a retention time of 17 days. When methanogenesis was inhibited with iodoform there was also a large improvement in dewaterability of between 10 and 19% based on the results of the CST test. Thus anaerobic fermentation of WAS and microalgae to produce VFAs may provide an alternate option to methane production.

The possible benefits with the integration of microalgae into the WWTP under both approaches were evaluated. It was evident that the co-digestion ratios influenced the corresponding flux of energy and organic matter. In regards to utilization of the existing digester headspace under the assumptions made in the research, every tonne of microalgae produced and digested within the EETP will lead to a potential energy gain of 1926 MJ (535 kWh).

The production of alternative biofuel such as acetic acid and ethanol using a modified AD system will lead to an energy profit of 3536 MJ/tonne (982 kWh) and 6654 MJ (1848 kWh) respectively per ton of microalgae produced and treated, suggesting an improvement by 1.8 – 3.4 times methane production.

Economic analysis of the proposed integration (per ton of microalgae produced and treated) suggests additional revenue provision from renewable energy (methane production) totalling £15,622 per annum however, alternative biofuel production such as ethanol can generate revenue (£53,990) which is 3.5 times more than methane revenue.

From the research study, the following recommendations can be suggested for future work:

- Additional work using other strains, and in particular a mixed culture of naturally occurring strains should be evaluated, as this might be a more realistic approach for integration of microalgae into the wastewater treatment flow sheet.
- The integration of MAS requires a great deal of technical expertise for design and maintenance of the process thus it is recommended to identify the optimal configuration which will put into account challenges that may occur in managing the growth conditions, culture compositions and harvesting.
- The carboxylate experiments in this study were carried out only in batch experiments. It is recommended for further studies, the possibility of these substrate/conditions in a continuous fermentation train as this can further enhance concentration of the VFAs thus helping to achieve a more detailed result from the approach (Chan and Holtzapple, 2003).
- Some technical bottlenecks still exist and require further studies with the full implementation of the carboxylate approach, some of these include: improving the final VFA concentration, productivity and yield. Also, complete inhibition of the methanogens at large scale will require in-depth study, as well as separation and purification cost of the final products.
- Most research carried out on MAS integration are laboratory based, making assumptions to be difficult for real life occurrences. For an extensive energy balance as well as cost benefit analysis, it is essential to incorporate real data available for microalgae cultivation (carried out especially using large scale plants) in terms of nutrient and energy use. This will provide a more detailed analysis for developing a standard cost benefit analysis of the intended integrated approach.

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