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Energy Engineering

School of Engineering

& Environmental Research Institute

University College Cork

Biogas Production from Novel Substrates

Eoin Allen B. E (Hons)

Thesis submitted for the degree of Doctor of Philosophy to the National University College Cork, Ireland

Supervisor: Professor Jerry Murphy

Head of School: Professor Nabeel Riza

May 2015

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Declaration

I hereby declare that this thesis is my own work and that it has not been submitted for another degree either at University College Cork or elsewhere. Where other sources of information have been used, they have been acknowledged.

Signature: ……..

Date: ……

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I would like to thank my parents, and family for their continued motivation and support.

My colleagues past and present at the time of my research who provided support and guidance in various aspects of my research; David Wall, Markus Volklien, Dr Ao Xia and Dr Christiane Herrmann.

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Abstract

Biogas production is the conversion of the organic material into methane (CH_4) and carbon dioxide (CO_2) under anaerobic conditions. Anaerobic digestion (AD) is widely used in continental and Scandinavian communities as both a waste treatment option and a source of renewable energy. Ireland however lags behind this European movement. Numerous feedstocks exist which could be digested and used to fuel a renewable transport fleet in Ireland. An issue exists with the variety of feedstocks; these need to be assessed and quantified to ascertain their potential resource and application to AD. Characteristics identified through the course of this research was the Carbon to Nitrogen ratio (C:N). From literature the ideal C:N ratio is between 25 and 30:1. Low levels of C:N (<15) can lead to problems with ammonia inhibition. Within the digester a plentiful supply of nutrients and a balanced C:N is required for stable performance. Feedstocks were sampled from a range of over 100 different substrates in Ireland including for first, second and third generation feedstocks. The C:N ranged from 81:1 (Winter Oats) to 7:1 (Silage Effluent). The BMP yields were recorded ranging from 38 $\pm$ 2.0 L CH_4 kg^{-1} VS for pig slurry (weaning pigs) to 805 $\pm$ 57 L CH_4 kg^{-1} VS for used cooking oil (UCO). However the selection of the best preforming feedstock in terms of C:N ratio or BMP yield alone is not sufficiently adequate. A total picture has to be created which includes C:N ratio, BMP yield, harvest yield and availability. Potential feedstocks which best meet these requirements include for Grass silage, Milk processing waste (MPW) and *Saccharina latissima.* MPW has a potential of meeting over 6 times the required energy for Ireland's 2020 transport in energy targets. *S. Latissima* recorded a yield of over 10,000 GJ ha^{-1} yr^{-1} which out ranks traditional second generation biofuels by a factor of more than 4. Grass silage has impressive all round characterises including a BMP yield of 400 L CH_4 kg^{-1} VS and 152 GJ ha^{-1} yr^{-1}. Grass silage has the added advantage of being grown on 91% of Irish arable land as well as being the cheapest crop to produce in this country. Further work included for batch testing and continuous operation. Using data from batch trials, feedstocks were combined in various mixes and operated for up to 10 months in continuous reactors. Continuous trials were used to simulate full-scale working conditions and determine which factors influenced stable operation, such as organic loading rate (OLR) and volatile fatty acid (VFA) concentrations. For example green macro-algae *Ulva lactuca (U. lactuca)* was trialled in 6 different reactors in dried and fresh form; it was co-digested with a varying percentage of dairy slurry (25, 50 and 75% by VS content). Dairy slurry increased the C:N ratio of the mixture; *U. lactuca* had a C:N ratio of 7:1. The optimum reactor mix was found to be 25% Fresh U. *lactuca* and 75% dairy slurry operated at an OLR of 2.5 kg VS m^3 d^{-1} with an average yield of 170 L CH_4 kg^{-1} VS.

Thesis output

Chapters which have been published or currently under review in peer reviewed journals:

Chapter 3: Allen, E., Wall. D., Herrmann, C., Murphy, J. (2015). A detailed assessment of variation in biomethane potential of first, second and third generation substrates. Submitted to: Renewable Energy journal.

Chapter 4: Allen, E., Browne, J., Murphy, J. (2014). Evaluation of the biomethane yield from anaerobic co-digestion of nitrogenous substrates. Environmental Technology 34 (13): 2027-2038.

Chapter 5: Allen, E., Wall. D., Herrmann, C., Xia, Ao., Murphy, J. (2015). What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? Energy 81 (0): 352–360.

Chapter 6: Allen, E., Browne, J., Hynes, S., Murphy, J. (2013). The potential of algae blooms to produce renewable gaseous fuel. Waste Management, 33 (11): 2425-2433.

Chapter 7: Allen, E., Wall. D., Herrmann, C., Murphy, J. (2014). Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel Bioresource Technology, 170: 436-444.

Co-authored peer reviewed publications

Appendix A: Browne, J., Allen, E., Murphy, J. (2014). Evaluation of the biomethane potential from multiple waste streams for a proposed community scale anaerobic digester. Environmental Technology 34 (13): 2059-2068.

Appendix B: Fitzgerald, J., Allen, E., Jackson, S., Dobson, A., Murphy, J. (2014). Microbial community analysis of anaerobic bacteria and the effect on biogas production stability on the digestion of macro algae and dairy slurry. Currently being prepared for submission.

Appendix C: Browne, J., Allen, E., Murphy, J. (2013). Assessing biomethane production from the organic fraction of municipal solid waste in batch and continuous operation. Applied Energy 128 (0): 307-314.

Appendix D: McEniry. D., Allen, E., Murphy, J., O'Kiely, P. (2014). Grass for biogas production: The impact of silage fermentation characteristics on methane yields in two contrasting biomethane potential test systems. Renewable Energy 63 (0): 524-530.

Appendix E:

Browne, J., Allen, E., Murphy, J. (2013). Improving hydrolysis in multiphase digestion of food waste. Waste Management. 33 (11): 24070-2477.

Murphy, J., Browne, J., Allen, E., Gallagher, C. (2013). The resource of biomethane, produced via biological, thermal and electrical routes, as a renewable transport fuel. Applied Energy 128 (0): 307-314.

Wall. D., Allen, E., P. O'Kiely., Murphy, J. (2015). Optimisation of digester performance with increasing organic loading rate for mono and co-digestion of grass silage and dairy slurry. Bioresource Technology 173 (0): 422-428.

Wall. D., Allen, E., P. O'Kiely., Murphy, J. (2015). The effect of trace element addition to mono-digestion of grass silage at high organic loading rates. Bioresource Technology 172 (0): 349-355.

Wall. D.,B. Straccianlini., Allen, E.,P. Nolan., C. Herrmann,. P. O'Kiely., Murphy, J. (2015). Investigation of effect of particle size and rumen fluid addition on specific methane yields of high cellulose grass silage. Bioresource Technology 192 (0): 266-271.

Wall. D., Allen, E., O'Shea, R., P. O'Kiely., Murphy, J. (2015). Investigating two-phase digestion of grass silage for demand-driven biogas applications: effect of particle size and rumen fluid addition. Submitted to Renewable Energy (11/5/2015) awaiting review.

Technical brochures:

Prepared and published for the International Energy Association, IEA. Algae: A source of renewable gaseous biofuel. Jerry D Murphy, Bernard Drosg, Eoin Allen, Jacqueline Jerney, Ao Xia, Christiane Herrmann. Edited by, David Baxter. Copyright © 2015 IEA Bioenergy, Task 37.

Conference papers and posters:

Allen, E., Browne, J., Hynes, S., Murphy, J. The mono-digestion of U. *lactuca* and the potential to produce renewable gaseous fuel (2012).Submitted and chosen to present at the, 4th international symposium on energy from biomass and waste, San Servolo, Venice Italy.

Allen, E., Wall. D., Herrmann, C., Xia, Ao., Murphy, J. BMP analysis on Ireland's available feedstock for anaerobic digestion and how to achieve renewable energy targets (2014). Submitted and chosen to present at, Biogas Science, Vienna, Austria.

Allen, E., Wall. D., Murphy, J. The effect of laboratory analysis on the design of a community digester (2012). The 22nd Irish Environmental Researchers Colloquium, NUIG Galway, Ireland.

Contributions to published papers

Chapter 3: I was the first author of this publication. I was responsible for the planning and gathering of most samples for laboratory experiments and conducting lab trials in conjunction with my colleagues. I was involved heavily in the experimental design of this experiment. I undertook the majority of writing of the paper and data analysis. This paper is a combination of all batch tests I conducted as part of my lab work with previous papers, additional samples collected and results completed with my colleagues.

Chapter 4: I was the first author of this paper which was co-published with the published paper from appendix A. I was responsible for the gathering of samples for the laboratory experiments and conducting continuously operated lab trials. I was involved heavily in the experimental design of this experiment and the decision making throughout the experimental trial period, which lasted 6 months.

Chapter 5: I was the first author of this publication. I was responsible for the gathering of samples for laboratory experiments and conducting lab trials. I was involved heavily in the experimental design of this experiment. I undertook the majority of writing of the paper and data analysis.

Chapter 6: I was the first author of this publication. I was responsible for the gathering of samples for laboratory experiments and conducting lab trials. I undertook the majority of writing of the paper and data analysis

Chapter 7: I was the first author of this publication. I was responsible for the gathering of samples for laboratory experiments and conducting lab trials. I was involved heavily in the experimental design of this experiment and the decision making throughout the experimental trial period, which lasted 9 months. I undertook the majority of writing of the paper and data analysis.

Appendix A: This was a joint publication of which I am a co-author of. This paper is a pre-cursor to the paper published in chapter 4. I conducted the laboratory analysis, contributed to the writing and data analysis of paper and shared co-authorship with my colleague.

Appendix B: I am a co-author of this paper with my colleague. I conducted all the continuous experimental biogas laboratory procedures of this paper. Microbial analysis was completed in conjunction with my colleague from a separate department (microbiology). This paper is a continuation from chapter 7. I contributed to the writing of the introduction, materials, methods and results sections in this paper.

Appendix C: I am a co-author of this paper with my colleague. I conducted all the continuous experimental laboratory analysis of this paper alongside the batch tests in this publication. I contributed to the writing of materials, methods and results sections in this paper.

Appendix D: I am a co-author of this paper with my colleague from another institution as part of an academic collaboration. I conducted batch laboratory experiments and analysis in this publication. I contributed to the writing of materials, methods and results sections in this paper.

Appendix E: These remaining 6 papers are a collection of published journal works which I have made minor contributions to. This also includes for a technical brochure currently in the final publication stages with the IEA.

Nomenclature

AD	anaerobic digestion
B_{ix}	biodegradability index
BMP	biochemical methane potential
CH_4	methane gas
CHP	combined heat and power
CNG	compressed natural gas
CO_2	carbon dioxide gas
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
DM	dry matter (equivalent to total solids in this thesis)
DS	dry solids (equivalent to total solids in this thesis)
EU	European Union
Eqn	equation
FW	food waste
H_2	hydrogen gas
H_2S	hydrogen sulphide gas
MSW	municipal solid waste
MPW	milk processing waste
NG	natural gas
NH_3	free ammonia
NH_3-N	ammonia nitrogen (equivalent to TAN in this thesis)

OFMSW	organic fraction of municipal solid waste
OLR	organic loading rate
pH	power of hydrogen
RES-T	renewable energy supply in transport
SD	Standard deviation
SHW	slaughter house waste
SMY	specific methane yield
t yr^{-1}	tonnes per annum
TA	total alkalinity
TAN	total ammonia nitrogen
TS	total solids
UCC	University College Cork
VFA	volatile fatty acids
VS	volatile solids
wwt	wet weight

1 Introduction

1.1 Introduction and background of thesis

Anaerobic digestion (AD) is an expanding renewable energy source throughout Europe. There were over 13,800 biogas plants across Europe in 2012 (European Biogas Association), however only 27 operational biogas plants were in Ireland. This number however includes for plants used as part of waste water treatment processes, which suggests the number of actual anaerobic digesters specifically built for biogas production is less. AD is a process where a consortium of anaerobic bacteria and achaea, degrade organic matter through four sequential stages to produce methane and carbon dioxide, the biogas is typically 50 – 60% methane. As the biogas industry grows in Europe, countries such as Ireland can take advantage of the advances made. A key issue with the design of an efficient biogas industry is identification of potential substrates. Ireland has a large agricultural industry, associated with a large export market. Farmers have established good practices in crop production and have identified the best yielding crops. With a large dairy and beef industry there are large volumes of wastes from the food processing sector, which may be combined with cattle manure to fuel biogas plants. The effective management of land use to avoid direct competition with existing food production and the establishment of waste pathways to treat organic waste are necessary for the successful development of a biogas industry in Ireland.

By 2020 according to The Renewable Energy Directive (European Commission 2012), 10% of energy use in transport should be renewable. In 2011 first generation biofuels provided for approximately 5% renewable energy supply in transport (RES-T) in the EU. In April 2015 the European Commission increased the cap on first generation food based biofuels to 6% RES-T, from 5% proposed in 2012. It was also stipulated that advanced biofuels, such as sourced from seaweed, should represent at least 2.5% RES-T by 2020. Combining the use of AD to treat organic wastes and produce biogas with Ireland's obligations set by the European Commission for RES-T, provides a sustainable platform to develop a biogas industry in Ireland. Also the as part of the ambition to meet RES-T, Member States shall ensure that compressed natural gas (CNG) refuelling points are available within at least 150 km of each other by 2020 (The Alternative Transport Fuel Directive). The development of CNG stations should facilitate the use of biomethane as a gaseous transport biofuel. What types of substrates would be best suited to allow Ireland satisfy 10% RES-T through biomethane systems?
Ireland, with over 7,500 miles of coastline and direct access to the Atlantic Ocean offers itself as an ideal location to utilise macro-algae (seaweed) as a source of biofuel. Algae can be either cultivated

in aquaculture farms or harvested from beaches or from the sea. *U. lactuca*, a green macro algae is a particular case in that it is detrimental to coastal environments that have long shallow bays which are subject to eutrophication. This has become more endemic in recent years in France, Denmark and Japan. It is seen as an algae bloom and can result in thousands of tonnes washing up on to beaches, forcing closures and threatening the amenity of the whole bay area. However the quantity of the bloom can lead to a cheap source of biofuel as it greatly reduces the harvesting costs. Seaweed (or macro-algae) biofuels are deemed to be third generation biofuels. They do not interfere with food production directly (they do not use food crops) or indirectly (they do not use agricultural land).

Industrial residues and wastes from municipalities can contribute to a biogas industry. Ireland produces over 35 m t yr^{-1} of bovine slurry alone, combined with a dairy industry which process 4.5 bn litres yr^{-1} of milk. Agriculture and industries linked to the agricultural sector produces vast quantities of wastes, which can all be processed by AD to produce biogas. A full review of all potential AD feedstocks is required to suitably identify potential yields, which could fuel an AD industry in Ireland. However one such test is not available to quantify all substrates and their biogas potential. An optimised approach or methodology needs to be designed to efficiently develop potential biogas yields through a combination of stoichiometric, batch and continuous laboratory experiments.

1.2 Thesis aims and objectives

The aims and objectives of this thesis are:

- Identify and classify available substrates, which may be used to produce biogas in Ireland.
- Compare mono and co-digestion of substrates and establish synergies between substrates which improve digestion efficiencies.
- Assess theoretical and actual biochemical methane potential (BMP) of macro algae substrates which are readily available around Irish coastlines.
- Analyse the suitability of macro algae *U. lactuca* to anaerobic digestion.
- Assess the energy yield per hectare per annum of optimum performing biofuel substrates.
- Relate substrate availability and potential biomethane production to RES-T targets.

1.3 Thesis outline and link between chapters

This thesis is comprised of 8 chapters and 4 appendices. Chapter 2 is a literature review of the biogas process and associated parameters, which are vital to understand successful operation of the biogas process. Chapters 3 through 7 are published or submitted papers for peer reviewed journals, which have been edited to fit a thesis format. They are the main body of work. There are 4 additional peer reviewed papers, which I contributed significantly to. A guideline to each chapter is detailed below.

Chapter 3: A detailed assessment of variation in biomethane potential of first, second and third generation substrates

This paper is an accumulation of assessments of various substrates. It includes for data which compile a matrix of potential BMP yields of available substrates in Ireland. As the biogas industry is starting to develop in Ireland, there is a need to quantify potential substrates for AD and understand associated parameters with these substrates. A standardised method was established to run BMP assays, in triplicate on the Bio-process AMPTS II unit. A total of 83 substrates from industry, agriculture, municipalities and marine sources were assessed. Homogenous samples were taken for each feedstock to ensure a representative sample was obtained. Using the same procedure each time, a blank and a cellulose control were assessed. This enabled each run to be compared to the previous run for statistical relevance and to maintain accuracy all BMP assays. Six primary categories were identified including: Agricultural wastes, Grass substrates, Root Crops, Food Processing Wastes, Municipal Wastes and Macro algae. Grass, received a category to itself as it is the most dominant arable crop grown in Ireland (91% of agricultural land is covered by grass). Each of the primary categories had a number of substrates trialled in an attempt to accurately identify that category. After each substrate was trialled the best performing substrates were chosen. The biomethane resource for Ireland was assessed, by factoring the quantity of substrate available by the specific methane yield (SMY) of that substrate. Crops and seaweeds an energy value per hectare was established. A route to reaching RES-T targets was then produced.

Chapter 4: Evaluation of the biomethane yield from anaerobic co-digestion of nitrogenous substrates.

This chapter is part of two papers submitted together to the same journal. The second paper is attached in appendix A. The biomethane potential from five major organic waste streams for a proposed community scale anaerobic digester in a rural town in Ireland were assessed. Batch tests, theoretical yields, kinetic and statistical modelling were all employed; this is a similar methodology as in Chapter 3. Five main waste streams were identified as possible substrates for biogas production, namely: abattoir waste, (consisting of paunch content and dewatered activated sludge); cheese waste effluent; commercial and domestic food waste; pig slurry; and waste water treatment sludge. From initial batch tests of each substrate individually, two co-digestion mixes were proposed; these were also run in batch tests. From these results only limited data was obtained. No information on organic loading rate (OLR), on levels of VFA, of total ammonia nitrogen (TAN) or other operating parameters are yielded from batch trials. This was the starting point of Chapter 4. The brief was to examine the relative merits of two mixes of the best performing substrates from the BMP trials. The three substrates used in the two mixes were: abattoir waste; cheese waste; and food waste. The two mixes comprised of: T1 (40% abattoir waste; 50% cheese waste; 10% food waste on a wet weight (w/w) basis) and T2 (30% abattoir waste; 40% cheese waste; 30% food waste). The C:N ratio of both mixes were below optimum. These low levels suggest that the production of free ammonia (NH_3) in semi-continuous digestion was of primary concern. Both mixes were digested in a semi-continuous process for 25 weeks. The aim of these continuous trials (as with chapter 7) was to reach a steady state of biomethane production at a yield as close or above the BMP yield of the mixes from batch tests. Final results recommended operating conditions for T1 as a loading rate of 3 kg VS m^{-3} d^{-1} at a retention time of 23 days. The biomethane yield was 305 LCH_4 kg^{-1} VS, which was 87% of the BMP value and indicated 61% biodegradability. For T2 (with a higher C:N ratio) a higher loading rate of 4 kg VS m^{-3} d^{-1} at a lower retention time of 15 days was recommended. The biomethane yield was 439 LCH_4 kg^{-1} VS (99% of the BMP value and 84% biodegradability). At these conditions levels of Total Ammonia Nitrogen (TAN) were 4109 and 4831 mg l^{-1} for T1 and T2 respectively. These values are on the large side according to the literature. The temperature was reduced to 35^{o}C to minimise toxicity associated with TAN. VFA inhibition did not occur as concentrations were well below permitted ranges.

Chapter 5: What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?

The use of third generation biofuels avoids competition between agricultural land and energy. Algae (both macro and micro) have been suggested as potential future sources of renewable energy in transport in Europe. The Renewable Energy Directive assigns a weighting of two to biofuel produced from algae. Thus in calculating renewable energy supply in transport (RES-T) targets the energy from algae biofuels may be doubled in considering the 2020 target of 10% RES-T. With the qualification of seaweeds for double credits, brown seaweeds in particular *Saccharina latissima* and *Laminaria digitata* have high concentrations of naturally occurring sugar such as alginate and lamanarian which can be readily degraded in a biogas digestion system and converted to methane. This chapter has the aims of identifying the most abundant brown macro algae varieties around Irish shorelines and developing a matrix of potential biomethane yields and characteristics associated with these seaweeds. A series of beaches and estuaries across the west Cork coastline were chosen to collect each seaweed variety to ensure a wide variety sample was collected. A full theoretical analysis was undertaken on ten different seaweeds to determine C:N ratios as well as C:S ratios to try and predict H_2S concentrations. From these results a series of batch tests were conducted to determine BMP yields. From the obtained yields and literary review a table of potential energy yields per hectare was developed to determine how much area would be required to meet RES-T targets. Results showed that *S. latissima* reported the highest BMP yield (ca. 342 L CH_4 kg^{-1} VS). *S. latissima* if farmed, may produce 10,250 m^3 CH_4 ha^{-1} yr^{-1} (365 GJ ha^{-1} yr^{-1}) which is in excess of all land based liquid biofuel systems.

Chapter 6: The potential of algae blooms to produce renewable gaseous fuel

Chapter 6 details the issue of a particular macro-algae species, which is both a residue and a third generation biofuel substrate. The macro algae or seaweed *U. lactuca* (commonly known as sea lettuce) is a green seaweed which dominates algae blooms. These blooms are collectively known as “Green Tides” and are caused by excess nitrogen from agriculture and sewage outfalls resulting in eutrophication in shallow estuaries. A solution to this problem is to remove the algae and use as a source of renewable energy in the form of biomethane. However as this seaweed is naturally occurring its growing and harvest conditions are hard to control compared to terrestrial crops like grass or maize. Samples of *U. lactuca* were taken from the Argideen estuary in West Cork for two consecutive years; these were analysed for biogas potential as well as physical characteristics. In batch tests fresh *U. lactuca* achieved a BMP yield 183 L CH_4 kg^{-1} VS. Fresh samples (sampled a year

later) produced results of 205 L CH_4 kg^{-1} VS. A series of pre-treatments were carried out in an attempt to improve biomethane yields. The best results were obtained for a dried, washed and macerated sample, yielding a BMP result of 250 L CH_4 kg^{-1} VS. The resource in this West Cork estuary is sufficient to fuel 260 cars. Some issues were highlighted with the seaweed in terms of its chemical makeup, which can lead to problems in digesting *U. lactuca*. The C:N ratio is low and the sulphur content is high. The sulphur content of *U. lactuca* is such that hydrogen sulphide (H_2S) is released when it anaerobically digests on the shore releasing rotten egg smells. It is recommended that *U. lactuca* is co-digested with a substrate which has a higher C:N ratio and also provides a source of minerals and trace elements to improve digester performance.

Chapter 7: Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel.

This chapter is a continuation of the work of the previous chapter. It deals with continuous digestion of *U. lactuca*. The green seaweed variety was found to have high levels of sulphur and a low ratio of carbon to nitrogen. Associated literature on the continuous digestion of a substrate with such characteristics illustrated that microbial failure could occur. These operational parameters, which cannot be analysed in batch tests, were assessed in continuous trials. Fresh and dried *U. lactuca* were continuously co-digested with dairy slurry at ratios of 25%, 50% and 75% (by volatile solid content) in 6 number 5L reactors for 9 months. Dried *U. lactuca* was chosen as it had the highest specific methane yield of all the pre-treatment options assessed in chapter 6. The reactors digesting a mix with 75% *U. lactuca* struggled to reach sustainable operating conditions. Failure was dominated by volatile fatty acid (VFA) inhibition. The levels of ammonia increased with percentage *U. lactuca* in the mix. Optimum conditions were observed with a mix of 25% fresh *U. lactuca* and 75% slurry. A yield of 170 L CH_4 kg^{-1} VS was achieved at an organic loading rate of 2.5 kg VS m^{-3} d^{-1}. The reactor with the lowest portion of *U. lactuca* preformed the best, this mix had the highest C:N ratio. There was a gradual decline in the biomethane yield for this reactor after it was increased to an OLR of 2.5 kg VS m^{-3} d^{-1} without any correlating rise in VFA or TAN concentrations. A detailed trace element and fingerprint rDNA analysis was conducted to establish the exact cause of the reduction in yields. The discussion and results of this data is presented in appendix B.

2 A review of anaerobic digestion and third generation biofuels

2.1 Early research in biomethane potential of substrates

Anaerobic digestion is a biological process, which degrades organic material via four sequential stages to produce a renewable natural gas in the form of biomethane. Hydrolysis, acidogenesis, acetogenesis and methanogenesis are the steps involved in breaking the volatile components of the substrates to methane and carbon dioxide. Initial studies conducted by Buswell and Neave [1], were conducted with the aim to fully understand the four stages of AD in treating sludge and stabilising waste. The premise of their work was to fully understand biochemical influences of carbonaceous and nitrogenous material on anaerobic degradation. Further studies in the same year by Neave and Buswell [2] developed the relationship to a stoichiometric equation to forecast potential methane and carbon dioxide yields from degradation of fatty acids. From these studies they showed that water acted as an oxidising agent in breaking down organic acids and a further relationship exists between the number of carbon atoms present in the acids and the number of molecules of water which exist during digestion.

Leading on from these trials Symons and Buswell [3] using previous methodology and results developed a method to determine the potential methane and carbon dioxide production of a substrate depending on its stoichiometric composition using an empirically derived formula. This formula is known as the *Buswell equation* and is used in this thesis as a first stage in the biogas analysis of substrates prior to biochemical methane potential (BMP) assays. However a drawback or constraint of the Buswell equation is that the stoichiometric equation does not take into account the organic material which is consumed by the anaerobic bacteria and archae, which need a source of energy for subsistence, reproduction and evolution. From this assumption made by Buswell and in a similar method published by McCarthy [4] that all electrons donated in the degradation process are exclusively used for metabolic energy and not for microbial growth, an overestimation in BMP yields can occur. This overestimation can vary widely from each substrate as additional factors can occur such as a substrate having a concentration of fibre or lignin which may not be readily degradable, yet are attributed to methane yield by the Buswell equation. Thus the Buswell equation is used only as a “ceiling value” or maximum theoretical yield to the total methane that may be produced and not the actual methane produced by a unit mass of a substrate. Labatut et all [5] attempted to determine a method to avert this issue and develop relationships between the concentration of volatile organic

matter of a substrate and stoichiometric composition. Results showed that their modified equation could predict the BMP of a substrate to above 90% accuracy. From this initial work and the development of equations to predict BMP yields, a detailed and precise understanding was established. However an initial BMP test would be required as a coefficient in Labatut's equation is the yield of the substrate itself.

2.2 Biochemical methane potential of substrates

There are a number of particular drivers which have led to a development of a biogas industry. These drivers can be defined as the treatment of various wastes streams, divert wastes from landfill, reduce CO_2 emissions, and meet renewable energy targets. Rather than just a process understanding, a full knowledge of the biogas cycle is required. One constraint with biogas systems is their dependence on local substrates. It is not economically viable to transport organic feedstocks great distances as this can affect the viability of the entire process; this is especially true for substrates with low percentage total solids (TS). Smyth et all [6] published results showing that the transport requirements of removal of the digestate 10km, alone were 2% of gross energy produced from a typical biogas plant treating grass and dairy slurry. This constraint has led to further research and development of optimised biogas production by further understanding the substrates and what is required to successfully digest the organic matter available.

Subsequent to theoretical yields, a laboratory method described as a batch test was successfully developed with a set of optimised parameters. Successful studies conducted by Chynoweth et all [7] and Owens et all [8] on batch tests developed vital parameters such as the feeding ratio of the inoculum and the substrate and the reactor vessel sizing. The batch test or BMP assay is conducted in a reactor vessel, which is fed once and sealed, allowing the biogas to escape through a designated outlet, which connects to a gas flow meter. It is difficult to make adjustments during the process. Some minor adjustments can be conducted such as pH adjustment but these adjustments have an impact on the final BMP yield. It is preferable to make any adjustments to the inoculum pH or trace element concentration, before the batch test is initiated. An optimised feeding rate was established. This is known as the Inoculum to Substrate ratio (I:S ratio). It is typically between 1:1 and 2:1 on a volatile solid (VS) basis [7, 8]. Volatile solids were determined to be the optimum substrate physical parameter rather than total solids which includes the ash content (AC). From these established results it was found that an I:S ratio below 1:1 would lead to inhibition within the batch vessel as there would not be a necessary amount of bacteria present within the inoculum to successfully digest the substrate. A retention time of over 30 days would also result, which compromises the

effectiveness of the test. An I:S ratio above 2:1 would produce an adequate result but the volume of the substrate required is quite small, which increases the percentage error in sampling a homogenous substrate.

A final conclusion was the reactor size of the batch tests. The use of the serum bottle batch tests which may allow high throughput, ultimately sacrifices the homogeneity and representativeness of the substrate as the serum bottle size can be under 100 ml. By using a reactor vessel this size, would mean a substrate sample of a significantly small size increasing percentage error. An optimised BMP system allows researchers an accurate method to determine the actual Specific Methane Yield (SMY in units of L CH4 kg^{-1} VS) of a substrate under optimised lab conditions as opposed to a theoretical yield from a stoichiometric equation. The AMPTS II system was the system used throughout these experimental trials. It uses a vessel of 400ml as a reactor. It is described in greater detail in chapter 3.

2.3 Continuous reactors

The specific methane yield (L CH_4 kg^{-1} VS) may be assessed using theoretical methods and using the BMP assay. However these methods fail to give adequate information on the allowable organic loading rate (OLR) in continuous digestion or the operational parameters of a biogas reactor. Such parameters are vital in establishing the viability and longevity of the AD process. These parameters include pH, total ammonical nitrogen (TAN), volatile fatty acids (VFAs) and FOS:TAC (ratio of acidity to alkalinity). The microbial fauna present in the digester are dependent on these parameters. Acetoclastic methanogens require a stable pH range between 7 and 8 [9]. If an accumulation of VFA occurs, a reduction in the pH of the reactor can occur. This reduction in pH leads to unfavourable conditions for the methanogens and may lead to biodegradability inefficiencies or worse total reactor failure. The presence of toxic levels of free ammonia in the form of NH_{3+} can also lead to reactor disturbances in biogas production [10]. These environmental characteristics however cannot be monitored through a period of time in a batch test. There is a requirement for continuous or semi-continuous laboratory experiments. By running a continuous experiment which is closely related to a large scale or industrial reactor these reactor parameters can be recorded for a particular substrate and possible causes of failures identified and prevented. This allows optimisation of the biogas process in the design of a commercial facility.

Various continuous reactor systems exist. They include for 1 stage continuous stirred tank reactors (CSTR) [11] and 2 stage CSTRs [12], which are distinguished by having one or more tanks are rector vessels. The CSTR can be horizontally or vertically operated. Horizontal CSTRs are commonly referred

to as plug flow reactors. Continuous reactors can also be double phase were the solid substrate is hydrolysed and undergoes acidogenisis in the first phase, and in the second phase conditions are optimised for methanogenisis possibly in an up-flow anaerobic sludge blanket reactor (UASB) [13]. The trialling of these various reactor configurations for a particular substrate will establish the optimum AD process.

2.4 Third generation biofuels

The technology includes for reactor configuration and substrate requirements. The substrate must be quantified in terms of proximate analysis and ultimate analysis. Existing first (crops) and second generation (residues and lignocellulosic substrates) biofuel substrates have received detailed examination [14, 15]. This may not be said for third generation (algae) biofuel substrates. The Carbon to Nitrogen (C:N) ratio is a primary indicator of how well a substrate may be digested [16]. An optimum range of the C:N ratio lies between 25:1 and 30:1 [17]. If the C:N ratio of a substrate lies outside this range it can make it difficult for the flora and fauna of the bacteria consortium to operate in a stable environment. The carbon to sulphur (C:S) ratio can forecast potential H_2S concentrations [18]. H_2S is a toxic gas, which can be produced alongside methane and carbon dioxide. It needs to be limited, as it causes harm to Combined Heat and Power (CHP) systems or upgrading equipment. It is also inhibitory to the AD process [19, 20].

An issue with third generation biofuels is that the C:N can vary significantly, far greater than terrestrial biofuel substrates currently used in AD. This large variance is combined with additional factors such as the composition of the substrates in terms of inhibitory concentrations of lipids or fats which rapidly degrade and produce an excess amount of VFAs [21, 22]. Substrates such as fish offal, food waste, used cooking oils and grease trap waste, have high lipid and soluble fat concentrations, which make continuous digestion difficult especially in a mono-digestion. Advanced continuous systems such as two phase digestion which cannot process high or shock loads of a high strength (COD concentration) liquor from rapidly degrading oily substrates such as food waste can lead to failure. This would not be the case for a lignocellulose substrate such as grass which has a lower rate of degradation [23]. When algae species are considered, there are two distinct categories: macro and micro algae. Micro algae are microscopic algae species and have a low total solids (TS) content (between 0.1% - 1% TS) as produced in open and closed bioreactors [24, 25]. Micro-algae like oil rich waste substrates are rich in lipids, which leads to high theoretical specific methane yields (SMYs) and actual yields obtained by BMP assays. A down side to these concentrations of lipids is the inhibitory effect on the AD process [21]. Macro algae are large algae species typically known as

seaweeds. Macro algae are typically separated into 3 groups: Red, Brown and Green macro algae. Green macro algae (similar to micro algae species) have low C:N ratios; they also have high sodium and sulphur concentrations, all of which make digestion challenging [26]. Brown seaweeds are found in all types of climatic conditions, while Red seaweeds are associated with warmer climates. The composition of these seaweeds can vary greatly in terms of carbohydrates and sugar content [27]. The literature available on the use of algae as a biogas substrate is sparse when compared to first and second generation biofuel substrates.

There is a great potential to be harnessed from directing third generation biofuel substrates to AD and producing renewable gaseous transport fuel. The EU awards double credits to renewable energy in transport derived from second and third generation biofuel substrates when assessing the 10% renewable energy supply in transport (RES-T) target for 2020 [28]. For instant Gosh et al. [29] outlined how a city of 1 million inhabitants could fuel between 5 - 9% of the city's natural gas demand from municipal refuse alone[29]. In an Irish context if the organic fraction of municipal solid waste (OFMSW) was diverted to AD (instead of landfilling) a biomethane quantity of 2.36 PJ yr^{-1} or 1.36% of RES-T could be satisfied. With double credits this could be 2.72% of the RES-T from OFMSW alone. A study carried out by Yokoyama et al. [30] showed that a potential of 18.66 PJ yr^{-1} of energy could be produced by growing a *Laminaria* species of macro algae off shore, in Japan [30]. All be it these are desktop calculations, there is existing data which can suggest that a large contribution of national and global energy targets and CO_2 reductions can be made by developing a biogas industry and substituting renewable gaseous fuel sources for fossil fuels.

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3 A detailed assessment of variation in biomethane potential of first, second and third generation substrates

A detailed assessment of variation in biomethane potential of first, second and third generation substrates

Eoin Allen[a,b,c], David M. Wall[a,b,c], Christiane Herrmann[a,b,c], Jerry D. Murphy[a,b,c]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

[c] Science Foundation Ireland (SFI) Marine Renewable Energy Ireland (MaREI)

Abstract

There are a myriad of substrates available for anaerobic digestion, which may be categorised as: first generation substrates (such as food crops); second generation (such as grasses and wastes) and third generation (such as seaweed). This paper recounts analysis of 83 samples of substrates through assessment of biochemical methane potential (BMP) and through kinetic analysis of the produced BMP curve. Significant variation in the BMP of a substrate may be found depending on for example, season and method of harvest. Grass samples ranged from 156 L CH_4 kg^{-1} VS hay to 433 L CH_4 kg^{-1} VS first cut baled silage. Dairy slurry from the same farm varied from 175 L CH_4 kg^{-1} VS in autumn (cattle fed on concentrate at end of farming year) to 239 L CH_4 kg^{-1} VS in the summer when cattle are fed fresh grass. Seaweeds such as S. *latissima* generated a higher volumetric yield 36.4 m^3 CH_4 t^{-1} wwt than summer dairy slurry 16 m^3 CH_4 t^{-1} wwt. It is likely that the most sustainable and cheapest source of biomethane will be from wastes, but this will not satisfy all energy demands. Biomethane from agri-food wastes could satisfy 65% of energy in transport in Ireland. Larger resources will require third generation substrates such as seaweed.

Keywords: Biomethane potential assays; first, second and third generation feedstocks.

3.1 Introduction

3.1.1 Anaerobic digestion industry in Europe and Ireland

Anaerobic digestion (AD) is an emerging renewable energy source throughout Europe. There were over 13,800 biogas plants across Europe in 2012, however there are only 22 operational biogas plants in Ireland and 14 of these are associated with wastewater treatment (IEA Task 37, 2015). Ireland has the advantage of utilising recent advancements in anaerobic digestion technologies (and learning from the mistakes and successes of others) prior to extending the industry to the scale of other European Countries such as Germany with 10,020 digesters and Austria with 337 (IEA Task 37, 2015).

3.1.2 Matching substrates to the biogas industry

A key issue with an efficient national biogas industry is identifying potential substrates available in that country. Ireland has a large food and agricultural industry and exports significant quantities of food; for example 85% of beef is exported [1]. High volumes of potential wastes from food processing may be combined with cattle manure to fuel biogas plants. This will minimise the usage of agricultural land and direct competition with food while establishing waste treatment pathways with production of renewable energy.

Diary slurry is seen as an excellent co-digestion feedstock in biogas facilities not alone in that Ireland has a significant dairy industry. It has an optimal Carbon to Nitrogen (C:N) ratio (between 20 and 30). It can reduce volatile fatty acids (VFA) within a biogas reactor, add to the microbial diversity in the digester and supply essential nutrients to encourage biogas production [2, 3].

Perennial rye grass (PRG) is the main forage crop grown in Ireland with 92% of arable land covered by grass. Grass silage also has an optimal C:N ratio [4]

3.1.3 Biomethane as a source of renewable transport fuel

Biogas may be readily converted to biomethane via upgrading technology. Ireland has made much progress in renewable energy supply in electricity (RES-E) mainly through wind turbines. But this is

not the case with renewable energy supply in transport (RES-T). The Renewable Energy Directive states that 10% of energy use in transport should be renewable by 2020, [5]. A recent amendment to this Directive suggests that first generation biofuels from food crops be limited to 7% of energy in transport (Council of European Union, 2014). This puts on onus on second generation biofuel substrates such as biomethane from residues to match the shortfall of 3% by 2020. This is reinforced by the Alternative Transport Fuel Infrastructure Directive [6], which states that Member States shall ensure that compressed natural gas (CNG) refuelling points are available within at least 150 km of each other by 2020. The development of CNG stations will facilitate the use of biomethane as a gaseous transport biofuel.

3.1.4 Assessment of biogas resource

The biomethane potential (BMP) of the substrate (measured in L CH_4 kg^{-1} VS) may be multiplied by the practical available resource to assess the energy that may be produced.

Of concern in a country initiating a biogas industry is that inexperienced consultants may take values from literature; this is not advisable as substrates differ from region to region and country to country. Food wastes (FW) from Ireland may differ from food waste in France or Germany due to different diets and inclusion (or not) of yard waste (grass cuttings and pruning). The BMP of substrates can vary over the year. Early cut grass silage yields a higher BMP than late cut grass silage [7]. Dairy slurry can vary in content over the year depending on the diet the animals are fed and stage of lactation.

The BMP may be evaluated through use of the Buswell Method [8] which uses the stoichiometric equation of the substrate to generate a maximum possible theoretical BMP (section 3.2.2.5). The BMP may ideally be evaluated through laboratory experiment (section 3.2.2.2). The laboratory BMP assay will be less than the theoretical maximum assessed using the Buswell method.

3.1.5 Aims and objectives

The aim of this paper is to identify and sample potential substrate's biogas production and to undertake BMP assays for each sample. The objectives of this paper are to:

- Identify and classify available substrates;

- Asses theoretical and actual BMP of each substrate;
- Analyse all tested substrates for biodegradability and apply kinetic modelling and statistical analysis to all BMP results;
- Assess the energy yield per hectare per annum of crop substrates;
- Determine a biogas route to facilitate compliance with RES-T targets.

3.2 Materials and methods:

3.2.1 Materials

Eighty-three substrates were sampled and assessed. Criteria for selection included the most abundant and available substrates in Ireland. The substrates included first generation substrates (food crops) such as: beets, maize, cereal crops, oil seed rape, potatoes and turnips. Second generation substrates included grass silage, agri-food waste streams, and agricultural and municipal wastes. Third generation substrates included marine biomass such as green seaweed (*U. lactuca*) and brown seaweeds (*Saccharina latissima*, *Laminaria digitata* and *Ascophylum nodosum*). Various pretreatments were assessed such as drying, ensiling and macerating to highlight differences in potential methane yields.

Specific substrates such as dairy slurry, food waste and grass silage were examined in closer detail due to their abundance and the variety of sub-streams. Nine different slurries were sampled as well as poultry manure and farmyard manure. Ten variations of grass silage were sampled. These represent the various methods Irish farmers used to preserve and cut silage and include baling, ensiling, hay and 1st (early and late stage cuts) and 2nd cuts of silage. Eleven variants of food wastes were sampled. Milk processing wastes (MPW) and abattoir wastes were also sampled as various wastes are produced from these processes. Appendix A and B in supplementary data includes for a full list of substrates.

3.2.2 Methods

3.2.2.1 Proximate and analytical methods

Each sample was analysed initially for total solids (TS) content and volatile solid (VS) content by drying to 105^{o}C for 24 hours and again placing samples in a furnace heated to 550^{o}C for 6 hours as described by APHA standards [9]. To determine a theoretical yield for each sample the Carbon, Nitrogen, Hydrogen and Oxygen percentage of each sample (<1mm particle size) was obtained from a dried sample of each substrate by a CE 440 elemental analyser in triplicate. The C:N ratio was also observed from the elemental analysis. To facilitate in the completion of successful BMP assays and to ensure homogeneity amongst each substrate, all samples were macerated to 5mm particle size by a lab scale macerator. Liquid samples were mixed by a lab scale blender prior to all analysis tests. Liquid samples were tested for soluble chemical oxygen demand (SCOD) using Hach Lange CLK 914 cuvettes. pH was obtained using a Jenway 3510 pH meter.

The biodegradability index (ratio of BMP assay yield to theoretical yield) was calculated for all substrates. This indicates the level of VS destruction of the substrate over 30 days.

3.2.2.2 BMP procedure

The same BMP apparatus was used on all of the substrates to ensure repeatability of results. Two Bioprocess AMPST® II units were used in tandem: all samples were run in triplicate. Inoculum and cellulose controls were conducted for each run; this allowed for 8 substrates to be analysed for each run of the BMP system. An inoculum to substrate ratio (I:S) on a VS basis, of 2:1 was chosen for these trials [10]. Nitrogen was used to flush the head space of the 400 ml reactors prior to commencing each BMP assay. The mixing system alternated between on and off for 60 seconds at 30 rpm. Reactor vessels were placed in a water bath and heated to 37^{o}C for the duration of the experiment. The biogas produced was then passed through a solution of 3M NaOH to remove CO_2, H_2S and other gaseous impurities. An electronic gas-tipping device recorded the volume of biomethane, which was produced from each reactor vessel. The total biomethane produced from the inoculum was averaged and subtracted from the volume of biomethane produced by the individual substrate, to determine the specific methane yield of the substrate. Automatic adjustment

was completed for standard temperature and pressure for all results. Overestimation in the flush gas was corrected for also by the AMPTS II system. In order to allow for the numerous substrates the inoculum was chosen from two different sources. One half was from a continuous lab scale reactor, which was fed grass silage, dairy slurry and macro algae. The other half of the inoculum came from the previous BMP assay run. Both inoculum sources were mixed to a homogeneous state and analysed for solids composition.

3.2.2.3 Statistical analysis

To compare a run of 8 BMP substrates to another run of 8 other substrates, the method of analysis of variance (ANOVA) was used to determine the influence of the substrate on biochemical yield. The BMP run was regarded as the block effect and the substrate as the main effect for the ANOVA test. The test procedure SIMULATE was used from the statistical analysis programme SAS 9.3. A significance of differences in methane yields between substrates was determined by multiple comparisons, with a significance level, α (set at $p \leq 0.05$).

3.2.2.4 Kinetic analysis

Kinetic analysis was performed on the cumulative production curves produced from each BMP assay. Kinetic modelling was used to create an insight into the biodegradability of each substrate. It is also used as a method of physical comparison for the cellulose BMP assays from each run. This can help to illustrate any differences between BMP runs and highlight any outlying results if kinetic values deviate from average results. A first order differential equation was used to determine the decay constant value, k (Eqn. 3.1). The modified Gompertz formula (Eqn. 3.2) was used to determine the remaining kinetic biodegradability values associated with BMP assays.

$$Y(t) = Y_m \,.\left(1 - \exp^{(-kt)}\right) \qquad \text{Eqn. 3.1}$$

$$M(t) = P \cdot \exp\{-\exp[\frac{R_{max} \cdot e}{P}(\Delta - t)] + 1\} \qquad \text{Eqn. 3.2}$$

Y(t) is the cumulative biomethane yield (L CH_4 kg^{-1} VS) at a digestion time t (days). Y_m is the maximum biomethane potential (L CH_4 kg^{-1} VS) of the substrate added. k the decay constant ($days^{-1}$). k is a measure of the rate that the substrate has been degraded. M(t) is the cumulative biomethane yield (L CH_4 kg^{-1} VS) at a given time t (days). P is the maximum biomethane potential (L CH_4 kg^{-1} VS) of the

substrate from the BMP test. R_{max} is the maximum biomethane production rate (L CH_4 kg^{-1} VS d^{-1}). Δ the lag phase is a measure of how long it takes (days) before biochemical methane production starts to occur. t is the time (days). T_{50} is the half-life (days) and is a measure of how long it takes to produce half of the maximum cumulative yield of biomethane. R^2 is a measure of how the kinetic equation model fits the curve of biomethane production (%).

3.2.2.5 Theoretical yields

Data for elemental compositions collected on each substrate was used to create theoretical methane yields using the Buswell equation (Eqn. 3.3). The Buswell equation is a method to determine the maximum BMP yield of substrates by converting all available VS to methane and carbon dioxide. This stoichiometric equation however assumes all donated electrons are used entirely for metabolic energy, which does not allow for the development of the anaerobic bacteria or losses within the system. This lends itself to an overestimation in the theoretical yield. A ceiling value however is established for the chosen substrates and a biodegradability index (BMP_{assay}/ $BMP_{Buswell}$) can be established which can indicate how well a substrate can be degraded.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \qquad \text{Eqn. 3.3}$$

3.3 Results and discussion

3.3.1 Proximate and elemental analysis

A wide range of substrates was sampled for these trials (Tables 3.1 to 3.6). Assessments included for: proximate analysis (TS and VS); ultimate analysis (the percentage of the dry solids which are Carbon, Hydrogen, Nitrogen, Oxygen); C:N ratio; specific methane yields (theoretical and laboratory BMP result); biodegradability index (ratio of BMP_{assay}/$BMP_{Buswell}$) and the results of the kinetic analysis. The solids content should be below 10% for a continuously mixed tank reactor (CSTR). The optimum C:N ratio is between 25 and 30:1 for a biogas reactor [11]. Below this range there is potential for inhibition from ammonia [12]. High percentages of TS and VS are preferable so as to reduce energy used in transportation and increase net energy yields per tonne wet weight (wwt). Substrates are

grouped into 6 main groups. The 6 wastes were: agricultural wastes; grass; crops; food processing residues; municipal wastes: and seaweed.

3.3.2 First generation biofuels

Twelve terrestrial feed crops were analysed from farms throughout County Cork, in the south of Ireland (Table 3.1). The crops included cereal crops, beets and potatoes. In Ireland cereal crops are dominated by barley and wheat. Spring and winter cereals were assessed. Whole crop (seed and stalk) was sampled for these trials. First generation biofuels have the distinct disadvantage of competing directly with land, which could be used for food. Provision of biofuels from crops are also capped at a maximum contribution to RES-T of 7% [6].

3.3.2.1 Cereal crops

No significant variation in BMP yields, were observed between the 4 samples of cereals: winter barley (harvested in July) and spring barley (harvested in August) or winter wheat (harvested in July) and spring wheat (harvested in September). BMPs of the samples ranged from 340.16 $\pm$ 9.56 L CH_4 kg^{-1} VS (spring wheat) to the maximum yielding crop of 366.53 $\pm$ 14.80 L CH_4 kg^{-1} VS (winter barley). Triticale and winter oats returned lower yields of 314.11 $\pm$ 7.61 L CH_4 kg^{-1} VS and 281.26 $\pm$ 4.57 L CH_4 kg^{-1} VS respectively (both harvested in August).

3.3.2.2 Oil seed rape

Oil seed rape (OSR) was initially introduced as a feedstock for biodiesel production in Ireland but has seen a switch over to a use as a feedstuff. It has a high lipid content and C:N ratio of 21:1 which makes it, potentially, a very high yielding biofuel. The BMP yielded 646.30 $\pm$ 7.39 L CH_4 kg^{-1} VS (macerated and harvested in July). The whole crop of OSR before it was sprayed off for harvesting (2 weeks prior to harvesting) yielded 318.92 $\pm$ 10.91 L CH_4 kg^{-1} VS. Un-macerated OSR however yielded a significantly lower yield of 215.17 $\pm$ 12.47 L CH_4 kg^{-1} VS. This can be attributed to the hard shell in which the OSR is enclosed. The crushing or pulverising of the crop as a pre-treatment, which increased the BMP yield by over 200% is a necessary step for biomethane production.

3.3.2.3 Root crops

Two varieties of potatoes sampled produced BMP yields of 351.01 ± 12.27 L CH_4 kg^{-1} VS (Rooster variety) and 337.56 ± 12.94 L CH_4 kg^{-1} VS (Kerr Pink variety). Both substrates had high C:N ratios (> 59:1). Turnips are easily tilled and grow well in Ireland. The BMP revealed a yield of 398.61 ± 8.62 L CH_4 kg^{-1} VS. Harvest dates occurred in October.

Three varieties of beets were assessed. Beets have been extensively studied in continuous mono-digestion and have been found to operate well over long periods of time without the supplementation of dairy slurry or trace elements [13]. Fodder beets are the easiest to grow in terms of fertilizer inputs and are popular with dairy farmers. All three beet varieties were harvested in December. The BMP yield was 332 ± 5.01 L CH_4 kg^{-1} VS. The second variety was a sugar beet sub variety termed energy beet. This was developed to produce higher levels of sugars and dry solids content to increase yield and profitability per hectare. This was reflected in the yield. Whilst sugar beet yielded 344.18 ± 12.36 L CH_4 kg^{-1} VS, energy beet yielded 375.13 ± 6.93 L CH_4 kg^{-1} VS. Beets had high C:N ratios, 54 – 65:1. Beets must be considered a good biofuel feedstock in Irish agriculture due to a strong knowledge of beet growing due to a large sugar industry, which has closed in recent years.

The whole beet was harvested for these trials. On average the beet tops made up 45% of the mass of the washed beet plant and yielded a BMP yield of 306 ± 12.75 L CH_4 kg^{-1} VS. The beet tops had a low C:N ratio of 12.9:1.

Table 3.1 Analysis of methane potential from first generation biofuel crops.

Substrate	TS %	VS %	C %	H %	N %	C:N	Theoretical **BMP** L CH_4 kg^{-1} VS	BMP assay L CH_4 kg^{-1} VS	SD $\pm$ L CH_4 kg^{-1} VS	Biodegradability index	Specific yield m^3 t^{-1} wwt	K value d^{-1}	Lag d	T_{50} d
Cereal crops														
Winter Barley	55.79	53.74	44.38	5.87	0.60	73.97	438	366.53	14.80	0.84	196.97	0.19	0.40	3.58
Spring Barley	68.64	67.08	44.17	6.32	0.64	69.37	439	361.81	22.81	0.82	242.70	0.15	0.16	4.50
Winter Wheat	59.52	54.07	44.03	6.42	1.12	39.20	505	354.48	6.16	0.70	191.67	0.18	0.48	4.01
Spring Wheat	66.95	63.70	43.92	6.23	1.15	38.19	446	340.15	9.56	0.76	216.68	0.15	0.04	4.66
Triticale	54.36	48.98	44.12	6.51	1.45	30.43	475	314.11	7.61	0.66	153.85	0.17	0.14	3.94
Winter Oats	63.89	61.90	44.39	6.38	0.55	80.70	450	281.26	4.57	0.63	174.10	0.15	0.12	4.60
Oil seed rape														
OSR (macerated)	92.62	87.55	58.83	8.69	2.80	21.01	772	646.30	7.39	0.84	565.84	0.090	2.90	7.57
OSR Whole Crop	88.49	84.92	50.68	7.43	1.90	26.63	590	318.92	10.91	0.54	270.83	0.13	0.12	5.30
OSR not macerated	92.42	88.60	59.98	8.51	2.54	23.65	762	215.16	12.47	0.28	190.63	0.09	1.80	7.36
Root crops														
Potatoes, Roosters	25.75	24.72	42.32	5.94	0.71	59.61	416	351.01	12.27	0.84	86.77	0.23	0.82	2.43
Potatoes, Kerr pinks	24.34	23.35	42.45	6.02	0.67	63.04	421	337.56	9.91	0.80	78.82	0.23	0.85	2.45
Turnips	10.65	9.99	41.10	5.65	1.80	22.83	416	398.61	8.62	0.96	39.82	0.130	1.00	5.20
Sugar beet	22.62	22.08	42.05	6.15	0.65	65.02	407	344.18	12.36	0.85	75.99	0.290	0.47	2.36
Energy beet	22.77	22.14	41.68	6.13	0.78	53.67	395	375.13	6.93	0.95	83.05	0.260	0.40	2.63
Fodder Beet	16.58	15.12	42.50	5.77	1.40	30.43	460	332.60	5.01	0.72	50.29	0.14	1.27	6.44
Sugar beet tops	12.06	9.67	41.06	5.12	3.17	12.94	544	306.01	12.75	0.56	29.59	0.230	0.33	3.01

Standard deviation (SD)

3.3.3 Second generation biofuels – Grasses

Fifteen samples of grass and maize substrates were collected. These include bales of silage, pit or clamp silage, hay, fresh grass and fresh maize. Different stages are included such as 1st cut silage, 2nd cut silage and a late 1st cut silage. Silage effluent and digestate from a biogas reactor fed 100% grass were also included (Table 3.2). Grass offers great potential as a biofuel for the AD industry in Ireland as it qualifies as a second generation biofuel (lignocellulosic material) and is liable for double credits in assessing the RES-T target for 2020. It is the most abundant crop in Ireland covering 92% of agricultural land [14].

3.3.3.1 First cut of silage grass

First cut clamp silage from perennial rye grass were sampled from three separate clamps in three different areas in Ireland during the early growth stage (May – June). Yields of 399.56 $\pm$ 4.12 L CH_4 kg^{-1} VS (Midlands), 389.61 $\pm$ 3.06 L CH_4 kg^{-1} VS (North) and 374.48 $\pm$ 29.69 L CH_4 kg^{-1} VS (South) were encountered. Statistically there was no significant difference. A late 1st cut sample was also assessed and yielded 392.55 $\pm$ 6.83 L CH_4 kg^{-1} VS (Midlands). This showed no significant difference from a regular first cut harvest. Fresh un-ensiled grass yielded a BMP of 367.83 $\pm$ 8.79 L CH_4 kg^{-1} VS (South). Grass, which was not ensiled, resulted in the lowest yielding of first cut substrates. It is well documented that ensiling can increase the yield of a feedstock in conjunction with being a cheap method of year round preservation [15]. All 1st cut grass silages are favourable substrates with optimal C:N ratios (>25:1) and Biodegradability indices of 0.79 to 0.90.

3.3.3.2 Baled silage

Baled silage was encased in a plastic wrap allowing for less effluent to escape. This may account for the higher observed yields as the sugar rich effluent doesn't escape. Yields of 432.85 $\pm$ 8.59 L CH_4 kg^{-1} VS (North, 1st cut) and 428.36 $\pm$ 7.79 L CH_4 kg^{-1} VS (North, 2nd cut) for baled silage from the same sources were achieved.

3.3.3.3 Other grass substrates

Second cut grass silage harvested in July, yielded 367.99 ± 6.19 L CH_4 kg^{-1} VS (South). Statistical analysis showed there was no significant difference between 1st and 2nd cut samples from the same origin despite the reduction in yields. A further observation made was the particle size of the grass and its effect on BMP values. A sample of late first cut PRG (Midlands) was only lightly cut to 30mm in size and produced a BMP value of 315.56 ± 20.46 L CH_4 kg^{-1} VS, a reduction of 77.0 CH_4 kg^{-1} VS (20%) on macerated late cut silage (Midlands). Other methods of grass preservation such as making hay were trialled. This produced a grass with a high dry solids content (87.4%) but caused a very large reduction in BMP yield, 156 ± 19.0 L CH_4 kg^{-1} VS (South). This may be expected as hay is left to dry for over 7 days which leads to a reduction in sugars present in the grass necessary for high BMP yields. An experimental grass (Szarvasi) yielded a BMP value of 311 ± 4.80 L CH_4 kg^{-1} VS. An advantage of Szarvasi is it is a perennial crop, which can be harvested twice yielding up to 15 t DM/ha (yields from sampled farm). Such a crop can help reduce GHG emissions and the carbon footprint of a grass based biofuel. This sample was sourced from South Germany but is readily adaptable to Irish growing conditions. The best preforming BMP yield however was from silage effluent, 553.68 ± 14.69 L CH_4 kg^{-1} VS. As it has a low C:N ratio (6.92) it may be only co-digested in low percentages.

3.3.3.4 Maize

Maize forage crop, which makes up a majority of German and Austrian biogas substrates were sampled from a farm in Cork, Ireland. The maize crop was grown using a Samco system©, which lays a layer of biodegradable plastic over the seed bed which encourages a green-house type effect. A fresh sample (harvested in September) producing 354.06 ± 12.94 L CH_4 kg^{-1} VS and an ensiled sample produced 394.08± 13.51 L CH_4 kg^{-1} VS. The ensiling process showed that an increase of BMP yield could be achieved. The specific yields of maize (121 – 127 m^3 t^{-1} wwt) showed that the maize whole crop could out preform the best grass substrates (107 m^3 t^{-1} wwt).

Table 3.2 Analysis of biomethane potential of grass.

Substrate	TS %	VS %	C %	H %	N %	C:N	Theoretical BMP L CH_4 kg^{-1} VS	BMP assay L CH_4 kg^{-1} VS	SD ± L CH_4 kg^{-1} VS	Biodegradability index	Specific yield m^3 t^{-1} wwt	K value d^{-1}	Lag phase d	T_{50} d
1st Cut grass silage														
Grass silage (Midlands)	29.27	26.84	43.32	5.88	1.67	25.94	443	399.56	4.12	0.90	107.24	0.11	1.94	7.65
Grass silage (North)	15.38	13.26	42.40	5.60	1.74	24.37	503	389.61	3.06	0.77	51.66	0.153	0.10	4.76
Grass silage (South)	12.22	11.25	44.47	5.65	1.02	43.74	472	374.48	29.69	0.79	42.13	0.10	3.20	8.86
Late cut silage (Midlands)	17.85	16.42	43.80	5.91	1.08	40.43	469	392.56	6.83	0.84	64.46	0.14	0.54	5.01
Fresh Grass (south)		20.42	43.14	5.84	1.92	22.51	472	367.83	8.79	0.78	75.11	0.16	0.60	4.50
Baled silage														
Silage bales (1st cut)	16.81	15.32	45.25	5.95	1.48	30.64	507	432.86	8.59	0.85	66.31	0.147	0.11	4.85
Silage bales (2nd cut)	17.35	15.90	44.43	5.94	1.75	25.34	493	428.36	7.79	0.87	68.11	0.166	0.15	4.50
Other grass substrates														
Silage (2nd cut, south)	24.96	23.14	44.30	5.96	1.27	34.97	477	376.99	6.19	0.79	87.23	0.153	0.08	4.80
Late 1st cut silage (Midlands NM)	22.16	20.42	43.80	5.91	1.08	40.43	598	315.56	20.46	0.53	64.44	0.110	0.79	6.04
Hay	87.38	82.17	44.86	5.77	1.59	28.15	470	156.03	19.00	0.33	128.21	0.09	4.84	10.37
Savazi grass (silage)	23.83	20.80	42.18	5.81	2.35	17.95	503	311.25	4.80	0.62	64.74	0.110	1.28	6.57
Silage effluent	4.55	3.09	34.76	4.79	5.02	6.92	599	553.68	14.69	0.92	17.11	0.29	0.00	2.32
Grass digestate	5.36	3.92	-	-	-	-	/	126.83	11.30	/	4.97	0.11	0.21	6.30
Maize substrates														
Fresh Maize	32.83	31.77	43.85	6.21	1.17	37.48	426	354.06	12.94	0.83	112.48	0.23	0.21	2.98
Maize silage	33.48	32.46	44.72	6.32	1.23	36.36	458	394.08	13.51	0.86	127.92	0.170	0.10	3.94

*NM = Not macerated, cut to 30mm. Data adapted from [4].

3.3.4 Second generation biofuels - Agricultural wastes

In total, 11 agricultural wastes were collected in these trials (Table 3.3). Slurries in particular are well documented as a co-digestion feed stock for various reasons such as: micro nutrient availability; high C:N ratio; year round availability; and reasonable BMP yields. However there is a variation in BMP values at different times of the year of dairy slurries sampled due to a production cycle that varies largely due to stopping and restarting of dairy cows lactation. Other farm wastes such as beef slurry, poultry manure, farm yard manure (FYM) and pig slurry do not have large variations in their compositions due to a constant year round production cycle without having to stop for calving and a drying off period, as seen in dairy farming.

3.3.4.1 Dairy slurries

Samples showed variations in TS and VS composition, ranging from 5.55 to 9.10 % TS and 4.44 to 6.69% VS. A relationship between the diet and production period of the dairy cows and dairy slurry BMP was established. Cows fed 2kg d^{-1} of concentrate feed (CF) at the end of the farming year (Slurry Autumn) grazing poor quality grass kept indoors for half a day yielded 175.45 $\pm$ 13.87 L CH_4 kg^{-1} VS. As the diet was stepped back up in terms of energy content, when the cows were fed good quality silage and CF, there was an observed increase in BMP yield. The increase in energy yield of the cows diet, was related to the stage of production, from a drying off period (Cows not milking) and not fed CF (December to January, A), calving period and transition to a higher energy diet (2 – 6 kg d^{-1} CF) (February to April, B) and finally a full milking diet with cows being fed a fresh grass diet supplemented by CF (2 – 3 kg d^{-1}) from April throughout the summer (Summer C), resulting in BMP yields of 199.97 $\pm$ 1.71 L CH_4 kg^{-1} VS (A), 214.01 $\pm$ 9.03 L CH_4 kg^{-1} VS (B), 238.11 $\pm$ 4.71 L CH_4 kg^{-1} VS (C, 2012), and 239.39 $\pm$ 9.46 L CH_4 kg^{-1} VS (C, 2013) respectively.

These observed results can be factored into assigning an average value for dairy slurry to accurately forecast yields for a biogas industry (Table 3.8). This average yield was 207.7 L CH_4 kg^{-1} VS.

Heifer animals 1-2 years in age fed a low energy diet (not being milked) produced the lowest BMP value 136.05 $\pm$ 11.97 L CH_4 kg^{-1} VS. The lowest observed solids percentage (5.55% TS) came from dairy parlour washings. This was expected due to level of water used to wash down collecting yards and milking parlours, causing dilution of the dairy slurry. Similar results were found by Hellwing,

Weisbjerg [16] where increased biogas yields were observed in slurry from dairy cows fed maize and grass silage, than when fed higher energy diets and they observed enteric methane emissions were lower from the cows when the biogas production was higher. One adverse trait of Dairy slurries is the low Biodegradability Index (0.26 – 0.62). This can mainly be attributed to the high percentage of fibre which is concentrated in cattle slurries [16].

3.3.4.2 Alternative agricultural wastes

Beef slurry had an observed TS and VS of 8.44 % and 6.76% respectively, which was higher than the average dairy slurry sample and yielded a BMP of 310.79 $\pm$ 6.08 L CH_4 kg^{-1} VS. Pig slurry was at the lower end of solids composition and C:N ratio. Pig slurry produced the lowest BMP from this grouping, 99.29 $\pm$ 8.79 L CH_4 kg^{-1} VS. C:N ratios were between 17.05 and 24.87 for dairy slurries; beef slurry had a C:N of 16.15. The remaining farm wastes had lower values with poultry manure producing a C:N ratio of 13.24, highlighting the poor yields and ammonia inhibition associated with poultry manure [17].

Table 3.3 Analysis of methane potential from agricultural waste.

Substrate	**TS %**	**VS %**	**C %**	**H %**	**N %**	**C:N**	**Theoretical BMP** L CH_4 kg^{-1} VS	**BMP assay** L CH_4 kg^{-1} VS	**SD** $\pm$ L CH_4 kg^{-1} VS	**Biodegradability index**	**Specific yield** m^3 t^{-1} wwt	**K value** d^{-1}	**Lag phase** d	**T50** d
							Dairy Slurries							
Slurry Autumn	6.50	5.10	42.25	5.36	1.98	21.34	575	175.45	13.87	0.31	8.95	0.13	1.99	6.55
Slurry (Dec – Jan (A)	7.11	5.75	42.80	5.88	2.51	17.05	606	199.77	1.71	0.33	11.49	0.09	4.40	9.26
Slurry Feb – Apr (B)	9.10	6.56	40.05	5.06	2.24	17.90	525	214.01	9.03	0.41	14.04	0.15	0.99	4.36
Slurry Summer (C, 2012)	6.10	4.78	39.31	5.22	1.90	20.72	525	238.11	4.71	0.45	11.38	0.15	0.85	4.25
Slurry Summer (C, 2013)	8.75	6.69	41.95	5.38	2.2	19.07	389	239.39	9.46	0.62	16.02	0.082	2.45	10.13
Slurry (dairy heifers)	7.84	5.51	43.26	5.84	2.19	19.75	516	136.05	11.97	0.26	7.50	0.12	1.56	4.29
Parlour washings	5.55	4.44	41.45	5.67	1.67	24.87	557	208.89	7.26	0.38	9.27	0.15	0.48	4.49
							Other agricultural wastes							
Slurry (beef)	8.44	6.76	42.49	5.78	2.63	16.15	529	310.79	6.08	0.59	21.01	0.093	1.62	7.44
Pig slurry (Mix)	5.42	4.16	39.65	5.22	2.97	13.35	330	99.29	8.79	0.30	4.13	0.07	1.98	5.11
Poultry manure	51.46	29.72	31.15	4.26	2.35	13.24	614	228.26	2.32	0.37	67.84	0.19	0.50	3.61
Farm yard manure	19.80	17.09	41.91	5.59	2.93	14.32	506	137.96	2.73	0.27	23.58	0.05	7.56	15.88

3.3.5 Second generation biofuels - Food processing wastes

3.3.5.1 Milk processing wastes

Wastes were sampled from a cheese and skimmed milk products processing plant, processing 160 m l yr^{-1}. These wastes are commonly referred to as milk processing wastes (MPW). Four samples were taken from separate stages of the waste water treatment process (WWTP) which gave varying results, ranging from waste activated sludge (WAS) 188.57 $\pm$ 3.57 L CH_4 kg^{-1} VS from an aeration basin, to the initial waste which first rises in a dissolved air floatation (DAF) tank which is high in sugars including lactose and pectin (787.36 $\pm$ 59.51 L CH_4 kg^{-1} VS). A final slurry mix, which is a combination of all present waste streams, which is maintained at between 8 to 10% TS was sampled producing a yield of 461.19 $\pm$ 31.20 L CH_4 kg^{-1} VS (8% sludge). This sludge stream may also be produced without a direct DAF input, which was also sampled for these trials. This waste is then mixed with a lime additive to produce a cake mixture which is roughly 35% TS and is land spread. Using the final slurry as an AD feedstock would avoid this final caking stage.

3.3.5.2 Abattoir waste

In 2011, 1.64m cattle and 5.31m sheep and pigs were slaughtered in Ireland [1]. This is a large potential for a country of 4.4 million people. Three separate wastes were evaluated from abattoirs: WAS; Paunch content (undigested residue from the animals); and green sludge (part of the processing waste from slaughtering). They produced BMP yields of 165.74 $\pm$ 13.37 L CH_4 kg^{-1} VS, 238.25 $\pm$ 16.31 L CH_4 kg^{-1} VS and 403.54 $\pm$ 18.78 L CH_4 kg^{-1} VS respectively. The green sludge had increased yields due to addition of fatty material added along the stages of production. A mix of the 3 streams was sampled, as all 3 streams are combined when land spreading the wastes; a yield of 336.45 $\pm$ 15.74 L CH_4 kg^{-1} VS was obtained. One issue associated with abattoir waste is the accumulation of ammonia within biogas reactors operated continuously due to its low C:N ratio (11.81 for the mixed sample). This may be overcome through co-digestion as is the case with MPWs [18].

3.3.5.3 Miscellaneous commercial wastes

Miscellaneous commercial wastes were assessed including for: bakery wastes, bread wastes, brewery stillage, grocery wastes, fish offal and city park/grass waste. Fish offal recorded the highest

BMP (591.82 ± 76.71 L CH_4 kg^{-1} VS). The high yield can be attributed to high levels of proteins and oils present in fish offal, however this can lead to adverse effects [19] due to its low C:N ratio (8.66). Bakery, grocery and bread wastes all produced high yields; 529.23 ± 27.26 L CH_4 kg^{-1} VS, 421.59 ± 44.47 L CH_4 kg^{-1} VS and 396.72 ± 7.53 L CH_4 kg^{-1} VS respectively. Brewing stillage was also collected from a local brewery in the city of Cork, Ireland. It produced a BMP yield of 331.93 ± 10.95 L CH_4 kg^{-1} VS.

Two final wastes from a local municipality were city park and grass waste (CPGW) and WWTP sludge. These had relatively low BMP yields: WWTP sludge yielded 247.41 ± 11.02 L CH_4 kg^{-1} VS; CPGW yielded 252.58 ± 26.03 L CH_4 kg^{-1} VS.

Table 3.4 Analysis of methane potential of food processing wastes.

Substrate	TS %	VS %	C %	H %	N %	C:N	Theoretical BMP L CH_4 kg^{-1} VS	BMP assay L CH_4 kg^{-1} VS	SD ± L CH_4 kg^{-1} VS	Biodegradability index	Specific yield m^3 t^{-1} wwt	K value d^{-1}	Lag phase d	T_{50} d
Milk processing waste														
WAS	15.92	8.85	24.90	4.10	4.60	5.41	530	188.57	3.57	0.36	16.69	0.13	1.2	10.8
8 % sludge (without DAF sub-stream)	8.63	7.3	55.1	8.62	3.46	15.92	508	454.28	19.41	0.89	33.16	0.11	3.32	11.2
8 % sludge	9.42	7.62	43.9	6.8	5.6	7.84	492	461.19	31.20	0.94	35.14	0.16	0.81	3.7
DAF	3.94	3.09	65.1	10.3	1.3	50.08	826	787.36	59.51	0.95	24.32	0.1	2.34	5.07
Abattoir wastes														
Abattoir waste (mix)	16.64	14.84	48.32	6.85	4.09	11.81	481	336.45	15.74	0.70	49.92	0.15	0.60	5.50
Abattoir waste (paunch content)	17.01	15.62	46.50	6.30	2.80	16.61	469	238.25	16.31	0.51	37.21	0.09	0.51	5.6
Abattoir waste (green sludge)	19.62	18.11	57.30	8.40	3.00	19.10	683	403.54	18.78	0.59	73.08	0.18	0.98	5.3
Abattoir waste (DAS)	13.3	10.78	41	5.8	6.5	6.31	408	165.74	13.37	0.41	17.87	0.17	0.55	4.2
Miscellaneous wastes														
Bakery waste	45.72	42.02	52.70	8.20	2.80	18.82	696	529.23	27.26	0.76	222.36	0.08	3	5.45
Brewing stillage	22.80	21.68	49.83	6.75	4.61	10.82	592	331.93	10.95	0.56	71.96	0.11	2.04	8.10
Grocery waste	16.29	15.28	49.48	6.88	2.60	19.01	584	421.59	44.47	0.72	64.42	0.22	0.52	3.19
Fish offal Mix	39.50	33.95	55.08	6.57	6.36	8.66	798	591.82	76.71	0.74	200.92	0.08	0.41	6.02
Bread waste	66.19	63.59	45.07	6.27	2.61	17.25	472	396.72	7.53	0.84	252.27	0.22	0.10	3.22
CPGW (City park and grass waste)	23.22	20.79	44.79	5.77	3.08	14.56	525	252.58	26.03	0.48	52.51	0.13	1.93	6.87
WWTS (municipal)	8.61	6.77	43.3	5.8	2.2	19.68	406	247.41	11.02	0.61	16.75	0.18	0.52	4.66

Data adapted from [21].

3.3.6 Second generation biofuels - Municipal wastes

Domestic and commercial food waste represent a large proportion of Ireland's municipal solid waste (MSW) fraction and in 2010 accounted for 0.82 m tonnes which was landfilled, incinerated or composted [20].

This resource currently is underutilised and if diverted to AD would offset GHG emissions from landfilling and incineration rather than add to GHG. Food waste is a readily digestible substrate due to the levels of fats and sugars present, especially in the liquor proportion.

Thirteen samples of food waste were collected to represent the various origins and variety of substrates. Values were adapted from previously published work completed in conjunction with this body of work to form a total range of potential municipal wastes substrates [21, 22]

3.3.6.1 Rural and urban domestic food waste

Rural food waste (RFW) and urban food waste (UFW) sourced from a domestic source segregated waste stream, yielded BMP values of 367.82 $\pm$ 6.32 L CH_4 kg^{-1} VS and 343.75 $\pm$ 2.91 L CH_4 kg^{-1} VS. During summer periods the addition of garden wastes is added to these waste streams from their source collection point. This had the effect of significantly reduced the BMP yield of RFW and UFW, by 93.66 and 47.04 L CH_4 kg^{-1} VS respectively. There was no significant differences observed between the RFW and UFW in terms of BMP yield, but there was a significant reduction in yields when the addition of grass cuttings was included.

3.3.6.2 Commercial and domestic streams of food waste

Food wastes from canteens and restaurants (CFW) were assessed in the summer and winter to represent the change in seasonal diet. CFW sampled in winter achieved a BMP of 490.97 $\pm$ 4.81 L CH_4 kg^{-1} VS. BMP yields increased to 534.55 $\pm$ 4.99 L CH_4 kg^{-1} VS for a summer sample. Further samples of food wastes were taken from a centralised collection centre (FWCCC), which consisted of food wastes from both rural and urban locations with only partial source segregation from separate domestic and commercial origins. These two substrates produced BMP yields of 419.62 $\pm$ 48.90 L CH_4 kg^{-1} VS and 535.27 $\pm$ 20.04 L CH_4 kg^{-1} VS for a domestic and commercial sample respectively. These

samples displayed a similar result where domestic sampled food waste produced a lower BMP value than a commercially sourced food waste. These two streams were also mixed before they left the centre, as is the processing procedure. The mixed sample recorded a yield of 508.44 ± 24.53 L CH_4 kg^{-1} VS. A dried sample of domestic food waste (FWCCC sample) was also trialled with a BMP value of 396.31 ± 5.14 L CH_4 kg^{-1} VS, highlighting the biogas potential and the soluble fats present in the liquor of food wastes.

3.3.6.3 Analysis of food wastes

Analysing the recorded parameters associated with food wastes substrates gave a further indication to which exact stream or sub-stream produced the best results as a feedstock. All sampled substrates had k values in the higher range of: 0.15 – 0.22 d^{-1} (except for the dried sample, where the kinetic value was 0.13 d^{-1}). Low C:N ratios were observed for all samples ranging from 12.6:1 to 17.3:1. This may contribute to inhibition when digested as suggested by Banks, Salter [23] due to VFA inhibition, even at low organic loading rates (OLR) making it a difficult substrate to mono-digest. A sample of recycled paper and cardboard waste was also analysed; this recorded a C:N ratio of 52.29, which make it an ideal substrate to co-digestate. It yielded a BMP of 254.19 ± 4.34 L CH_4 kg^{-1} VS, which was greater than the maximum yielding dairy slurry.

3.3.6.4 Alternative wastes

Two final substrates associated with food wastes and direct food consumption are, used cooking oil (UCO) and grease trap waste (GTW). UCO had the highest of all substrates sampled, 804.61 ± 57.0 L CH_4 kg^{-1} VS, largely due to its high lipid content as an oil and saturated fats collected during the cooking process. GTW yielded a BMP value of 416.59 ± 6.85 L CH_4 kg^{-1} VS. Elemental analysis could not be conducted on these 2 substrates due to the high percentage oil content, which was not compatible with the elemental analyser used. Values from literature were used in their absence [3].

Table 3.5 Analysis of methane potential of municipal wastes

Substrate	TS %	VS %	C %	H %	N %	C:N	Theoretical BMP L CH_4 kg^{-1} VS	BMP yield L CH_4 kg^{-1} VS	SD ± L CH_4 kg^{-1} VS	Biodegra-dability index	Specific yield m^3 t^{-1} wwt	K value d^{-1}	Lag phase d	T_{50} d
Rural and Urban domestic food waste														
RFW (with grass)	33.40	27.49	43.30	5.90	2.70	16.04	577	274.16	4.78	0.48	75.36	0.16	2.20	8.87
RFW (without grass)	30.6	27.05	44.90	6.60	3.10	14.48	566	367.82	6.32	0.65	99.50	0.18	2	8.66
UFW (with grass)	25.66	18.89	41.30	5.20	2.60	15.88	625	296.71	6.14	0.47	56.04	0.17	1.3	6.71
UFW (without grass)	31	29.08	46.50	7.30	3.70	12.57	564	343.75	2.91	0.61	99.96	0.15	2.2	9.32
Commercial and domestic streams of food waste														
CFW (summer)	32.8	30.37	49.00	7.00	3.40	14.41	620	534.55	4.99	0.86	162.36	0.21	2.3	9.34
CFW (winter)	23.8	21.42	48.20	70.00	3.60	13.39	620	490.97	4.81	0.79	105.17	0.19	2.5	10.74
FWCCC (mix)	28.72	25.14	48.80	7.30	3.25	15.02	537	508.44	24.53	0.95	127.82	0.17	6.75	12.80
FWCCC (domestic)	21.91	19.96	46.80	6.30	2.70	17.33	471	419.62	48.90	0.89	83.76	0.17	1.35	4.92
FWCCC (commercial)	35.4	30.11	49.00	7.00	3.40	14.41	550	535.27	20.04	0.97	161.17	0.22	3.26	8.42
FWCCC (domestic & dried)	95.12	87.51	50.57	7.21	3.68	13.74	569	396.31	5.14	0.70	346.81	0.134	1.1	4.12
Alternative wastes substrates														
Recycled paper & cardboard	90.15	77.81	41.66	5.46	0.80	52.29	476	254.19	4.34	0.53	197.78	0.14	2.02	6.43
UCO	99.90	99.12	-	-	-	-	986	804.61	57.00	0.82	797.53	0.091	3.57	9.71
Grease trap waste	27.36	26.01	-	-	-	-	975	416.59	6.85	0.42	108.35	0.22	0.11	3.56

Data adapted from [21, 22].

3.3.7 Third generation biofuels

In recent years research into macro algae (seaweed) as an AD feedstock has gained popularity and is been seriously examined as a potential biogas feedstock. Seaweed has been partially disregarded as an AD feedstock in countries of cooler climates for various reasons including the inhibitory concentrations of salts, high levels of nitrogen (in green seaweeds) and sulphur [24, 25]. Brown seaweeds such as kelps have TS percentage content comparable to energy crops and C:N ratios which can be as high as 31.9:1 [26]. Ireland has a coastline of over 7,500 km which makes both growing seaweed off shore and collecting beach cast seaweed very accessible [27]. Seaweeds also qualify as 3rd generation biofuels when used to produce biomethane as a transport biofuel. In assessing RES-T targets algal biomethane qualifies for double credit (of the energy production), with the added advantage of not competing with land based food production [28].

3.3.7.1 Seaweed and biomethane potential

Ten separate seaweed varieties were collected from the southern coastline of Ireland (51N -9E) in August 2013. Nine samples were categorised as brown seaweeds and 1 as green seaweed. From available literature three particular sugars which are present in brown seaweeds, which contribute to their BMP: alginate; laminarin; and mannitol [29]. The highest yielding BMP seaweed was *S. latissima* (also known as Sugar Kelp) with a BMP 341.46 $\pm$ 36.40 L CH_4 kg^{-1} VS and a C:N ratio of 23.9:1. The next best yielding seaweed was *S. polyschides* (263.25 $\pm$ 4.23 L CH_4 kg^{-1} VS) followed by *H. elongate* (260.81 $\pm$ 2.05 L CH_4 kg^{-1} VS) both having high C:N ratios (above 22:1). *L. digitata* and *A. nodosum* were the only remaining seaweeds with C:N ratios above 23:1.

No statistical significant difference was observed between samples of *U. lactuca* collected from two separate years from the same beach location, where the 2012 sample had a BMP yield of 205.27 $\pm$ 8.65 L CH_4 kg^{-1} VS and 2011 sample yielding 183.20 $\pm$ 5.83 L CH_4 kg^{-1} VS [27]. Four seaweeds yielded in excess of 30 m^3 CH per t wet weight (ww). These were (in descending order): S.*polyschides* (34.5); S.*latissima* (34.5); F.*spiralis* (32.7); and A.*nodosum* (32.3).

One aspect, which may encourage the use of seaweeds in the production of biogas is the high yields per hectare which could be achieved if farmed off shore. A yield of 30 tVS ha^{-1} yr^{-1} for *S. latissima* was reported [30]. Combined this with yields from these trials, a potential yield of 10,250 m^3 ha^{-1} yr^{-1} (363 GJ ha^{-1} yr^{-1}) for this seaweed variety, which is in the upper range of existing bio-energy crops (Allen et al., 2015).

Table 3.6 Analysis of methane potential of seaweed

Substrate	TS %	VS %	C %	H %	N %	C:N	Theoretical BMP L CH_4 kg^{-1} VS	BMP yield L CH_4 kg^{-1} VS	SD ± L CH_4 kg^{-1} VS	Biodegradability index	Specific yield m^3 t^{-1} wwt	K value d^{-1}	Lag phase d	T_{50} d
Brown seaweed														
H. elongate	12.65	8.10	30.81	4.07	1.38	22.33	334	260.81	2.05	0.78	21.13	0.18	1.17	4.24
L. digitata	14.20	10.34	34.19	4.78	1.46	23.42	479	217.56	4.14	0.45	22.54	0.19	0.79	3.85
F. spiralis	19.72	13.92	36.11	4.72	2.08	17.36	540	234.76	9.43	0.43	32.74	0.16	0.74	4.85
S. latissima	15.49	10.09	29.14	3.78	1.22	23.89	422	341.46	36.40	0.81	34.47	0.16	1.23	4.55
A. nodosum	23.16	19.44	40.38	5.3	1.62	24.93	488	166.52	20.00	0.34	32.33	0.12	0.32	7.48
F. serratus	19.72	13.92	37.08	4.76	2.38	15.58	532	101.71	9.37	0.19	14.16	0.18	1.62	3.84
F. vesiculous	21.18	16.11	26.81	3.24	1.54	17.41	249	126.34	11.38	0.51	20.35	0.22	0.50	3.10
S. polyschides	15.25	13.11	36.11	4.99	1.56	23.15	386	263.25	4.23	0.68	34.51	0.19	0.45	3.85
A. esculenta	18.72	11.91	29.3	4.24	1.89	15.50	474	225.98	5.66	0.48	26.91	0.19	0.50	3.61
Green seaweed														
***U. lactuca* 2013**	18.03	10.88	30.00	4.40	3.50	8.57	495	190.15	3.10	0.38	20.69	0.13	0.96	5.30
***U. lactuca* 2012**	17.75	10.35	33.8	2.61	3.71	9.11	514	205.27	8.65	0.40	21.25	0.06	6.3	16
***U. lactuca* dried 2012**	77.94	46.36	29.30	2.95	4.14	7.08	525	225.71	3.58	0.43	104.64	0.23	0.40	3.00

Data adapted from [27, 31].

3.3.8 Kinetic and statistical analysis

One issue involved with using kinetic analysis on numerous BMP assays and when comparing kinetic values with values from literature is the variance, which occurs due to the inoculum not always being the same. This can also be an issue when trialling large numbers of BMPs over a long period of time as the inoculum may change. This variance was measured by assessing the kinetic results of each of the 12 BMP runs undertaken. The twelve cellulose BMP assays yielded an average of 362.04 L CH_4 kg^{-1} VS with a SD of 13.83 L CH_4 kg^{-1} VS (3.82% SD variation). The cellulose achieved an average biodegradability index of 0.87 when compared to the theoretical yield of 414 L CH_4 kg^{-1} VS; this indicated a healthy and productive inoculum throughout these trials. The kinetic decay constant (K) averaged 0.18 d^{-1}.

3.3.8.1 Range of kinetic decay contestants

The kinetic values are related to the composition of the substrate in terms of carbohydrates, proteins and lipid concentrations, which degrade at varying rates, resulting in different BMP curve profiles, T_{50} values and K values for each substrate [32].

The K values observed in these trials may be divided between high (>0.2 d^{-1}), medium (<0.2 - >0.1 d^{-1}) and low (<0.1 d^{-1}). Three high yielding BMP substrates had low K values, UCO 0.09 d^{-1}, OSR 0.09 d^{-1} and fish offal 0.08 d^{-1}. This can be attributed to the high lipid content associated with these oily substrates, which is an inhibitor to the AD process [19]. Substrates, which performed well in terms of K values, were fresh Maize and potato varieties, with K values of 0.23 d^{-1}; this is high due to the high starch content. Sugar beet varieties had K values of 0.26 – 0.29 d^{-1}; due to the high sugar content. Silage effluent had a K value of 0.29 d^{-1}; this can be directly attributed to volatile fatty acid and lactic acid content. These high ranging substrates also had good biodegradable rates ranging from 0.84 (potatoes) to 0.93 (energy beet), indicating that continuous digestion will require shorter hydraulic retention times and digest very well. The majority of substrates fell into the medium range of K values group.

Kinetic values can be linked to the biodegradability index where substrates with easily degradable carbohydrate concentrations produce higher BMP yields and decay quickly. Kinetic analysis can also highlight such issues in digesting substrates such as their biological

composition, which relates to reduced biogas yields or biodegradability index. This may be seen with lower K values for grasses (0.09 – 0.17 d^{-1}) which is lignocellulosic and slurries (0.07 – 0.15 d^{-1}) which contain fibrous material [4].

3.3.8.2 Statistical results of BMP assays

Statistical analysis conducted on each of the six groups of substrates identified any significant differences between substrates. Similar substrates such as Wheat and Barley varieties showed no significant difference between each other. Neither did first cut grass silages. Conversely significant differences were observed in substrates, which were assessed for pre-treatment effects such as maceration, drying and some sampling dates. Sampling dates for Commercial Food Waste, for summer and winter resulted in no significant difference in BMP yields; the same was observed for the seaweed *U. lactuca*. However drying specific substrates such as food waste (FWCCC) and grass exhibited significant differences for both increased (FWCC) and decreased BMP (hay) yields. However no significant difference was observed in drying *U. lactuca*. Where commercial food waste and grass showed a reduction in BMP yields in a dried sample, *U. lactuca* exhibited increases in BMP yields in a dried sample. This highlights the BMP potential in the liquor fraction of the food waste and grass silage. Maceration also exhibited significant differences in BMP yields when trialled on selected substrates such as grass (PRG) and oilseed rape (OSR), in particular OSR where the hard outer shell once broken allowed greater degradation of the substrate. A full list of results for statistical variance between substrates can be seen in supplementary data section.

3.3.9 Bio-resource associated with best preforming substrates

The availability of substrates was assessed to determine which substrates would minimise land use and maximise energy yield per hectare. Crops and seaweeds maybe compared in terms of a yield of biomethane per hectare per annum (m^3 ha^{-1} yr^{-1}) as in table 3.7. Substrates such as wastes and residues require no land (Table 3.8) and digestion provides a feasible waste to energy pathway. All substrates can be compared to each other on a

specific yield per ton wet weight (m^3 t^{-1} wwt). A selection of the best preforming substrates were chosen from each group of substrates and assessed in terms of quantities and yields (Tables 3.7 and 3.8). Yields in table 3.7 are based on yields from harvest and results from BMP assays. Average figures for BMP and TS collected for all samples collected throughout the year were used for residues (Table 3.8).

Some figures were difficult to establish, as literature was not found for quantities of specific waste streams. In these cases average figures for dry matter yields were established from literature where available and cross referenced with waste yields obtained from sources of substrates (the industry operators producing MPW and abattoir waste). For example the quantity of fish offal (0.073 M dry tonnes) was assessed as follows: The volume of fish produced in Ireland in 2004 (latest available figure) was multiplied by 0.6; according to Kołodziejska, Skierka [33] waste fish offal can equate to 60% of the live weight. It was then factored by the percentage dry weight obtained from the collected fish offal sample in these trials. Dairy slurry yields were averaged throughout the year from samples taken.

Table 3.7 Energy yields for crop biomethane systems

Substrate	Grass silage (midlands)	Energy beet	Winter Barley	Maize (silage)	S. *latissima*	Potatoes (Roosters)
Yield (Dry t ha^{-1} yr^{-1})	11.0[a]	17.2	12.2	19.1	30[b (VS)]	35.6
CH_4 yield (L CH_4 kg^{-1} VS)	400	375	367	394	341	351
CH_4 yield (m^3 ha^{-1} yr^{-1})	4,030	6,274	4,308	7,297	10,244	11,996
Gross energy (GJ ha^{-1} yr^{-1})	152	237	163	276	387	453
Specific yield (m^3 t^{-1} wwt)	107.3	83.1	197.0	127.9	34.5	86.8
Area required to meet RES-T (ha^{-1})	123,684	79,325	115,337	68,116	48,579	41,501

A, [4]. B, [30]. Higher heating value used for Energy conversion of 1 m^3 CH_4 = 37.77MJ. Energy required to satisfy 10% renewable energy in transport (10% RES-T = 18.8 PJ) ignoring any weighting from Directives

Table 3.8 Energy yields for residue biomethane systems

Substrate	FW (average)	Slurry dairy (average)	Slurry Beef	Fish offal	MPW slurry	Abattoir waste
Available yield (Dry t yr^{-1}) M	0.155[a]	1.264[b,c]	2.43[b,c]	0.073[c,d]	8.71[c,]	0.028[c]
CH_4 yield (L CH_4 kg^{-1} VS)	419	208	311	592	461	336
CH_4 yield (m^3 yr^{-1}) M	56.6	204.8	604.1	37.1	3217.6	8.4
Gross energy (PJ yr^{-1})	2.14	7.73	22.82	1.40	121.55	0.32
Specific yield (m^3 t^{-1} wwt)	107.9	11.5	21.0	200.9	35.1	49.9
% RES-T met by waste substrate	1.1	4.1	12.14	0.74	64.65	0.17

a, [20] b, [34] c, [1] d, [33].

Potatoes displayed a high gross energy yield per hectare (Table 3.7) however they are up to 5 times more expensive to produce per tonne and have higher energy inputs than maize or beet crops [35]. MPW provided the best available waste substrate in terms of gross energy per annum for a biogas system (Table 3.8).

Seaweeds such as *S. latissima* have yet to trialled in long-term continuous trials and have expensive harvesting costs, which will prevent immediate commercial use. Technology readiness level (TRL) concepts would suggest residues and first generation biofuel crops such as Energy Beet and Maize will dominate biogas systems. However first generation biofuels are limited to 7% of energy in transport (when used as a transport fuel) and conflict with direct land use for food production. Residues are sustainable but their resource is finite.

3.4 Conclusions

In examining substrates from anaerobic digestion and assessing a resource for renewable energy in Ireland, care has to be taken in understanding the substrate and its biomethane yield. There is a significant variation in methane potential of substrates. Grasses vary by time of harvest and method of harvest. Dairy slurry has less methane potential at the end of the season when the animals are fed a reduced diet as compared to milking season. Milk processing wastes such as from the creamery and cheese production facilities show the largest resource with potential to satisfy 65% of energy demand in transport in Ireland.

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3.5 Supplementary data

Lower case letters after each substrate tittle indicate their significance to all other substrates. Where a substrate shares at least one similar letter to another, they are said to be significantly similar to a 95% (α, $p \leq 0.05$) confidence factor. Otherwise substrates display a significant difference in terms of BMP yield from one another.

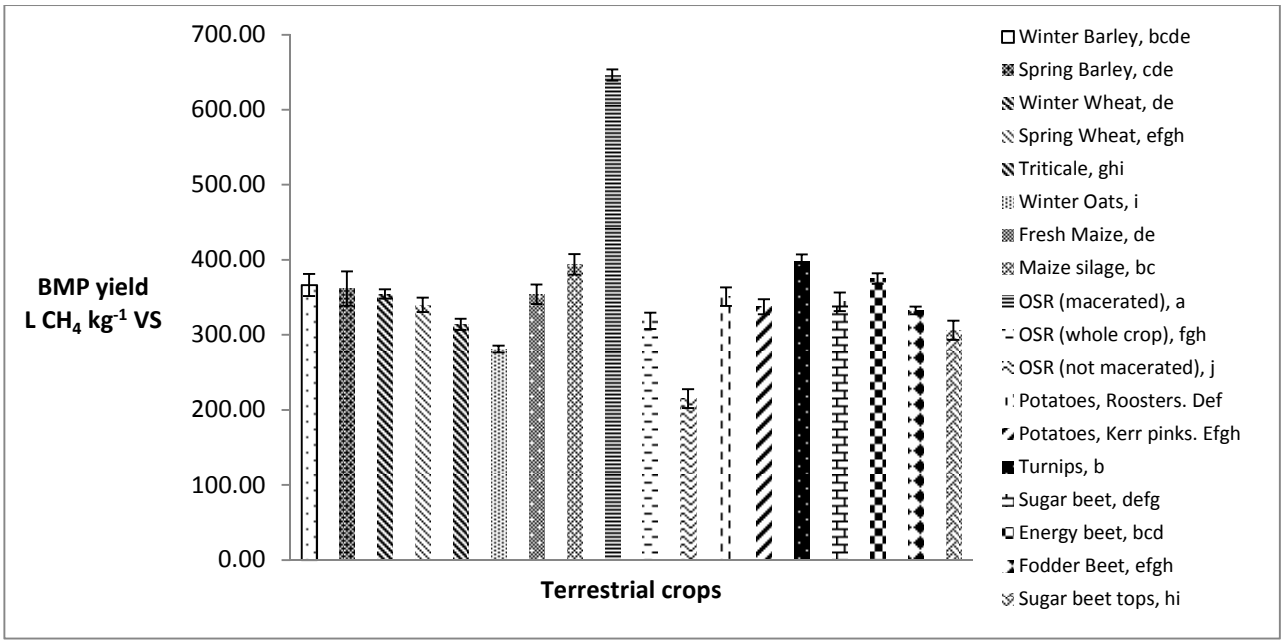


Figure S3.1 Statistical analysis of terrestrial biofuel crops and BMP yields.

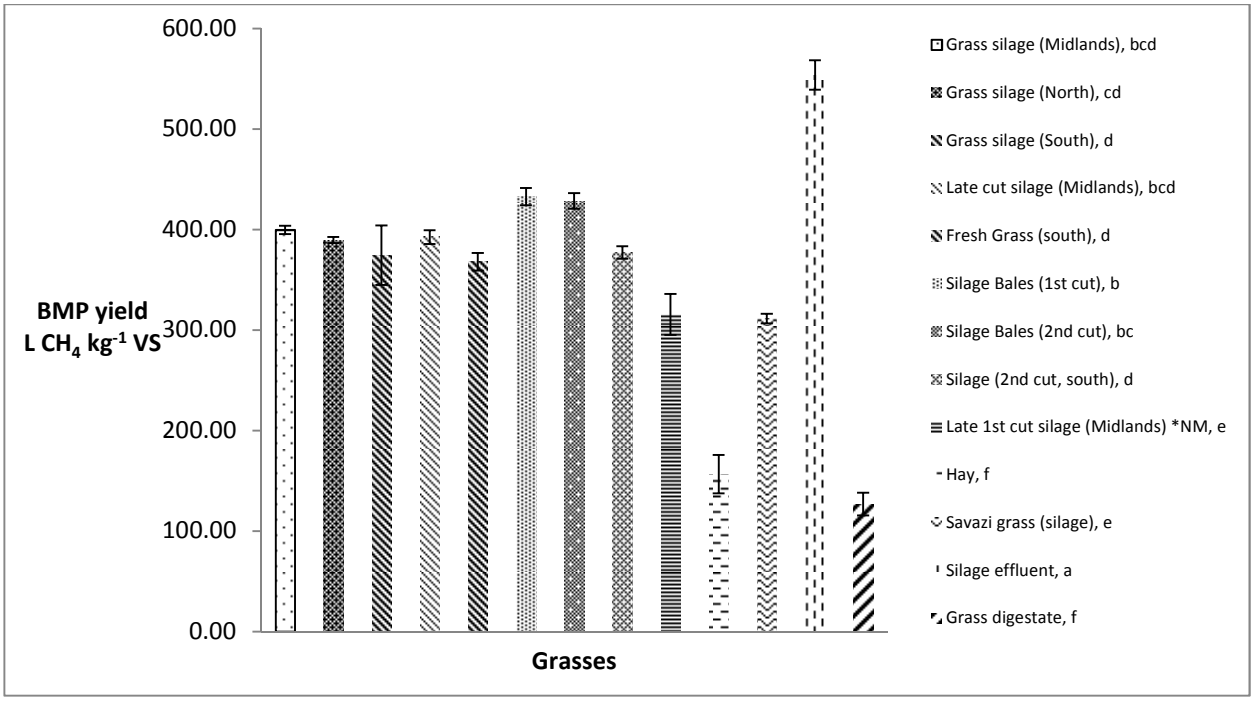


Figure S3.2 Statistical analysis of grass substrates and BMP assay results.

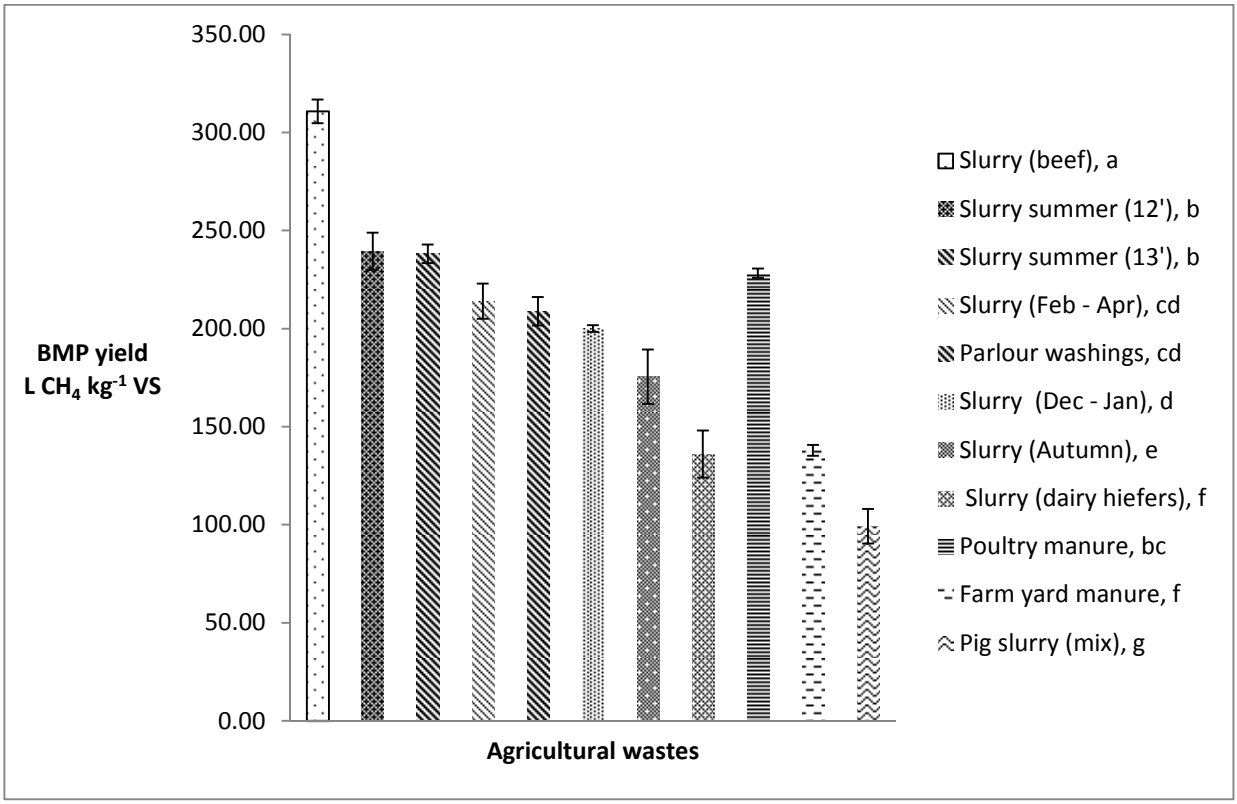


Figure S3.3 Statistical analysis of agricultural wastes and BMP assay results.

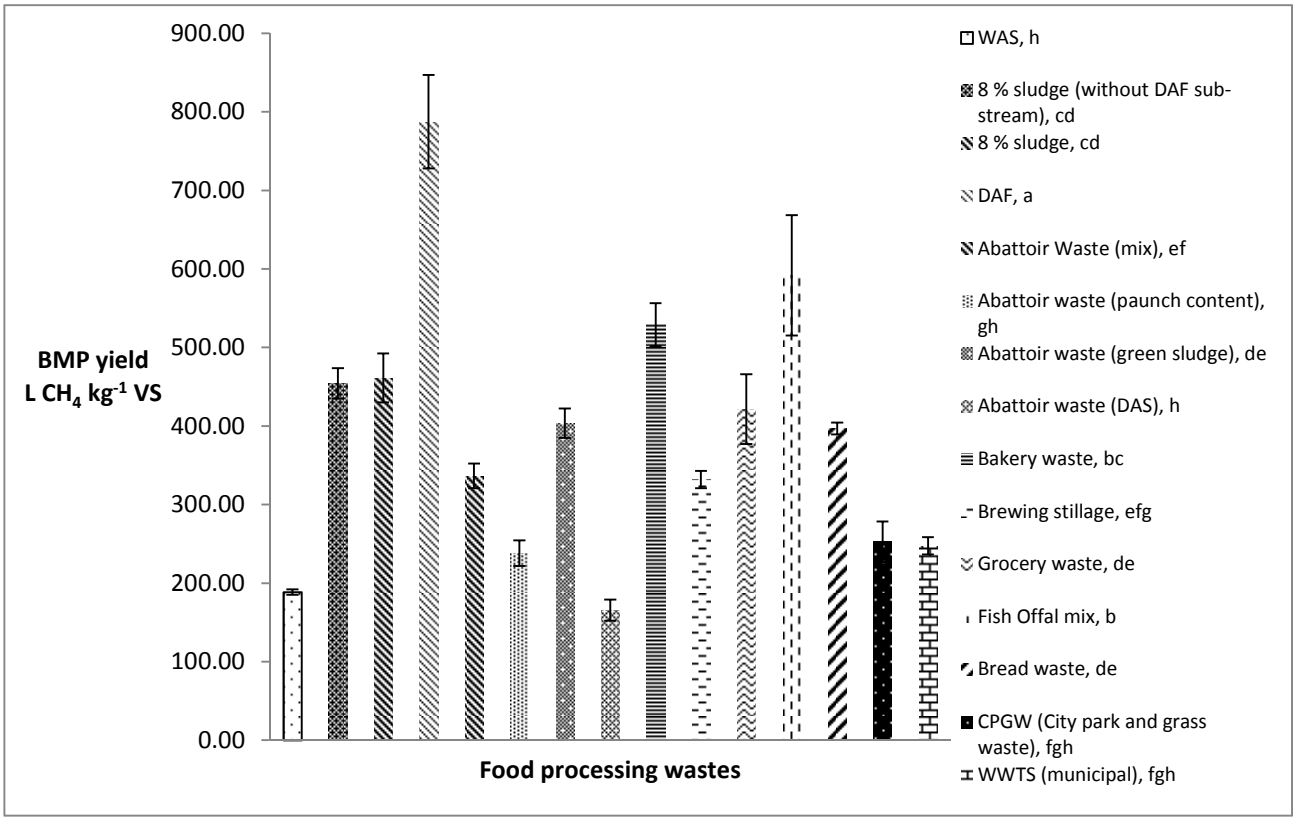


Figure S3.4 Statistical analysis of food processing wastes and BMP assay results.

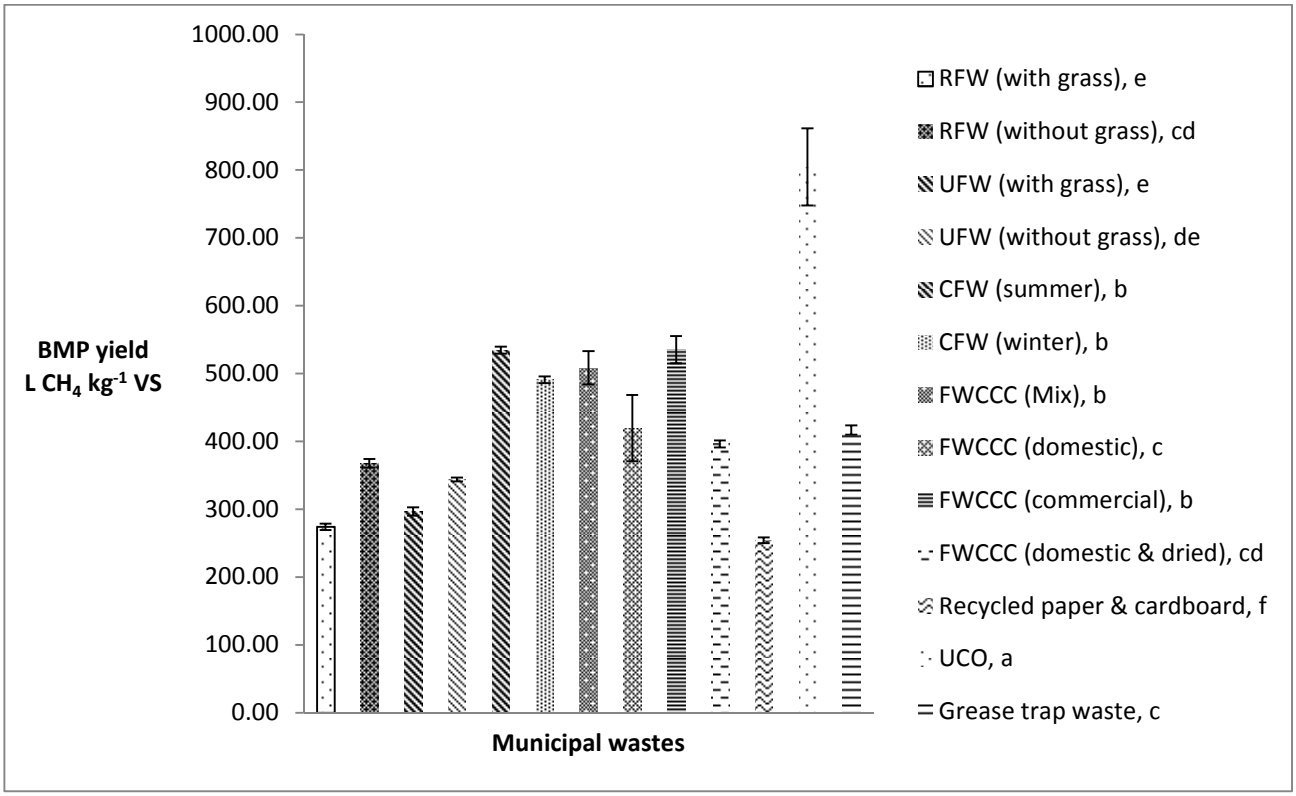


Figure S3.5 Statistical analysis of municipal wastes and BMP assay results.

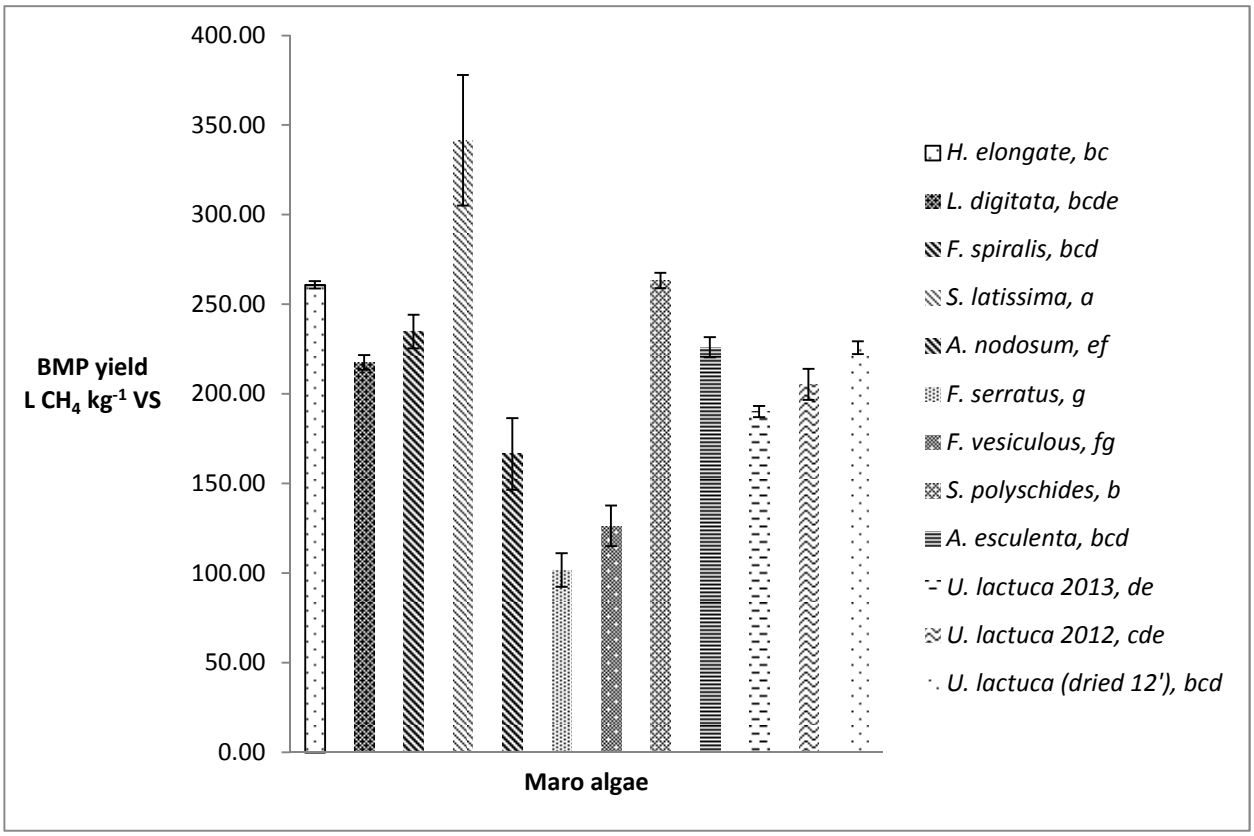


Figure S3.6 Statistical analysis of macro algae and BMP assay results.

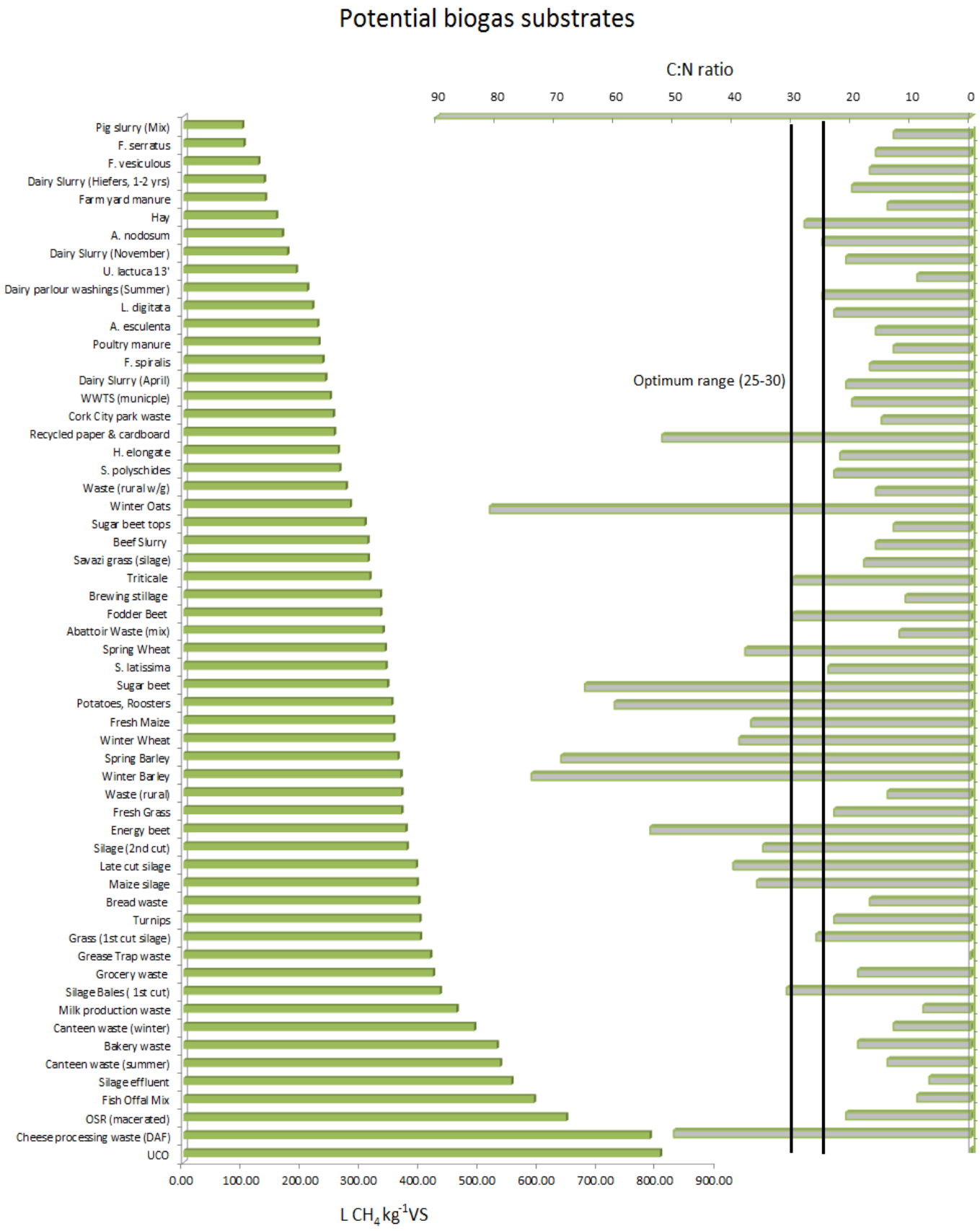


Figure S3.7 Comparison between a selection of BMP yields and C:N ratios.

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4 Evaluation of the biomethane yield from anaerobic co-digestion of nitrogenous substrates

Evaluation of the biomethane yield from anaerobic co-digestion of nitrogenous substrates

Eoin Allen [a,b], James Browne [a,b], Jerry D. Murphy [ab]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract

This paper examines three substrates for anaerobic co-digestion: abattoir waste; cheese waste and food waste. These substrates were assessed in detail for suitability for biomethane production. Biomethane potential (BMP) assays were carried out in mono and co-digestion for the three substrates and two mixes: T1 (40% abattoir waste; 50% cheese waste; 10% food waste on a wet weight (w/w) basis) and T2 (30% abattoir waste; 40% cheese waste; 30% food waste). The C:N ratio of both mixes was below optimum. Low levels suggest that the production of free ammonia (NH_3) in semi-continuous digestion was of primary concern. Both mixes were digested in a semi-continuous process for 25 weeks. The recommended operating condition for T1 was a loading rate of 3 kg VSm^{-3} d^{-1} at a retention time of 23 days. The biomethane yield was 305 LCH_4 kg^{-1} VS which was 87% of the BMP value and a biodegradability index of 0.61. For T2 (with the higher C:N ratio) a higher loading rate of 4 kg VS m^{-3} d^{-1} at a lower retention time of 15 days was recommended. The biomethane yield was 439 LCH_4 kg^{-1} VS (99% of the BMP value and 0.84 biodegradability index). At these conditions levels of Total Ammonia Nitrogen (TAN) were 4109 and 4831 mg l^{-1} for T1 and T2 respectively. These values are on the large side according to the literature. The temperature was reduced to 35°C to minimise toxicity associated with TAN. Ratios of volatile acids to total alkalinity were typically in the range 0.2 to 0.3 suggesting stable operation.

Keywords: biomethane; biogas; abattoir waste; food waste.

4.1 Introduction

4.1.1 Importance of biofuel production from residues

The EU Renewable Energy Directive [1] allows a double credit to biofuels produced from residues. In October 2012 the European Commission [2] published a proposal to limit food-based biofuels to 5% of renewable energy in transport. Biofuel production at present is very close to this level and as such the Commission is placing barriers to the development of further first generation liquid biofuel systems. Their objective is to stimulate second generation biofuels from non-food substrates such as wastes which do not interfere with food production. Greenhouse gas emissions from biofuels must effect a 60% reduction in greenhouse gas emissions on a whole life cycle basis as compared to the fossil fuel displaced [1, 2]. Typical values are given in The EU Renewable Energy Directive for biofuel systems, including for: 83% for compressed biomethane generated from residues; 32% for wheat ethanol and 45% for rapeseed biodiesel [1, 3]. This paper interrogates the optimum production of biomethane from residues available in a rural community and builds upon a paper by Browne et al [4].

4.1.2 Outline of scenarios to be investigated

Browne et al. [4] outline of scenarios to be investigated and examined the potential for biomethane production from a community from five substrates: abattoir waste; cheese waste; food waste; pig slurry and wastewater treatment sludge. They highlighted the requirement for detailed sampling of the various components of the substrates. For example abattoir waste had three components (paunch grass, green sludge and waste activated sludge). These three components yielded different specific biomethane production (L CH_4 kg^{-1} VS) rates and were available in different quantities. Based on the analysis of fifteen BMP assays Browne and co-workers [4] suggested that pig slurry and wastewater treatment sludge should be omitted from this community digester if optimisation of gas production per unit substrate was required. Food waste was shown to have the highest yield per volume of substrate (131 m^3 CH_4 t^{-1}). This substrate is also beneficial as it generates a gate fee [5]. At present source segregation of food waste only allows for 1000 t yr^{-1} of available substrate in this community. It is very possible with new

legislation that 5000 t yr^{-1} will be available in the short term. Thus two trials will be investigated as outline in Table 4.1.

4.1.3 Operational parameters of concern for semi-continuous digestion

A limitation with preliminary design of anaerobic digesters using the results of BMP assays is that little information is given on organic loading rate (OLR), hydraulic retention time (HRT) or parameters which indicate the stability of the process, such as: levels of total ammonical nitrogen (TAN); and the ratio of volatile fatty acids (VFAs) to alkalinity. A low C:N ratio is an indication of a nitrogen rich substrate and the potential for significant ammonia production within the digester when digested. The un-dissociated form of ammonia nitrogen, NH3 is the toxic component. The concentration of NH3 is temperature and pH dependent. Inhibition starts somewhere between 1500 and 3000 mg l^{-1}TAN, but higher concentrations (up to 8500 mg l^{-1}*) can* be tolerated [6] but often with a reduction in biomethane production. It is important to monitor the ratio of VFAs to alkalinity. Typically, a ratio greater than 0.3 indicates that the process is beginning to become unstable and levels at 0.8 suggest that the process is in failure.[7] In this paper, the ratio of volatile organic acids (VOA) measured in gHAceq L^{-1} (which is equivalent to the measurement of acetic acid), to alkalinity measured in mg CaCO3 L^{-1} was also assessed. This is referred to as Fos:Tac, the German translation as per the industry standard.

4.1.4 Literature on digestion of food waste

Food waste is not a homogenous substrate and its composition varies from place to place. It also depends on whether the food waste has been source segregated or is from a co-mingled source, separated at a materials recovery facility. The organic fraction of municipal solid waste (OFMSW) is another source of food waste which includes for more refractory material (paper, cardboard and textiles) and potentially is a poorer source of biomethane.[5] Data on biomethane production from source segregated food waste found in the literature include: 401–489 L CH_4 kg^{-1} VS,[8] 455 L CH_4 kg^{-1} VS [9] and 467–529 L CH_4 kg VS^{-1}.[5] Lower values are encountered for source segregated OFMSW. Cecchi et al. [8] quote values of 158–397 L CH_4 kg^{-1} VS while Davidsson et al. [10] quote values of 300–400 L CH_4 kg^{-1} VS.

4.1.5 Literature on digestion of abattoir waste

Banks et al. [11] highlighted the high level of nitrogen (and the corresponding low C:N ratio) in kitchen waste leading to high levels of ammonia in the digester which may be responsible for accumulation of VFAs. For similar reasons anaerobic digestion of slaughterhouse waste may be problematic. A digester in Austria [12] digests floatation fat, pig blood, hind gut of pig and bovine rumen content. TAN levels of between 4500 and 7500 mg $l^{-1}$are documented; at the higher levels, gas production decreased. NH3 which is temperature dependent is the toxic form of ammonia nitrogen; at lower temperatures, lessNH3 is produced.[7] The slaughterhouse waste digester in Austria maintained the temperature of the digester at or below 35◦C to minimize production of NH3 and maximize production of biomethane.[7] Edstrom et al. [13] document the problems in mono-digestion of 3 slaughter wastes (stomach and intestinal content, animal low risk excluding blood, and blood). Again the primary issue is the significant production of TAN, accumulation of VFAs and limiting methane production. To successfully operate a pilot scale facility they co-digested the slaughter waste with food waste and eventually operated at 3 kg VS m^{-3} d^{-1}. Ammonia nitrogen levels were of the order of 4500 mg l^{-1}. The biomethane yield was 560 L CH_4 kg^{-1} VS with a methane concentration of 70%.[13]

4.1.6 Literature on digestion of cheese waste

Waste from cheese production is also a high nitrogen content substrate, typically with a C:N ratio below 15.[4,14] In a trial experiment to establish an optimum loading rate for cheese waste Jihen et al. [14] added biological waste from a dairy farm in order to increase the C:N ratio. This resulted in both higher levels of biodegradability and increased methane content.[14]To overcome high ammonia levels, Comino et al. [15] reduced the operating temperature to 35◦C in co-digesting cheese waste and cattle slurry (1:1 mix). Biomethane yields of 343 L CH_4 kg^{-1} VS were obtained.[15]

4.1.7 Inhibition associated with TAN

Ammonia (NH3*)* is a compound that can be present in both gaseous and soluble form.

$$NH_3(aq) + H_2O(l) => NH_4^+(aq) + OH^-(aq).$$ Eqn 4.1

Gerardi [16] reported on the relationship of ammonia in an anaerobic digester as follows: Ammonia is released through the degradation of amino acids and proteins and comes in the form of either ammonium ions (ionized ammonia NH+ 4) or dissolved ammonia gas (free ammonia NH3*)*. The release of ammonia increases the digester alkalinity which is an important buffering step in the digestion process. However, at certain concentrations ammonia can become toxic to methanogens and may result in digester failure. The dissolved ammonia gas (free ammonia NH3*)* is the more toxic component specifically to acetoclastic methanogens. Ammonium ions (NH+ 4) are less toxic and are used by the bacteria as a nutrient source for nitrogen. Both free ammonia (NH3*)* and ammonium ions (NH+ 4) are reduced forms of nitrogen. The two forms are in equilibrium as the conversion of free ammonia to ammonium ions is pH dependent. A higher pH results in the production of free ammonia (NH3*)*, while lower pH results in the production of ammonium ions (NH+ 4). Dropping the pH in a reactor can convert much of the free ammonia to the less harmful ammonium ions. Deublein and Steinhauser [17] similarly stated that the equilibrium relationship is also temperature dependent and that a rise in temperature will shift the equilibrium in favour of free ammonia (NH3*)*, thereby increasing the chances of inhibition. Dropping the temperature by a few degrees Celsius can improve reactor stability. Banks and Heaven [18] described an equation relating production of free ammonia to the pH and temperature:

$$\frac{\text{Free } NH_3}{\text{Total } NH_3} = \left(1 + \frac{10^{-pH}}{10^{-(0.09018+(2729.92/T(K)))}}\right)^{-1}.$$ Eqn 4.2

4.1.8 Aims and objectives of paper

An ambition of this paper is to evaluate the relevance, and highlight the limitation of the BMP assay as a method for undertaking design of a community digester facility. To facilitate this ambition, semi-continuous digestion was undertaken of two mixes of substrates (with different C:N ratios as outlined in Table 4.1) over 175 days. The objectives of the paper are to:

- Compare theBMPassays of these substrates in monodigestion and anaerobic co-digestion
- Assess the ideal operational parameters (OLR and HRT) for the two mixes (Table 4.1) in semi-continuous digestion.
- Assess the performance of the reactors at these operational conditions, in particular specific methane yields (L CH_4 kg^{-1} VS), ratio of organic acids⁄alkalinity (Fos:Tac) and levels of TAN.
- Compare the biomethane yield to that obtained using BMP assays.
- Compare the BMP assays of these substrates in mono-digestion and anaerobic co-digestion.
- Assess the ideal operational parameters (OLR and HRT) for the two mixes (Table 4.1) in semi-continuous digestion.
- Assess the performance of the reactors at these operational conditions, in particular specific methane yields (L CH_4 kg^{-1} VS), ratio of Fos:Tac and levels of TAN.
- Compare the biomethane yield to that obtained using BMP assays.

Table 4.1 Individual substrates, proposed mixes and BMP results.

	C:N ratio	**TS (%)**	**VS (%)**	**C (%)**	**H (%)**	**N (%)**	**BMP mono-digestion** (L CH_4 kg^{-1} VS)	**Trial T1 proportion of mix**		**Trial T2 proportion of mix**	
								wwt basis (%)	VS basis (%)	wwt basis (%)	VS basis (%)
Abattoir	13.6	12	10.6	47.2	5.8	3.4	239	40	42	30	24
Cheese factory	14.8	8.3	6.9	48.5	8.0	3.3	515	50	34	40	21
Food waste	15	28	24.5	48.8	7.3	3.3	535	10	24	30	55
C:N ratio								14.3		14.6	
TS (%)								11.7		15.3	
VS (%)								10.1		13.2	
BMP weighted mono-digestion L CH_4 kg^{-1} VS								403		459	
BMP actual co-digestion L CH_4 kg^{-1} VS								350		443	

4.2 Materials and methods

4.2.1 Materials

The three substrates were sourced in significant quantities to allow representative samples to be taken and to allow for 25 weeks of laboratory assessment. The samples were macerated to a particle size of less than 2mm and placed in a freezer set at -20◦C. The samples were previously described in Browne et al. [4] and summarized below: *Abattoir waste:* Slaughter wastes containing 53% grass like paunch; 32% dewatered activated sludge and 15% green sludge. *Cheese waste*: Liquid sludge which includes 83% biologically treated effluent and 17% dissolved air floatation sludge. *Food waste*: Source segregated domestic and commercial waste. Present levels of 1000 ta^{-1} are expected to rise to 5000 ta^{-1} over the next four years. Of issue with the substrates is the low C:N ratio (Table 4.1). Ideally, the C:N ratio of the substrates in an anaerobic process should be in the range of 20:1–30:1.[7] The levels here (14.3:1 for Trial 1 and 14.6:1 for Trial 2) suggest excess nitrogen and as such elevated levels of TAN.[6,11]

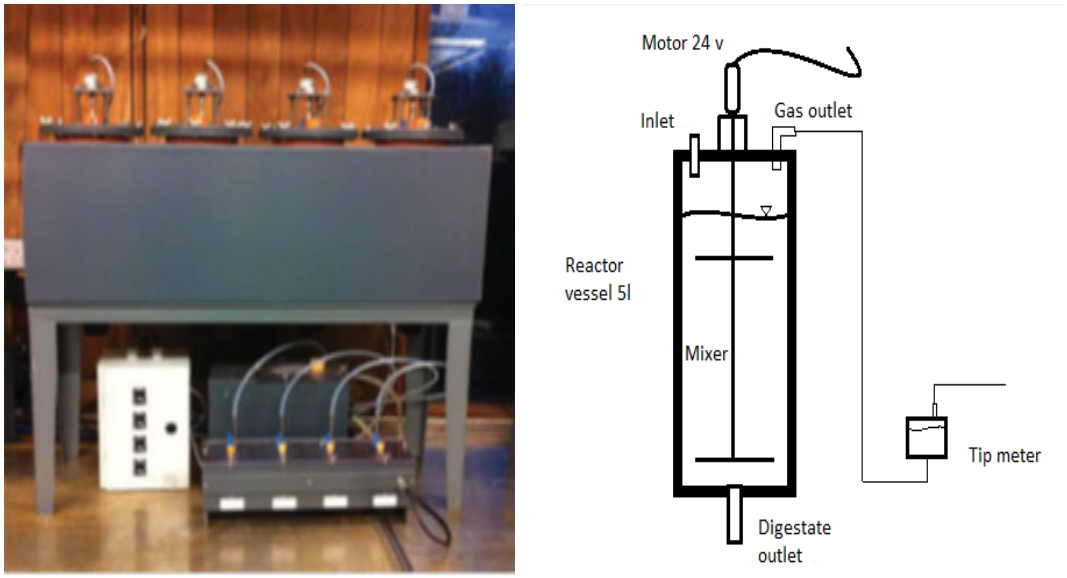


Figure 4.1 Semi-continuous digestion system consisting of 5L reactors and tipping bucket measuring device.

4.2.2 BMP assays

BMP assays are in essence a batch digestion process. Inoculum at a ratio of 2:1 or greater to feedstock on a volatile solids (VS) basis is recommended in laboratory BMP trials.[19]

This reduces the chances of process inhibition from excess VFAs or ammonia. The same process was used here as in Browne et al. [4] using the automatic methane potential test system developed by Bioprocess™. All assays were carried out in triplicate. The assays were run until biogas production was minimal (in this case less than 5ml day−1*)*. Glass bottles with a working volume of 400 ml mixed by electric stirrers are maintained at a constant temperature. Carbon dioxide is removed from the biogas by passing through a solution of 1Msodium hydroxide. Individual gas tippers automatically count and record biomethane flow. BMP assays were performed on both the individual substrates (mono-digestion) and the mix of the substrates (co-digestion). BMP assays were also carried out on the digestate removed from the semi-continuous reactors at the end of the process to quantify the BMP remaining in the digestate.

4.2.3 Semi-continuous digestion trials

Semi-continuous trials were carried out in two parallel continuously stirred tank reactors. The reactors were referred to as T1 (Tank 1 used for Trial 1 mix) and T2 (Tank 2 used for Trial 2 mix). The trials ran for a period of 25 and 24 weeks. The reactors were initially maintained at 37 $\pm$ 1◦C and continuously stirred at a rate of 100 rpm. The temperature was reduced to 35 $\pm$ 1◦C at the start of week 13, when the OLR increased to 3 kg VS m^{-3} d^{-1}. The reactors were constructed out of thick walled plastic with a vertically mounted stirring mechanism. The tank volumes were 5 L with a working volume of 4 L. Each reactor was placed inside a coiled copper pipe frame which was heated by a thermo-circulator; an insulated cover was placed over the system to reduce heat loss (Figure 4.1).

4.2.4 Inoculum, start-up and feeding and operation

Inoculum for both the BMP assays and the semi-continuous trials were sourced from a working reactor which co-digested dairy and poultry manure and food waste. For the semi-continuous trials, the inoculum was placed in the reactors two weeks before the start of the trial; this was done to de-gas, and digest any residual VS in the inoculum. An OLR of 2 kg VS m^{-3} d^{-1} was chosen as a start-up feeding rate. The substrate was macerated and weighed and placed in 100 ml containers for each of the two systems. An ultimate analysis (percentage carbon, hydrogen and oxygen) and a proximate analysis (total solids (TS), VS and ash content), was carried out for both mixes in the containers to insure minimal

variations. Each reactor was fed five days a week (not on Saturdays or Sundays). In order to reduce HRT and to minimize stress on the stirring mechanism, the substrates were reduced to a maximum of 10% TS content. This was achieved by recirculation of liquor digestate from the reactor output to the inlet. The OLR was determined by analysing the VS in all substrates. The two mixes (Table 4.1) were based on a wet weight (WW) basis. To determine accurate destruction rates and maintain a constant liquor level in both tank reactors, a mass balance was conducted including for biomethane yields; this allowed calculation of the amounts of digestate to be removed daily from both T1 and T2 (Table 4.2).

4.2.5 Gas measurement in semi-continuous trials

The cumulative gas yield for the full week was recorded and divided by the grams of VS fed over the week (only five days of feeding). Biogas was collected in Teflor gas bags and analysed for composition (percentage CH_4, CO_2 and H_2S in ppm). The measuring system used incorporated gas tipping buckets. A set volume of gas (ca. 78 ml) causes the tipping mechanism to tip. The number of tips was recorded and translated into volume of biogas. Measurement of the percentage of methane in the biogas allowed calculation of biomethane production.

Table 4.2 Mass balance of T1 at organic loading rate of 2 kg VS m^{-3} d^{-1}

Mix T1 (40% Abattoir Waste, 50% Cheese Waste and 10% Food Waste)
11.7% TS and 10.1% VS
Feeding and recirculation
OLR 2 kg VS m^{-3} d^{-1} * 4L effective volume = 8g VS d^{-1}
8g VS d^{-1} at 10.1% VS = 79 g wwt d^{-1}
Expect 90% destruction of volatiles; 7.2 g VS converted to methane d^{-1}
79 g wwt d^{-1} with destruction of 7.2 g d^{-1} implies addition of 71.8 g d^{-1}
To keep liquor level constant remove, 71.8g of digestate d^{-1}
DS of liquor is 6%:
79g wwt T1 mix at 11.7% TS plus 34g liquor at 6% TS = 113 g wwt at 10% DS
Hydraulic Retention Time
4000 L of effective volume equates to ca. 4000g of mass
HRT including recirculation is 4000 g/113g d^{-1} = 35 days
HRT excluding recirculation is 4000 g/79g d^{-1}= 51 days

4.2.6 Analysis and parameter calculations

The composition of the biogas was measured using two hand-held gas measuring devices which were checked with a standard solution of calibration gas each week for accuracy to ±1% CH_4 (1,171,580) using a 35% CO_2 in CH_4 balance. Two infrared analysers were used: a Drager X-AM 7000 and a Status Scientific Control ComBI-R Biogas analyser. All biogas and biomethane yields were reported in L CH_4 kg VS^{-1} and adjusted for standard temperature at 273K and pressure at 1013 mbar. The VOAs were measured in mg HAceq l^{-1}. Alkalinity was measured inmgCaCo3 l^{-1}. The Nordmann titration method [20] was used to output both the VOA and alkalinity, using a sample of 0.1n sulphuric acid using a Titronic Universal titrator. The ratio of VOA to alkalinity was measured using the Fos:Tac method as described by Weiland [21]. The titration is first carried out until a pH of 5.0 (bicarbonate alkalinity) and then until 4.4 (alkalinity caused by organic acids). TS and VS were determined by APHA standards. [22] Samples were taken twice weekly. pH was measured daily on samples of digestate using a Jenway 3510 pH meter. TAN was measured weekly using a Hach DR 3900 spectrophotometer (Hach phial number CLK 303).

4.3 Results

4.3.1 BMP results

The three substrates underwent mono-digestion in BMP assays and co-digestionin mixes with results as outlined in Table 4.1. BMP based on actual co-digestion varied from the calculated specific methane yield based on weighted mono-digestion. For mix T2 there was a slight reduction in yield (3.5%). In mix T1 there was a variance of 52 L CH_4 kg VS^{-1} or a 13% reduction in the BMP from co-digestion as compared with the expected yield based on weighted mono-digestion.

4.3.2 Initial loading and retention time for semi-continuous trials

The two systems operated in parallel. They were initially run on a low OLR (2 kg VS m^{-3} d^{-1}*)* to allow a period of acclimatization and ensure a healthy start-up for the reactors. The TS contents of the two mixes for T1 and T2 were 11.74% and 15.31%, respectively. The calculated quantities of liquor return were added to dilute the solids content of the feed to a level of ca. 10%. This had the added effect of reducing HRT from 51 to 35 days for reactor T1 and from 66 to 31 days for T2. Table 4.2 outlines the loading regime for T1.

4.3.3 Results of semi-continuous trials at an OLR of 2 kg VS m^{-3} d^{-1}

For a period of 13 weeks both reactors were operated at a temperature of 37 $\pm$ 1◦C and an OLR of 2 kg VS m^{-3} d^{-1} to allow for an adequate start-up phase (Table 4.3). This equated to three HRTs. The maximum yield recorded for T1 (378 L CH_4 kg VS^{-1}) over the entire 25-week experimental period was recorded in the first retention time of the OLR of 2 kg VS m^{-3} d^{-1}. There was a decline in yields from the first HRT to the second HRT (Figure 4.2). The third HRT was a more stable period for biomethane production. The methane production ranged $\pm$22 L CH_4 kg VS^{-1} for HRT 3 as compared with $\pm$129, and $\pm$52 L CH_4 kg VS^{-1} for HRT 1 and HRT 2, respectively. T2 did not produce any significant levels of biogas for the first two weeks of operation; biogas production in T2 started in week 3 (Figure 4.2(a)). There was a similar trend for T2 as for T1 (Figure 4.2(a)). A sharp rise in biomethane levels were recorded in the first retention period, followed by a decline to lower levels in the second HRT and a steady state in the third HRT as indicated by a smaller deviation in biomethane yields. The Fos:Tac ratio of in T1 and T2 was predominately below 0.2 only

rising above this limit for 2 weeks out of a total of 13 weeks in the first reactor (Table 4.5). T2 had higher levels of a Fos:Tac ratio but was at the upper bound of acceptable limits, reaching 0.37 at its maximum. This suggests that steady state had been reached. Biomethane production values in table 4.3 exclude the initial period of start-up (the first three weeks). The methane content in the biogas (Figure 4.2(b); Table 4.4) indicates the time to stable operation is of the order of five weeks.

Table 4.3 Summary of results of biomethane yields for T1 and T2

Method	**T1** (L CH_4 kg^{-1} VS)	**T2** (L CH_4 kg^{-1}VS)
Theoretical maximum based on Buswell Equation		
	501	525
BMP		
Weighed based on mono-digestion	407	438
Co-digestion	350	443
Results from 25 weeks of continuous trials		
Average (25 weeks)	267	378
Average, after start up	312	413
OLR 2 kg VS m^{-3} d^{-1}		
HRT 1 after start up	266	189
HRT 2	267	366
HRT 3	281	398
Average after start up	280	380
OLR 3 VS m^{-3} d^{-1}		
HRT 1	267	386
HRT 2	334	440
Average	305	410
OLR 4kg VS m^{-3} d^{-1}		
HRT 1	291	469
HRT 2	290	420
Average	291	439

4.3.4 Results of semi-continuous trials at an OLR of 3 kg VS m^{-3} d^{-1}

The OLR was increased from 2 to 3 kg VS m^{-3} d^{-1} in both reactors and the temperature was dropped to 35 ± 1◦C to reduce the toxic effect of free ammonia. With return of liquors the HRT was calculated as 23 days and 20 days for T1 and T2, respectively (Table 4.5). An initial decrease in biomethane yields was recorded for both reactors, but this levelled out (Figure 4.2(a)) and a more stable production of biomethane was observed. T1 yields for this OLR averaged 305 L CH_4 kg VS^{-1}; this may be compared with an average of 280 L CH_4 kg VS^{-1} for the lower OLR. The average yield raised from 267 to 334 L CH_4 kg VS^{-1} from retention period 1–2 (Table 4.3). The Fos:Tac ratio averaged 0.21 for this period (Table 4.5) indicating a lack of stress on Figure 4.2. (a) Biomethane yield, (b) methane content and (c) NH3-N for T1 and T2. the system. The pH rose somewhat from 7.63 to 7.89 (Table 4.5). TAN levels (Figure 4.2(c)) dropped off somewhat from 4518 (at an OLR of 2 kg VS m^{-3} d^{-1}*)* to 4109 mg l^{-1} (at 3 kg VS m^{-3} d^{-1}*)*. This may be explained by the drop in temperature to 35◦C. Trends for T2 were similar to T1. Biomethane production averaged 410 L CH_4 kg VS^{-1} as compared with an average of 380 L CH_4 kg VS^{-1} for the lower OLR. The average yield raised from 386 to 440 L CH_4 kg^{-1} VS from retention period 1–2 (Table 4.3). Fos:Tac ratio averaged 0.29 for this period (Table 4.5) down from 0.37 from the previous loading rate. The pH rose from 7.69 to 7.91 (Table 4.5). TAN levels (Figure 4.2(c)) dropped from 5501 (at an OLR of 2 kg VS m^{-3} d^{-1}*)* to 4834 mg l^{-1}(at an OLR of 3 kg VS m^{-3} d^{-1}) again explained by the drop in temperature.

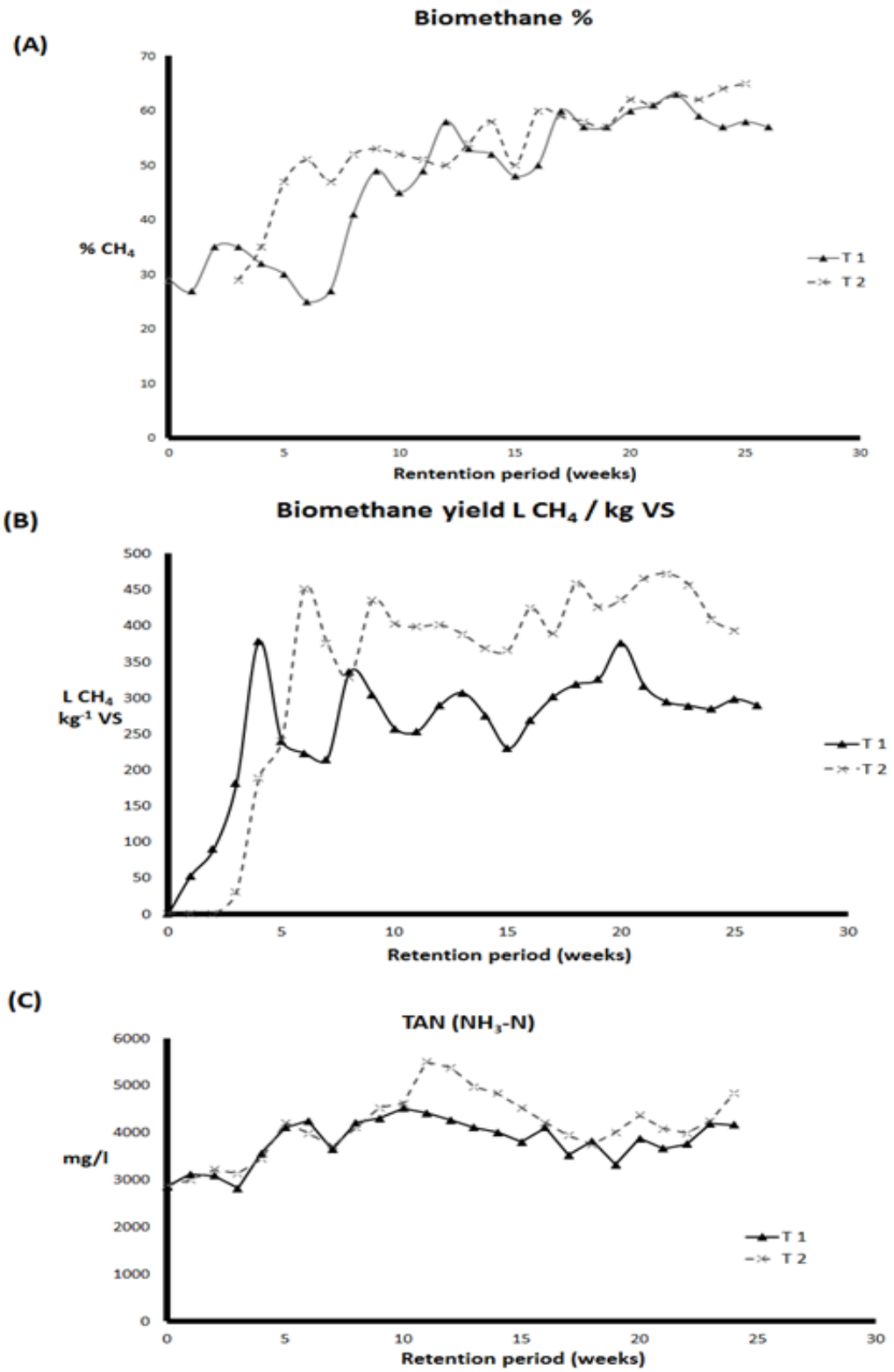


Figure 4.2 Biomethane yields and TAN concentrations for T1 and T2.

(a) Biomethane yield, (b) methane content and (c) NH_3-N for T1 and T2.

Table 4.4 Efficiency of biomethane production

Reactor	T1			T2		
OLR (kg VS m^{-3} d^{-1})	2	3	4	2	3	4
Yield/ BMP	0.80	0.87	0.85	0.86	0.95	0.99
Biodegradability index	0.56	0.61	0.60	0.72	0.79	0.83
CH_4 %	63.11	63.45	66.93	63.48	64.07	66.85
Specific CH_4 yield (L CH_4 kg^{-1} VS)	279.7	305.3	291.0	380.0	409.8	439.0
Specific yield m^3CH_4 t^{-1}	28.3	30.9	29.44	50.46	54.42	58.30

Table 4.5 Analysis of operation of T1 and T2

Reactor	T1			T2		
OLR (kg VS m^{-3} d^{-1})	2	3	4	2	3	4
HRT (ignore recirculation) d	51	34	25	66	44	33
HRT (with recirculation) d	35	23	17	31	20	15
Operating parameters						
TAN (mg l^{-1})	4518	4109	4187	5501	4834	4831
Free Ammonia (mg l^{-1})	412.89	375.94	400.10	340.98	441.56	478.12
pH	7.63 $\pm$ .11	7.89 $\pm$ .09	8.00 $\pm$ .07	7.69 $\pm$ .16	7.91 $\pm$ .12	8.03 $\pm$ .09
VOA (Fos) (g HAceq l^{-1})	1205	653	1322	1687	1109	956
TIC (Tac) (mg CaCo3 l^{-1})	5239	3110	5508	4559	3824	4780
Fos:Tac ratio	0.23	0.21	0.24	0.37	0.29	0.20

4.3.5 Results of semi-continuous trials at an OLR of 4 kg VS m^{-3} d^{-1}

Again the systems were operated for two retention times. Reactor T1 averaged 291 L CH_4 kg VS^{-1} which was a decline of 4.6% from the previous average production at the lower OLR (Table 4.3). The biomethane yield was quiet stable; variation in average yield between the first and second retention period was only ±1%. TAN was very similar to the lower OLR (4187 compared with 4109 mg l^{-1}*).* Fos:Tac ratio was low at 0.24 (up from 0.21). The pH did rise to 8 which is high; ammonia is more toxic at higher pH.[7] The authors believe that this system is stable with slightly less biomethane production than at the lower OLR (3 kg VS m^{-3} d^{-1}*).* This would suggest that for T1 the optimum OLR lies somewhere between 3 and 4 kg VS m^{-3} d^{-1}. Reactor T2 increased biomethane yields; from an average of 410 L CH_4 kg^{-1} VS at 3 kg VS m^{-3} d^{-1} to 439 L CH_4 kg^{-1} VS at 4 kg VS m^{-3} d^{-1} (an increase of 7.3%; Table 4.3). Fos:Tac was recorded at 0.2. TAN was at levels of 4831 mg l^{-1} (Table 4. 5). pH was recorded in excess of 8 which is high and of issue when associated with high ammonia levels.[7] The biomethane level achieved is very similar to the BMP result. Using the weighted BMPs for the individual substrates a value of 459 L CH_4 kg VS^{-1} is calculated. The BMP of the substrate mix T2 was recorded at 443 L CH_4 kg VS^{-1}. The semi-continuous system has a specific methane yield very close to these values (Table 4.6). The result of the semi-continuous trial for an OLR of 4 kg VS m^{-3} d^{-1} is within 1% of the BMP of the mixture. The authors would suggest that 4 kg VS m^{-3} d^{-1} is very close to optimum performance. The retention time is low at 15 days including for recirculation.

Table 4.6 Summary of evaluation of methane yield from multiple waste streams

Method	T1	T2
Theoretical maximum based on Buswell Equation (L CH_4 kg^{-1} VS)	501	525
BMP weighted based on mono-digestion (L CH_4 kg^{-1} VS)	403	459
BMP co-digestion (L CH_4 kg^{-1} VS)	350 $\pm$ 12	443 $\pm$ 14
Recommended OLR (kg VS m^{-3} d^{-1})	3	4
Hydraulic Retention time without recirculation (d)	34	33
Hydraulic Retention time without recirculation (d)	23	15
Corresponding biomethane production (L CH_4 kg^{-1} VS)	305	439
Biomethane production as a ratio of BMP	0.87	0.99
Biodegradability index	0.61	0.84
NH_3-N (mg l^{-1})	4109	4831
Fos:Tac	0.21	0.2
pH	7.89	8.03

4.4. Discussion of results

4.4.1 Level of efficiency

Efficiency of the reactor was estimated (Table 4.4) using two different metrics: · Dividing the biomethane produced in semi-continuous trials by the maximum theoretical yield derived from the Buswell equation.[23] This is known as the biodegradability index and is expressed as a ratio. Dividing the biomethane produced in semi-continuous trials by the BMP yield recorded in BMP assays. Many believe that the BMP is an upper limit on specific

biomethane yield and as such the value should not be greater than 1. Reactor T1 had its highest rates of biomethane production (Table 4.4) at an OLR of 3 kg VS m^{-3} d^{-1} (0.87 (ratio of BMP) and 0.61 (biodegradability index)). Reactor T2 had the highest biomethane production (Table 4.4) at an OLR of 4 kg VS m^{-3} d^{-1} (0.99 (ratio of BMP) and 0.83 (biodegradability index)). The BMP is often considered the upper level of biomethane production, but a number of researchers [24–26] have recorded methane yields from semi-continuous processes in excess of values obtained in BMP assays. Table 4.4 outlines the specific yields of the mixes for the different OLRs and also list these values in yields per unit mass on a wwt basis. For example, at an OLR of 4 kg VS m^{-3} d^{-1} T1 yields 29 m^3 CH_4 t^{-1} as compared with 58 m^3 CH_4 t^{-1} for T2. This is almost double the yield. This highlights the higher methane potential and dry solids content of food waste (535 L CH_4 kg VS^{-1} at 24.5% VS = 131 m^3 t^{-1}*)* as compared with abattoir waste (239 L CH_4 kg VS^{-1} at 10.6% VS = 25 m^3 t^{-1}).

4.4.2 Ammonia levels

The Hach Lange cuvette test results yield the reduced form of nitrogen (TAN). According to Section 1.7, Banks and Heaven [18] described an equation relating production of free ammonia to the pH and temperature. TAN levels in both T1 and T2 were measured weekly (Figure 4.2(c)) and reached their highest level after 10 weeks in T1 and 11 weeks in T2 (4518 mg $l^{-1}$and 5501 mg l^{-1}, respectively). These levels are considered high with respect to the scientific literature [6, 7]. Drosg et al. [6] suggest inhibition can start at 3000 mg l^{-1}. A slaughter waste digester in Austria [12] operated with TAN levels of between 4500 and 7500 mg $l^{-1}$and experienced reduced biomethane production at the higher levels. Initially (up to week 13), the temperature was set at 37◦C, but as ammonia levels began to rise, the temperature dropped to 35◦C and maintained at this level for the remainder of the experiment. The objective of this was to reduce the toxic effect of free ammonia (NH3*)* and maintain stability as recommended by Hansen et al. [27] The inoculum used in this experiment was taken from a commercial scale digester operating on poultry manure and food waste. This inoculum would be expected to have high levels of TAN even before feeding commenced. Levels of TAN in the inoculum before a period of de-gassing took place were 3368 mg l^{-1}. At the start of the experiment, the level was 2860 mg l^{-1}. However, after a suitable period of acclimatization had been allowed to take place the ammonia

decreased in concentration. At week 13 when the OLR was increased and the temperature dropped the concentration of TAN reduced to just over 4106 mg l^{-1}in T1 and 4966 mg l^{-1}in T2. It continued to decrease to a level of 3316 mg l^{-1}in T1. At the OLR of 4 kg VS m^{-3} d^{-1} it averaged 4187 mg l^{-1}. T2 had a similar curve profile but at a slightly elevated level; it reached a lower level of 3750 mg l^{-1}at week 18 and averaged 4831 mg l^{-1}at an OLR of 4 kg VS m^{-3} d^{-1}. Free ammonia concentrations are reported in table 4.4 For stable anaerobic digestion at high ammonia concentrations, the following parameters are a prerequisite [6]: · good adaptation of the microbes, · good trace element availability and · low-to-medium hydrogen sulphide concentrations.

4.4.3 Stability of process

The Fos:Tac ratio was not high. Levels remained steadily in the range of 0.15–0.3 in both reactors. An average level of 0.37 was experienced in T2 at the lowest OLR but this dropped as the system evolved. This suggests that both consortia of microbial bacteria were healthy and not under undue pressure. pH in both reactors was at satisfactory levels (7.5 for T1 and 7.6 for T2) for the entire period with an OLR of 2 kg VS m^{-3} d^{-1}. The pH rose to above 8.0 at an OLR of 4 kg VS m^{-3} d^{-1} for both T1 and T2. This is problematic when coupled with high ammonia levels.[6,7]

4.4.4 Biogas composition

Biogas composition showed increases in volume of CH_4 from an average of 63% CH_4 (after start-up) in T1 and T2 at an OLR of 2 kg VS m^{-3} d^{-1} to 64% CH_4 in T1 and T2 for an OLR of 3 kg VS m^{-3} d^{-1} (Table 4.4). This further increased to 67% CH_4 for the final OLR. Methane composition in the biogas was predominantly higher on the day after the two-day feeding lull (Saturday and Sunday). Percentages reached on average 2–3%CH_4 higher on Mondays as compared with the weekly average. Biogas composition was similar in both reactors. H_2S levels did not register in the biogas composition until week 9 in both reactors (Figure 4.3). It remained under 500 ppm until week 20 when the OLR was increased to 4 kg VS m^{-3} d^{-1}. The initial device (Status Scientific Control ComBI-R Biogas analyser) could not measure levels in excess of 500 ppm. A new device (Drager X-AM 7000) was purchased (in place in week 23) with a larger measuring range for H_2S. Levels of up to 860 ppm were recorded in T1 and 980 ppm in T2 (Figure 4.3). The Drager recorded levels of hydrogen over 2000 ppm from

week 23 when it was purchased to week 25 (termination of experiment). Figure 4.3. Levels of H_2S (ppm) in biogas from T1 and T2.

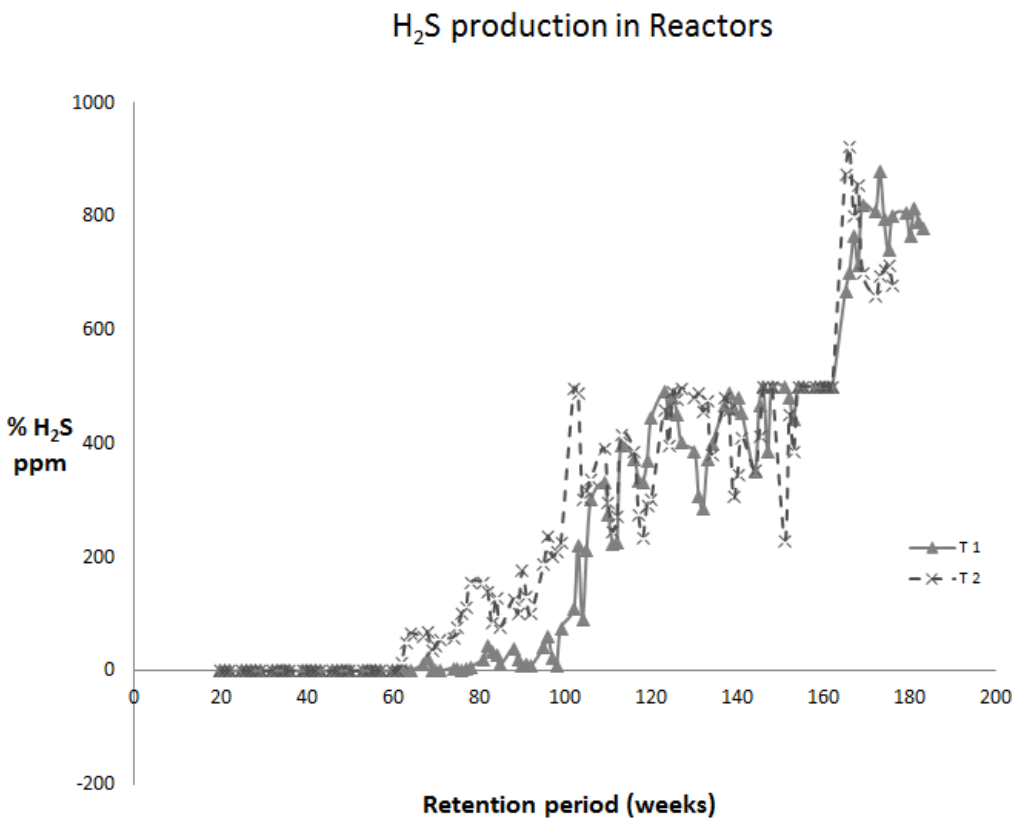


Figure 4.3 Levels of H_2S (ppm) in biogas from T1 and T2.

4.5 Conclusions

The results of this paper and previous work [4] allow a comparison of biomethane yields using four methodologies including for three different laboratory procedures. The methods include:

· Theoretical maximum calculated using the Buswell equation based on the ultimate analyses of the substrates.

· The first laboratory procedure is based on mono-digestion BMP trials.

· The second laboratory procedure is based on BMP trials of actual mixes.

· The third laboratory procedure included for 25 weeks of semi-continuous digestion at three different OLRs. The results are summarized in table 4.6. The result of the BMP assay from Co-digestion is not the same as would be calculated using a weighting of mono-digestion results. There is actually a small decrease for T2 (3%) and a more significant decrease for T1 (13%). The recommended OLR is lower (3 kg VS m^{-3} d^{-1}*)* for T1 than for T2 (4 kg VS m^{-3} d^{-1}*)*. The HRT (with recirculation) is recommended at 23 days for T1 and 15 days for T2. The ratio of VOC/alkalinity is typically below 0.3 for both trials. This suggests stability though the pH is on the high side at 7.89 and 8.03, respectively, for T1 and T2. The ratio of the biomethane yield from the semi-continuous trials to that obtained using BMP assays is 0.87 for Trial 1 and 0.99 for Trial 2. It is suggested that using a BMP for a preliminary design of an anaerobic reactor does not yield sufficient data for choosing operating conditions.

Acknowledgements

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5 What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?

What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?

Eoin Allen[a], David M. Wall[a], Christiane Herrmann[a], Ao Xia[a], Jerry D. Murphy[a,b]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract

Seaweed may be a source of third generation gaseous biofuel, in the form of biomethane. The scientific literature is sparse on the relative suitability of different varieties of seaweed to produce biomethane. This paper assesses the biochemical methane potential (BMP), ultimate analysis and theoretical yields of ten species of seaweed which may be found in commercial quantities around the coastline of Ireland. Saccharina latissima reported the highest BMP yield (ca. 342 L CH_4 kg^{-1} VS). S. latissima if farmed, may produce 10,250 m^3 CH_4 ha^{-1} yr^{-1} (365 GJ ha^{-1} yr^{-1}) which is in excess of all land based liquid biofuel systems.

Key words: Anaerobic digestion; Biogas; Macro-algae; third generation biofuels.

5.1 Introduction

5.1.1 Why use algae to generate gaseous biofuel?

The use of third generation biofuels avoids competition between agricultural land and energy. Algae (both macro and micro) have been suggested as potential future sources of renewable energy in transport in Europe. The Renewable Energy Directive [1] assigns a weighting of two to biofuel produced from algae. Thus in calculating renewable energy supply in transport (RES-T) targets the energy from algae biofuels may be doubled in considering the 2020 target of 10% RES-T.

5.1.2 Micro-algae biofuels

Much work has been published on micro-algae as a potential source of biofuel. A disadvantage associated with production of liquid biofuel from micro-algae (micro-algae biodiesel) is the low total solids (TS) content of the micro-algae (0.1% to 1%TS) produced in open and closed bioreactors [2, 3].The requirement to utilise only dry lipids in the bioesterification process leads to a requirement for energy intensive thickening, dewatering and drying processes [4]. Prajapati and co-workers, have shown that gaseous biofuels produced by anaerobic digestion may overcome these disadvantages, as the algae can be digested wet [5, 6]. Micro-algae are rich in lipids which leads to high theoretical specific methane yields (SMYs) as assessed by biochemical methane potential (BMP) assay, however these lipids can cause inhibitory conditions when digested [7]. Micro-algae have low carbon to nitrogen (C:N) ratios, high sodium and sulphur concentrations, all of which make its digestion challenging [8]. Solutions to these problems include: co-digestion of micro-algae with substrates rich in carbon such as cassava [9], optimisation of microbial growth to reduce protein content and increase C:N ratio, efficient pre-treatments and combined hydrogen fermentation and anaerobic digestion [10].

The literature on gaseous biofuel production from macro-algae (also known as seaweed) is less abundant than from micro-algae.

5.1.3 Seaweed as a source of gaseous biofuel

5.1.3.1 Characteristics of seaweeds

The characteristics of seaweeds are such that they have no lignin, low levels of cellulose and low levels of lipid content [11, 12]. Jard and co-workers, break down seaweeds into three broad types: brown, red and green seaweeds. Brown seaweeds are very prevalent on the Irish Coast and include for *Saccharina latissima, Ascophylum nodosum* and *Laminaria digitata* [12, 13]. Green seaweeds such as *U. lactuca* tend to be associated with eutrophication and algae blooms [14]. Red seaweeds (such as *Gracilaria verrucosa*) are not prevalent in Ireland.

Seaweeds tend to have high solids content. The solids content for seaweeds are documented in the range 8.3 to 22% [12, 15, 16]. As such there is no requirement for any energy intensive thickening, dewatering or drying processes.

The volatile solids content expressed as a percentage of dry solids for brown and red seaweeds ranged from 44.6% to 73.8% [12]. Green seaweed (in particular *U. lactuca*) has reported values in the range 57 to 82.1% [14, 17].

5.1.3.2 Benefits of biofuels produced from seaweeds

Brown seaweeds (or kelps) have significant potential as a farmed feedstock for anaerobic digestion in temperate oceanic climates. It is suggested that seaweed farms be situated in close proximity to sources of pollution (such as salmon farms) to optimise their growth rate whilst simultaneously cleaning the water of excess nutrients.

A further advantage of seaweed as a biofuel feedstock is the sequestration of CO_2 associated with cultivation. Typically one tonne of kelp sequesters six times the amount of CO_2 that is emitted during transport and maintenance of the kelp stock [18]. The majority of greenhouse gas (GHG) emissions (and costs) are associated with harvesting of seaweed. A value added product from digestion of seaweed is the rich level of macro elements in the digestate, which can be beneficial as a bio-fertiliser [19].

5.1.3.3 Suitability of seaweed to anaerobic digestion

The C:N ratio for optimal anaerobic digestion is in the range 20:1 to 30:1 [14]. If the C:N ratio is less than 15 excess levels of ammonia can lead to unstable digestion [20]. Protein concentrations are low in brown seaweeds but can be high in green sea weeds [12]. The nitrogen associated with high protein content thus leads to low C:N ratios in *U. lactuca* (green seaweed); values of less than 10 have been recorded [14]. Brown seaweeds on the other hand, contain high levels of carbohydrates in the form of polysaccharides (mannitol, laminarin and alginate) which are easily degradable [21]. Jard and co-workers recorded a C:N ratio of *Saccharina latissima* of 22 [12].

Ascophylum nodosum (a brown seaweed) contains polyphenols which are difficult to degrade and can inhibit anaerobic digestion [22]. *U. lactuca* can have a sulphur content of up to 5%; as a result hydrogen sulphide (H_2S) is prevalent in the biogas and can inhibit anaerobic digestion [23].

5.1.3.4 Biomethane potential and energy yields per hectare per annum

An essential element of a biofuel system is the gross energy output per hectare per annum. These have been calibrated for an assortment of biofuel systems and tabulated in table 5.1. The authors have not found literature on gross energy yields for seaweed biofuels. This figure is dependent on the biomethane potential of the seaweed species (L CH_4 kg^{-1} VS), the volatile solids (VS) expressed as a percentage of wet weight and the growth of the seaweed per hectare of sea.

BMP values found in the literature vary from 200 to 335 L CH_4 kg^{-1} VS for *Himalthalia elongate* in France and *Saccharina latissima* in Ireland respectively [12, 13]. Seaweeds can be either collected off the beach (beach cast) or farmed at sea (aquaculture). Growth rates can be as high as 45 t TS ha^{-1} yr^{-1} for green seaweeds; these are predominantly beach cast and are not as suitable for aquaculture as brown seaweeds maybe. Yields for farmed brown seaweed are suggested in the range 26 – 80 t volatile solids (VS) ha^{-1} yr^{-1} [17, 24]. This may be compared to land cultivated biomass such as perennial ryegrass, which has yields up to a maximum of 15 t TS ha^{-1} yr^{-1} [25].

Table 5.1: Gross energy yields of biofuel

Biofuel System	**Gross energy** (GJ ha^{-1} yr^{-1})
Rape seed biodiesel	46
Wheat ethanol	66
Palm Oil biodiesel	120
Sugarcane ethanol	135
Grass biomethane	122 – 163
Willow biomethane	95 - 130

Expressed as GJ ha^{-1} yr^{-1}; data from [25-27].

5.1.4 Objectives of paper

There is little research documented in the scientific literature at present into either the availability of seaweed stocks or their potential to produce biomethane.

The objectives of this paper are to:

- Classify a number of species of seaweed common in Ireland through proximate and ultimate analysis;
- Assess the BMP of these species;
- Assess the energy yield per hectare per annum of third generation gaseous biofuel produced from seaweed.

5.2 Materials and methods

5.2.1 Materials

Ten varieties of seaweed were collected from beaches in Cork, in the south of Ireland (51°N, -9°E). The seaweeds were beach cast and harvested from their natural environment. The samples were taken in August to represent peak growing conditions [28]. Nine of the seaweeds were brown, one was green. The brown species were: *Ascophylum nodosum, Himalthalia elongate, Laminaria digitata, Saccorhiza polyschides, Fucus spiralis, Fucus serratus, Fucus vesiculosus, Alaria esculenta* and *Saccharina latissima*. The green species

was *Ulva lactuca.* Approximately 25 kg of each species was collected. These seaweeds are the most commonly occurring around the coast of Ireland.

Inoculum was sourced from a combination of numerous lab scale reactors processing grass silage, dairy slurry and macro-algae; all of these reactors operated at 37^{o}C.

5.2.2 Methods

5.2.2.1 Method design

Proximate and ultimate analysis and sample preparation for BMP assay

A representative sample of each seaweed was sampled for TS and VS using the standard method of drying (105^{o}C for 24 hours and further baking at 550^{o}C) [29]. Samples were prepared for ultimate analysis by drying for 24 hours at 105^{o}C and then finely grinding to pass through a 600 µm sieve. Samples were analysed for C, H, N, and O (O calculated by difference) on an ash free basis using a CE 440 elemental analyser.

Prior to digestion, samples were macerated using a Buffalo macerator to a particle size of less than 4 mm. The pH of the samples, were measured using a Jenway 3510 pH meter. The prepared samples were placed in sealed containers and frozen at -20^{o}C; they were defrosted prior to BMP assessment.

5.2.2.2 BMP assay

The Bioprocess AMPST II® system was used to conduct BMP assays. All samples were assessed in triplicate. In each run of the system one of the samples was inoculum and one was cellulose. An inoculum to substrate ratio (I:S) on a VS basis, of 2:1 was used [30]. The reactor vessels had a working volume of 400 ml with a total head space of 250 ml. After the calculated inoculum and substrate amounts were placed in the reactor vessels, nitrogen was flushed through the system to create anaerobic conditions. Each reactor vessel was placed inside a water bath constantly maintained at 37^{o}C. A mixing system was connected to each reactor vessel and was continuously operated at a speed of 30 rpm, alternating between on and off for 60 second periods. The biogas produced was passed through a solution of 3M NaOH to remove CO_2, H_2S and other impurities. The biomethane was then

passed through a gas tipping device which recorded the volume of gas produced for each reactor vessel. This data was constantly recorded and logged for each day. Each BMP assay was ran in triplicate and assessed for standard deviation. The total average biomethane produced from the inoculum was subtracted from the average biomethane produced by each sample to determine specific biomethane production. All results were automatically adjusted for standard temperature and pressure and overestimation error was eliminated for the flush gas.

In total 14 BMP assays in triplicate were conducted for this experimental procedure. These included for the 10 species of seaweed. The assays were carried out in two runs; thus a BMP of the inoculum and of cellulose were carried out twice.

5.2.2.3 Kinetic analysis

The kinetic analysis allows a viewpoint on the biodegradability and the rate of biodegradability of the substrate. Kinetic and statistic modelling was applied to the output of the BMP system. Data was taken from cumulative production curves and input to a Matlab code to output kinetic values. A first order differential equation was used to determine the decay constant values (Eqn. 5.1). The modified Gompertz formula (Eqn. 5.2) was used to develop a list of variables to describe the decay process of organic matter in batch tests [31].

$$Y(t) = Y_m \,.\left(1 - \exp^{(-kt)}\right) \qquad \text{Eqn. 5. 1}$$

$$M(t) = P \cdot \exp\{ - \exp[\,\frac{R_{max} \cdot e}{P}(\Delta - t)] + 1\} \qquad \text{Eqn. 5. 2}$$

Where,

Y(t) is the cumulative biomethane yield (L CH_4 kg^{-1} VS) at a digestion time, t (days).

Y_m is the maximum biomethane potential (L CH_4 kg^{-1} VS) of the substrate added.

k the decay constant (days $^{-1}$) is a measure of the rate that the substrate has been degraded.

M(t) is the cumulative biomethane yield (L CH_4 kg^{-1} VS) at a given time t (days).

P is the maximum biomethane potential (L CH_4 kg^{-1} VS) of the substrate from the BMP test.

R_{max} is the maximum biomethane production rate (L CH_4 kg^{-1} VS d^{-1}).

Δ the lag phase is a measure of how long it takes (days) before biochemical methane production starts to occur.

t is the time (days).

T_{50} is the half-life (days) and is a measure of how long it takes to produce half of the maximum cumulative yield of biomethane.

R^2 is a measure of how the kinetic equation model fits the curve of biomethane production (%).

5.2.2.4 Theoretical biomethane calculation

The ultimate analysis data allows the theoretical biomethane yield to be calculated. Using the Buswell equation (Eqn. 5.3) values are input to give a maximum potential methane yield through conversion of VS to methane and carbon dioxide [32]. The molar volume of the gases is taken as 22.414 L at 0°C and 1 atm. However a short coming of using the Buswell equation is that it does not take into account maintenance and anabolism of the microbial community. Also some of the VS content present in macro-algae consists of proteins and fibres which are difficult to break down. This leads to a reduction in BMP yields when compared to yields derived from the Buswell equation. Therefore an over estimation of biomethane yields occur which leave theoretical yields as ceiling values for BMP assays.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \qquad \text{(Eqn. 5.3)}$$

5.2.2.5 Statistical analysis

Statistical analyses were performed using the software SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was carried out in order to assess the influence of

substrate on biochemical methane yield with the BMP run regarded as a block effect and substrate regarded as the main effect. The significance of differences in methane yield between substrates was determined by multiple comparisons applying the test procedure SIMULATE in SAS [33]. The significance level α, was set to $p \leq 0.05$.

5.3 Results

5.3.1 Proximate and ultimate analysis

The results of a proximate and ultimate analysis of the seaweeds and the inoculum are reported in table 5.2. The C:N ratio is quiet low for the green seaweed (*U. lactuca*). Many of the seaweeds are in, or close to the ideal range of 20:1 to 30:1. Theoretical biomethane yields were calculated from this analysis for each substrate using the Buswell equation (column 4: table 5.3).

5.3.2 BMP batch results

The substrate significantly influenced the BMP yield (F=96.17, P <0.0001) whereas no significant effect was found for the BMP run (F=2.06, P=0.165). BMP yields of seaweed ranged from 101.7 to 341.7 L CH_4 kg^{-1} VS (Figure 5.1). The BMP yield of *S. latissima* significantly exceeded yields of all other seaweed investigated. The lowest BMP yields were analysed for *F. serratus* and *F. vesiculosus*. Cellulose controls recorded BMP yields within recommended ranges from literature.

The biodegradability index is defined as the BMP yield divided by the theoretical biomethane yield. This value gives an indication of how well the substrate was degraded and how the BMP yield compared to the theoretical biomethane yield. The highest biodegradability index (0.81) was found for *S. latissima*.

F. serratus, *A. nodosum* and *U. lactuca* displayed low biodegradability (0.19, 0.34 and 0.41 respectively). Pre-treatment technologies will have significantly more applicability where the biodegradability is low [34]. The specific yield (Table 5.3) is defined as the yield of methane per tonne wet weight; this indicates the biomethane yield per tonne collected

and may be more instructive to the biogas developer. *S. latissima* and *S. polyschides* have the highest values at 34.5 m^3 CH_4 t^{-1} wwt.

Table 5.2 Characteristics of raw seaweeds and inoculum used in experimental trials.

Substrate	TS % of wwt	VS % of wwt	Ash % of TS	C % of TS	H % of TS	N % of TS	O* % of TS	C:N ratio
A. nodosum	23.2	19.4	16.1	40.4	5.3	1.6	36.6	26.0
H. elongate	12.65	8.10	36.0	30.8	4.1	1.4	27.7	21.4
L. digitata	14.20	10.34	27.2	34.2	4.8	1.5	32.3	22.3
F. spiralis	19.72	13.92	29.4	36.1	4.7	2.1	27.7	17.3
F. serratus	20.07	14.74	26.6	37.1	4.8	2.4	29.1	15.5
F. vesiculosus	21.18	16.11	24.0	26.8	3.2	1.5	44.5	17.6
S. polyschides	15.25	13.11	14.0	36.1	5.0	1.6	44.3	23.2
S. latissima	15.49	10.09	34.9	29.1	3.8	1.2	31.0	24.0
A. esculenta	18.72	11.91	36.4	29.3	4.2	1.9	28.2	15.5
U. lactuca	18.03	10.88	39.7	30.0	4.4	3.5	22.4	8.5
Inoculum	2.97	1.88	36.7	33.4	4.1	1.8	24.0	18.4

* O was not measured but evaluated assuming that Total Solids is comprised entirely of Ash, C, N, H and O. wwt = wet weight.

Table 5.3 Biomethane production for seaweed using results of BMP analysis and theoretical analysis.

Substrate	**BMP yield** (L CH_4 kg^{-1} VS)	**Theoretical composition of biogas** (CH_4 %)	**Theoretical yield** (L CH_4 kg^{-1} VS)	**Biodegradability index**	**Specific yield** (m^3 CH_4 t^{-1} wwt)
A. nodosum	$166.3^{bc} \pm 20$	53	488	0.34	32.3
H. elongate	$260.9^{f} \pm 2.05$	36	334	0.78	21.1
L. digitata	$218.0^{de} \pm 4.14$	53	479	0.46	22.5
F. spiralis	$235.2^{ef} \pm 9.43$	55	540	0.44	32.7
F. serratus	$101.7^{a} \pm 9.37$	54	532	0.19	13.5
F. vesiclosus	$126.3^{ab} \pm 11.38$	37	249	0.51	19.4
S. polyschides	$263.3^{f} \pm 4.23$	48	386	0.68	34.5
S. latissima	$341.7^{g} \pm 36.40$	50	422	0.81	34.5
A. esculenta	$226.0^{def} \pm 5.66$	53	474	0.48	26.9
U. lactuca	$190.1^{cd} \pm 3.10$	48	465	0.41	20.9
Cellulose	$357.4^{g} \pm 15.20$	-	414	0.86	-

Different superscript letters ^{abcdefg} Indicate significant differences between BMP yield means of substrates ($P \leq 0.05$, adjustment = SIMULATE). wwt = wet weight.

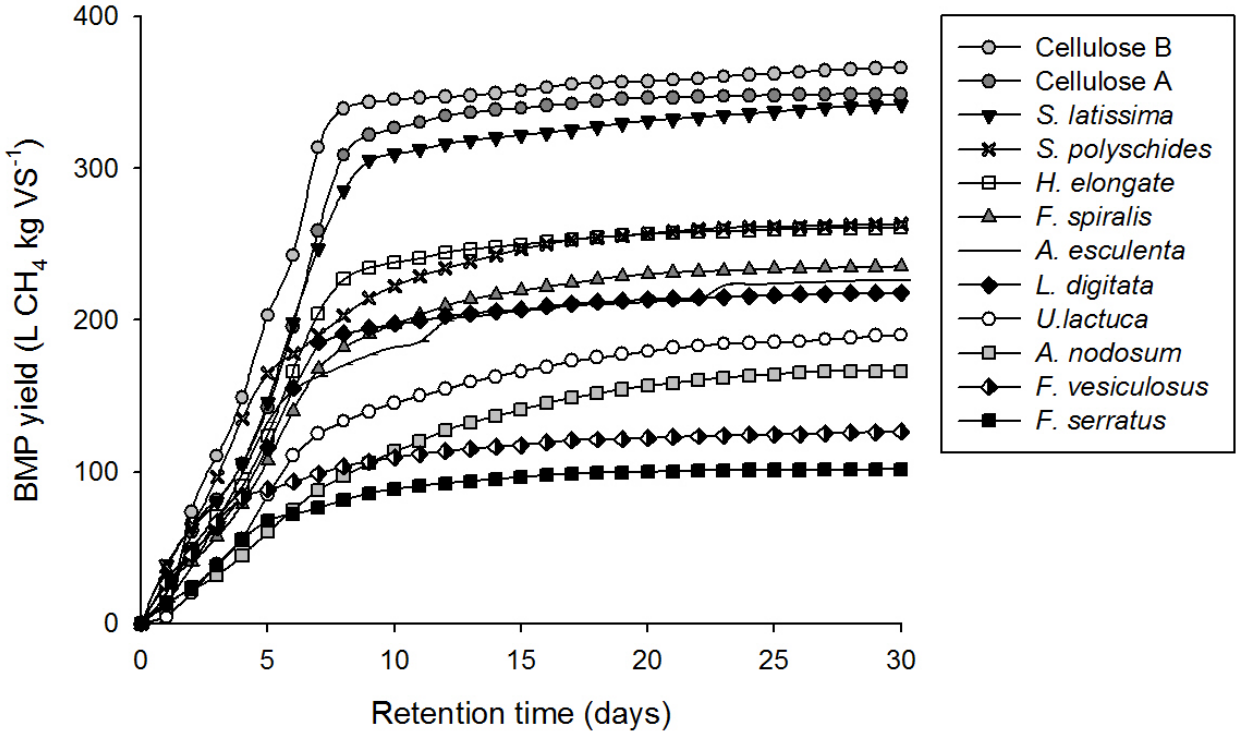


Figure 5.1 BMP cumulative yield curves.

5.3.3 Kinetic analysis

The kinetic and statistical analysis results are described in table 5.4. The modified Gompertz equation showed very good correlation as values of R^2 were within acceptable tolerance (defined as greater than 0.95). Cellulose values showed the least correlation, though still within tolerance. This is largely due to the BMP profile curve of the control substrate where there is an elongated lag phase, which disrupts the potential accuracy of the kinetic assessment. This is commonly observed when using cellulose as a control substrate.

In general all seaweeds performed well with good kinetic decay values; *F.vesiclosus* had the highest value at 0.22 d^{-1}. As a perspective, analysis in the same laboratory highlighted a k value for perennial ryegrass of 0.11 d^{-1} [25] whilst food waste had a k value of 0.17 d^{-1} [35]. The lag phase for all seaweeds were less than or equal to that of cellulose. The half-life for all substrates were relatively low suggesting a retention time of less than 20 days would be more than sufficient in a full scale reactor. The lag phase observed was substantially less

than observed by Gurung and co-workers (6 - 9 days) who also digested brown seaweeds [36]. This may be explained by the low I:S ratio of 0.8:1; as compared to 2:1 which is used in these trials and others [14, 35].

Table 5.4 Kinetic and statistical analysis of seaweeds and cellulose

Substrate	**k** (d^{-1})	**R^2**	**Δ** (d)	**T_{50}** (d)
A. nodosum	0.12	0.98	0.32	7.48
H. elongate	0.18	0.95	1.17	4.24
L. digitata	0.19	0.96	0.79	3.85
F. spiralis	0.16	0.97	0.74	4.85
F. serratus	0.18	0.99	1.62	3.84
F. vesiculosus	0.22	0.99	0.50	3.10
S. polyschides	0.19	0.99	0.45	3.85
S. latissima	0.16	0.95	1.23	4.55
A. esculenta	0.19	0.98	0.50	3.61
U. lactuca	0.13	0.98	0.96	5.30
Cellulose A	0.17	0.94	1.75	4.54
Cellulose B	0.19	0.95	1.25	3.57

5.4 Discussion of results

5.4.1 Brown seaweed

5.4.1.1 *S. latissima*

The best yielding seaweed was *S. latissima* (figure 5.2 A) which produced a BMP yield of 341.7 $\pm$ 36.4 L CH_4 kg^{-1} VS or 34.5 m^3 CH_4 t^{-1} wwt. *S. latissima* had the highest standard deviation of all the samples. When compared to literature these results matched the highest available values for *S. latissima*; 333 $\pm$ 64.1 L CH_4 kg^{-1} VS reported for a washed and

macerated sample harvested in Denmark (harvest date not given) [37]. However a lower value was reported (223 ± 61 L CH_4 kg^{-1} VS) for *S. latissima*, harvested in August in Norway [38]. The variability in yields highlights the effect of location of harvest, and time of harvest, on yield. Handå and co-workers reported a trial where *S. latissima* was cultivated in two locations: an off shore salmon farm and a near shore location [39]. Large variations were observed in C:N ratios throughout the year: from 7 to 21 for the near shore location; and 7 to 16 for species harvested from the salmon farm. The C:N ratio was 24 in this study. Alginate concentration varied between 6% and 27% TS [39]. The high sugar content of *S. latissima* leads to the elevated specific yields of biomethane when compared to other seaweed varieties, but when alginate levels are reduced due to specific geographic or harvest conditions, the seaweed will have a reduced specific biomethane yield.

5.4.1.2 *L. digitata*

L. digitata (Figure 5.2B) has been widely reported as a potential biofuel feedstock due to the levels of laminarin (up to 30% TS) and mannitol (up to 25% TS). These sugars can be readily converted to biomethane [40]. Significant research has been conducted on the seasonal variation of *L. digitata* in terms of the variation in these digestible sugars and in the C:N ratio; both of these parameters dictate the specific methane yield (SMY). The C:N ratio of *L. digitata* ranged from 10.9 in January to a peak of 31.9 in August harvested in the UK [28]. In this study, *L. digitata* collected in August with a C:N ratio of 22.5 generated a BMP yield of 218.0 ± 4.1 L CH_4 kg^{-1} VS; this yield was higher than *L. digitata* harvested in May (184 L CH_4 kg^{-1} VS) [41] and in January (103.3 ± 19.8 L CH_4 kg^{-1} VS) [42] both in Ireland.

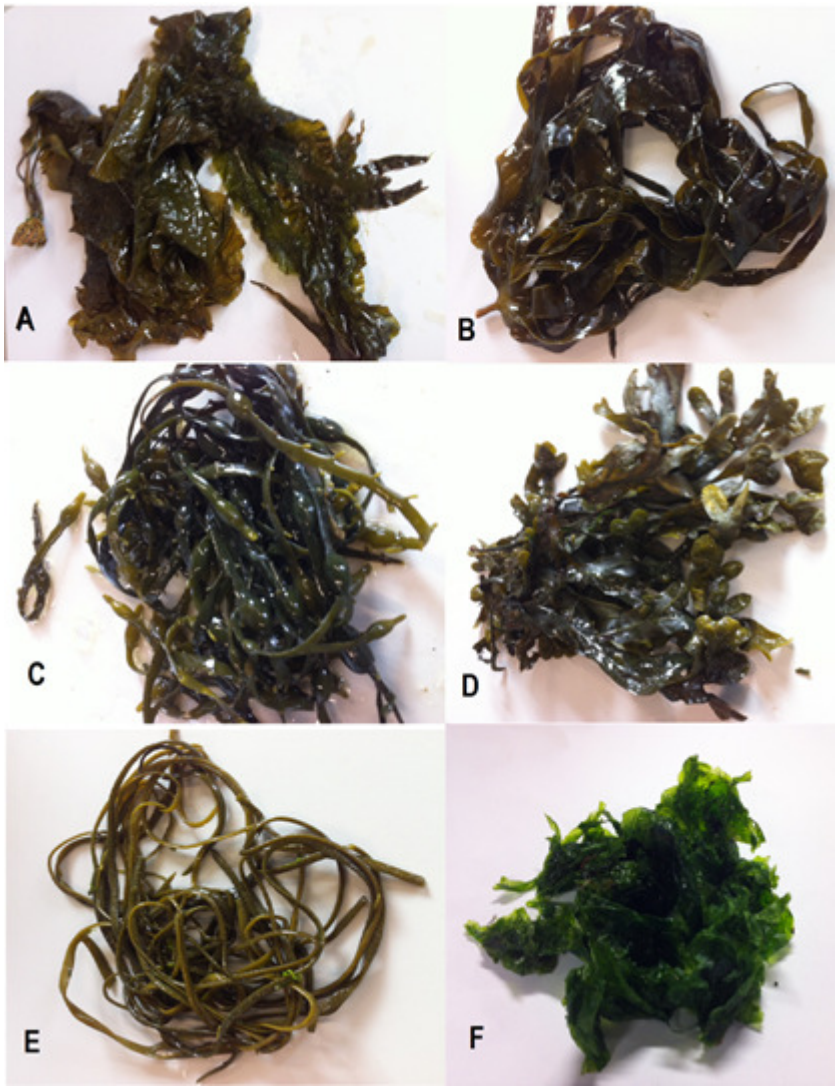


Figure 5.2 A selection of six varieties of the macro algae sampled in these trials.

A: *S. latissima*; B*: L. digitata*; C: *A. nodosum*; D: *F. spiralis*; E: *H. elongate*; F: *U. lactuca.*

5.4.1.3 *A. nodosum*

A. nodosum (Figure 5.2C) is the most abundant seaweed on Irish and Nordic coastlines [43]. *A. nodosum* shares its family type with the macro-algae *Fucus* spp. (Figure 5.2D) and shares similar growing conditions and appearance characteristics. It consists of bladder like nodules which contain a gel substance. *A. nodosum* can be rich in calcium alginate gels as well as containing alginic acid. Alginate concentrations can reach up to 33% of TS [44].

These high concentrations of degradable carbohydrates, provide a good base to support the use of *A. nodosum* as a substrate for biomethane, but it also can contain high levels of polyphenols, up to 14% of TS, which are natural inhibitors of the anaerobic digestion (AD) process [22]. *A. nodosum* sampled in this work produced a BMP yield of 166.3 $\pm$ 20 L CH_4 kg^{-1} VS when compared to 110 L CH_4 kg^{-1} VS harvested in September in Norway [45]. The low BMP yield, combined with the low biodegradability index (0.34: Table 5.3) and the low kinetic decay values (0.12 d^{-1}: table 5.4) indicate that high polyphenol levels may be present. However *A. nodosum* may be a prime candidate for a pre-treatment process, especially one that could remove polyphenols.

5.4.1.4 *F.* species

Three species of *Fucus* were sampled in these trials. *F. vesiculosus* and *F. spiralis* (Figure 5.2D) share a particular physical resemblance to *A. nodosum* (Figure 5.2C)*;* both have bladders attached to the leaf section of the plant. All three *Fucus* species exhibited low C:N ratios when compared to other brown seaweeds, ranging from 15.5 to 17.6 (Table 5.2).

F. vesiculosus is also reported as having a high polyphenol content; as high as 13% of TS content [22]. In this work *F. vesiculous* had a low biodegradability (0.51) and a poor BMP yield (126.3 $\pm$ 11.4 L CH_4 kg^{-1} VS); this yield was higher than the BMP yield of 71.5 $\pm$ 4.9 L CH_4 kg^{-1} VS of *F. vesiculosus* harvested in January in Ireland [42].

F. serratus shared little physical characteristics with the two other *Fucus* species sampled. It has one of the lowest C:N ratios of brown seaweed sampled. *F. serratus* is a low density material which grows along rocky shores and piers, and is difficult to harvest in large volumes. A BMP yield of 101.7 $\pm$ 9.4 L CH_4 kg^{-1} VS was recorded, which was the lowest of all sampled seaweeds. A yield of 65 L biogas kg^{-1} VS was observed for *F. serratus* collected in September, Ireland [13].

F. spiralis however produced a BMP yield of 235.2 $\pm$ 9.4 L CH_4 kg^{-1} VS and a specific yield of 32.74 m^3 CH_4 t^{-1} wwt, which made it one of the better preforming macro-algae species trialled.

5.4.1.5 *S. polyschides*

S. polyschides has a good polysaccharide concentration, with particularly high alginate concentrations (up to 16% TS) [12]. *S. polyschides* has similar growth and geographical patterns to both *L. digitata* and *L. hyperborea*. They attach to rocky coastlines and could be readily cultivated along artificial rock beds or along rope lines. A BMP yield of 263.3 $\pm$ 4.2 L CH_4 kg^{-1} VS was recorded in these trials. Due to its high volatile solids content, it produced the highest yield per weight wet (34.5 m^3 CH_4 t^{-1} wwt). In the literature yields of *S. polyschides* include for 216 $\pm$ 16 L CH_4 kg^{-1} VS in July in France [12] and 255 L CH_4 kg^{-1} VS in September in Ireland [13]. It would be considered a good seaweed for digestion with a k value of 0.19 (in excess of food waste) and a C:N ratio in the optimum range (23.2). It has the highest volatile solid content when expressed as a percentage of wet weight and a good biodegradability index ratio (0.68).

5.4.1.6 *H. elongate*

There is little literature associated with biomethane production from *H. elongata* (Figure 5.2E). It has a long string like appearance and grows along shallow rock faces and is easily accessed at low tide. This makes the harvest intensive and potential for up-scaling difficult. *H. elongate* recorded a BMP yield of 260.9 $\pm$ 2.1 L CH_4 kg^{-1} VS compared to a BMP yield recorded in July in France of 202 $\pm$ 0.03 L CH_4 kg^{-1} VS [12]. *H. elongate* has a C:N ratio in the optimum range (21.4).

5.4.1.7 *A. esculenta*

Again the literature on *A. esculenta* is sparse. It was not found to be in abundance when sampling was conducted for these trials. There is little evidence of significant resource to satisfy a biomethane industry. A BMP yield of 226.0 $\pm$ 5.7 L CH_4 kg^{-1} VS was recorded. *A. esculenta* also had the lowest C:N ratio (15.5) of brown seaweeds.

5.4.2 Green macro-algae – *U. lactuca*

U. lactuca, commonly known as sea lettuce, is a green seaweed. Algae blooms (or green tides) are a direct result of eutrophication. These blooms of *U. lactuca* accumulate over the

summer months and spoil the amenity of beaches [14]. This species has attracted particular attention in recent years, particularly in France where it has become an increasing problem for both shell fish production and amenity of beaches. *U. lactuca* on the low tide can result in depths of 300mm or more of rotting sea lettuce giving off the idiosyncratic rotten egg smell of H_2S gas. *U. lactuca* contains almost no lignin (< 0.03 g kg^{-1}) and has rich glucose concentrations in the form of uronic acid and xylose which make up its sugar content [46]. H_2S is produced due to the high sulphur content of the *U. lactuca* [47]. In these trials the sulphur content of *U. lactuca* species was analysed; a value of 2.95% of TS was recorded. BMP results recorded were 190.1 $\pm$ 3.1 L CH_4 kg^{-1} VS (this work) and 183 L CH_4 kg^{-1} VS in June the previous year from the same beach [14]. This compares well to *U. lactuca* assessed in Denmark at 200 L CH_4 kg^{-1} VS [17].

5.4.3 Additional macro-algae species

Additional species worth mentioning are *Laminaria hyperborea* which shares its genus with *L. digitata* and is also similar in physical appearance. No samples of *L. hyperborea* were available for collection over the period of trials in the selected beaches from which the seaweeds were collected. However this species potentially offers a good biofuel potential as it is reported to grow well from rock beds. A yield of 260 L CH_4 kg^{-1} VS was observed from continuous digestion from *L. hyperborea* harvested in July in the UK [47].

Laminaria japonica is a species of *Laminaria* which is heavily cultivated in Japan and eastern Asia for food production. Over 5 x 10^6 wet tonnes of *L. japonica* were harvested globally; 36% from aquaculture [48]. A yield range of 260 – 280 L CH_4 kg^{-1} VS was achieved for *L. japonica* which puts it in the higher range of brown seaweed chosen for trials in Ireland [30]. However *L. japonica* is not native to Irish waters but does cope well in cold waters, which make it a potential species for large scale aquaculture.

Macrocystis pyrifera commonly referred to as “giant kelp” is the only macro-algae species that has been harvested at large scale in the USA. *M. pyrifera* has the potential to be harvested at large scales for two reasons: its maximum growth size, up to 43 meters in length; and growth rate of 50 t VS ha^{-1} yr^{-1} [49]. *M. pyrifera* also has a high concentrations of both polysaccharides, mannitol (5 - 6% TS) and alginate (13-24% TS) which make it a very suitable feedstock for anaerobic digestion [50]. Reported yields are also high, in the range

of 390 – 410 L CH_4 kg^{-1} VS. However low C:N values (in the range 11.7 – 17.5) were reported [24]. These lower ranges of C:N cause difficult operation of continuous digestion at high loading rates due to ammonia toxicity. The optimum growing temperatures stated for *M. pyrifera* lie between 13 – 15^{o}C [51], which make it possible to grow such a species in Irish waters for approximately 5 months of the year (June – October).

5.4.4 Requirement for continuous digestion trials

Batch tests such as BMP assays are a first step in the design of an anaerobic digestion system. Full design requires a continuous digestion trial to highlight operating parameters such as levels of volatile fatty acid (VFA) and ammonia. Sulphur can be problematic in continuous digestion if the C:S ratio is less than 40 [52]. *U. lactuca* sampled as part of these trials had a C:S ratio of 10.2. C:S ratios for *S. latissima* was recorded as 24:1 [12] while for *L. digitata,* values of 29 - 60.3 were documented [28].

5.4.5. Harvesting potential

The harvest of seaweed can be by manual collection from naturally occurring stocks either by physical harvest or from beach cast. The data on yields is limited. For example only one data source was found for *L. Digitata* which suggested 5 t TS ha^{-1} yr^{-1} [57]. This is very small when considering the other species of *Lamanaria (hyperborea and japonica)* are attributed yields in the range of 30 to 90 t TS ha^{-1} yr^{-1}.

A significant issue arising from harvesting of natural stocks is the cost per tonne of seaweed. Harvest of *A. nodosum* is reported anecdotally from industry sources as costing €330 dry t^{-1} in Ireland. This may be compared to the cost of grass silage of €79 dry t^{-1} [53]. *A. nodosum, H. elongate, F. spiralis, F. serratus* and *F. vesiculosus* are all seaweeds which would share similar harvesting cost, due to their growing environment along piers and rocky shorelines. The cost may be rationalised by considering the road infrastructure to costal rural beaches, the separation between beaches and the manual nature of the work.

U. lactuca falls into a different category in that it accumulates as a bloom in such volumes that it can be mechanical cleared off of beaches. The reported arising of *U. lactuca* in Lannion Bay, Brittany, France are of the order of 100,000 t wwt yr^{-1} [54]. In West Cork an

area of 85 ha of *U. lactuca* covered to a depth of 300mm is visible on low tide; 10,000 t wwt yr^{-1}, is cleared off this beach each year [14]. As it is detrimental to the amenity of a bay and there are little opportunities for reuse or disposal, it is possible to be given a gate fee to process this substrate. It is likely that this may be the cheapest source of seaweed for biofuel production.

The second option involves aquaculture. *L. digitata, S. polyschides, S. latissima and A. esculenta* are all species which can be cultivated at larger scales and added to existing aquaculture systems. These seaweeds may benefit from a poly-culture system, producing the seaweed in association with mussels, or in the vicinity of a salmon farm (cleaning the water through removal of excess nutrients). Seaweed farms may be associated with off shore wind turbine towers, or wave or tidal turbines, which have existing infrastructure to act as a growing medium for the seaweed. Large financial costs are involved in the development and maintenance of a seaweed farm. Alvarado-Morales, Boldrin [18] undertook a life cycle analysis of a seaweed biofuel project in Nordic conditions and found the production stage was the most energy intensive, requiring 57% of energy input. Developing a low cost, high productivity (large yields of VS ha^{-1} yr^{-1}) and high biomethane yielding seaweed is essential to optimising gaseous biofuel industry produced from seaweed.

5.4.6 Energy yields from seaweed biomethane

The EU recommend 2.5% renewable energy supply in transport (RES-T) from advanced biofuels such as from seaweed [55]. According to Allen and co-workers, one beach in West Cork (generating 10,000 t wwt yr^{-1} of *U. lactuca*) has the potential to provide biomethane to fuel 264 cars on a year round basis from a single digester [14]. Potential biomethane yields per hectare of seaweed on an annual basis are presented in table 5.5. To put these figures in context Smyth and co-workers, assessed the gross energy per hectare for a range of terrestrial crop liquid biofuel systems [27]. The best results were obtained for palm oil biodiesel at 120 GJ ha^{-1} yr^{-1} and sugarcane ethanol at 135 GJ ha^{-1} yr^{-1}. Biogas systems tend to produce more energy per hectare from crops than liquid biofuel systems. Murphy et al. assessed a range of energy crop biogas systems. Maize and perennial ryegrass have

biomethane yields of between 1,660 - 12,250 and 2,682 - 6,400 m^3 ha^{-1} yr^{-1} respectively [56]. This corresponds to 60 to 441 GJ ha^{-1} yr^{-1}. From table 5.5, seaweeds have a higher upper bound in the energy production per hectare range, potentially yielding over 700 GJ ha^{-1} yr^{-1}. What is crucial however is that this area required to grow the macro algae is at sea and is not taking land from food production.

Table 5.5 Potential gross energy production per hectare per annum based on a variety of species of seaweed

Substrate	**Yield (harvest)**		**Biomethane yield**	**Biomethane yield**	**Gross Energy**
	tTS ha^{-1} yr^{-1} (*tVS ha^{-1} yr^{-1})	t wwt ha^{-1} yr^{-1}	m^3 CH_4 t^{-1}wwt	m^3 ha^{-1} yr^{-1}	GJ ha^{-1} yr^{-1}
L. digitata	5.0[a]	35.2	22.5	792	28
S. polyschides	22.5[b]	147.5	34.5	5090	181
S. latissima	30.0*[c]	297.3	34.5	10,260	365
A. esculenta	36.0*[d]	302.2	26.9	8130	289
U. lactuca	45.0[e]	249.6	20.9	5216	186
L. hyperborea	30.0 – 90.0[f]			6630 – 19,890	239 – 716
L. japonica	31.0*[c] – 80.0*[g]			8060 – 20,800	290 – 749
M. pyrifera	34.0*[d] – 50.0*[h]			13,260 – 19,500	477 – 702

* = VS basis, a = [57], b = [43], c = [58], d = [59], e = [17] , f = [60], g = [61], h = [24].
Biomethane yields (m^3 ha^{-1} yr^{-1}) are calculated from data in table 5.3.

5.5 Conclusions

Seaweeds are a source of third generation gaseous biofuel. They do not require agriculture land and do not interfere with food production. The market however is not as yet commercial and there are many variables to consider in proposing an optimal model for

this proposed industry. Of the beach cast seaweeds *U. lactuca* appears most profitable as it gathers in blooms that need to be removed from beaches. Thus harvest cost is cheap and it may even attract a gate fee. Gross energy yields are of the order of 186 GJ ha^{-1} yr^{-1}, which are higher than the optimal first generation liquid biofuel systems such as sugarcane ethanol (135 GJ ha^{-1} yr^{-1}) or palm oil biodiesel (120 GJ ha^{-1} yr^{-1}).

It is suggested that *S. latissima* has the highest specific methane yield of seaweeds available in Ireland. If used in aquaculture yields of 365 GJ ha^{-1} yr^{-1} may be achieved. It is suggested that seaweed farms be situated in close proximity to sources of pollution (such as salmon farms) to optimise their utility.

Other species of seaweed not indigenous to Ireland such as *L. japonica* and *M. pyrifera* may provide higher energy yields.

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6 The potential of algae blooms to produce renewable gaseous fuel

The potential of algae blooms to produce renewable gaseous fuel

Eoin Allen [ab], James Browne [ab], Jerry D. Murphy [ab]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract:

U. lactuca (commonly known as sea letuce) is a green sea weed which dominates *Green Tides* or algae blooms. *Green Tides* are caused by excess nitrogen from agriculture and sewage outfalls resulting in eutrophication in shallow estuaries. Samples of *U. lactuca* were taken from the Argideen estuary in West Cork on two consecutive years. In year 1 a combination of three different processes/pretreatments were carried out on the *U. lactuca*. These include washing, wilting and drying. Biomethane potential (BMP) assays were carried out on the samples. Fresh *U. lactuca* has a biomethane yield of 183 L CH_4 kg^{-1} VS. For dried, washed and macerated *U. lactuca* a BMP of 250 L CH_4 kg^{-1} VS was achieved. The resource from the estuary in West Cork was shown to be sufficient to provide fuel to 264 cars on a year round basis. Mono-digestion of *U. lactuca* may be problematic; the C:N ratio is low and the sulphur content is high. In year 2 co-digestion trials with dairy slurry were carried out. These indicate a potential increase in biomethane output by 17% as compared to mono-digestion of *U. lactuca* and slurry.

Keywords: macro-algae; biomethane; gaseous biofuels.

6.1 Introduction

This paper investigates the potential to convert *U. lactuca* (a sea weed commonly known as sea lettuce) into biomethane, a renewable gaseous fuel. Annually, large amounts of *U. lactuca* are washed up on shorelines where it decomposes, generating malodours, and reducing the amenity of the bay. *Green Tides* (blooms of *U. lactuca*) occur in shallow estuaries or bays, which are subject to eutrophication from excess run off of nitrogen [1] from non-point sources (septic tanks and spreading of slurries on agricultural land) and point sources (sewage outfalls). Mono-digestion of *U. lactuca* is difficult due to the high sulphur content and the low C:N ratio [2]. The C:N ratio was assessed at less than 10:1, which is significantly less than the ideal range of 20:1 to 30:1 [3].

6.1.1 Worldwide use of algae

Algae may be split into two groups: micro algae and macro algae. Both algae types were investigated as potential fuel sources during the oil crises of the 1970's in Japan and the USA [4] However over the last 15 years research is dominated by micro algae biodiesel [5] whilst research on digestion of digestion of micro algae is very limited partly due to the poor returns in biomethane production [6].

Research on macro algae (or sea weed) receives less attention. Digestion of macro algae has been shown to produce significant levels of biomethane [7] . Macro algae may be harvested from natural stocks (cast sea weed) or cultivated [8]. The FAO [9] reported that there was approximately 1.29 million wet tonnes of macro algae harvested from natural stocks; this is about 1/8th of the 10.1 million wet tonnes of marine biomass cultivated (with a net value of $6 billion). In 2010 the FAO [10] reported a harvest of 1.5 million wet tonnes from natural stocks and 15.4 million wet tonnes cultivated.

Ireland, Denmark, France, Italy and Japan suffer greatly from *Green Tide* and the associated deposition of *U. l*actuca (sea lettuce) on the shore line. Anaerobic digestion of this resource is beneficial to the marine environment and a source of third generation renewable gaseous biofuel.

6.1.2 Macro algae as a source of gaseous biofuel

Biofuels from sugars, starches and oil crops may be considered first generation biofuels. Biofuels from lingo cellulosic biomass and residues are considered second generation. Biofuels from algae, are considered third generation. There is a significant call to limit the production of first generation biofuels; second generation biofuels from lignocellulosic biomass (such as *Willow* or *Miscanthus*) require agriculture land and are as such, still an issue in the food fuel debate [11]. The energy balance of micro algae biodiesel (due to the need to separate the lipids from the micro-algae solution) is poor [12].

Biogas production from macro algae may be a sustainable gaseous biofuel. It is free from the food fuel debate and it does not suffer from the energy balance issues of biodiesel micro algae. Macro algae consist of polysaccharides (alginate, laminarin and mannitol), with lignin content as low as 0.03g/kg dry matter [13]. This may be compared to the lignin content of grass (a substrate used for biogas production) of between 3-7% [14, 15]. Seaweeds have low cellulose content, which make them an easy material to convert to methane by anaerobic digestion processes [16]. *U. l*actuca is both a third generation feed stock for biofuels and a residue requiring treatment. A Sea Lettuce Task Force [17] stated that a: *"do-nothing" approach is not an option given the potential serious health concerns associated with accumulations of decaying sea lettuce.* The report further stated that in 2009 some 10,000 tonnes of sea lettuce (13kg sea lettuce per m^2 of estuary) accumulated around the Argideen estuary, in West Cork (the source of seaweed used in the experimental analysis in this paper).

6.1.3 Eutrophication, *U. lactuca* and green tides

The Irish EPA issued reports on eutrophication [18, 19] and identified nine sites as eutrophoric (Figure 6.1) and susceptible to *Green Tide*. *Green Tide* is also an issue in Japan and France [20, 21]. Two factors influence growth of *U. Lactuca*.

The first is the physical characteristics of the bay; shallow basins with protected inlets are most susceptible; the shape of these bays prevents the blooms and nitrate concentrations being discharged to the sea. Coastal lagoons due to their topography (generally being shallow) lend themselves to growth of macro algae and sea grasses such as *U. lactuca* rigida [22]. Along with preventing wash out of algae; it also keeps the pollutant Urea content of

other nitrogen causing materials to stay within the estuary initiating the algal growth [23]. Macro algae blooms are produced by nutrient enrichment of estuaries in which the sea floor can still receive levels of light penetration known as the photic zone [24].

The second factor in macro algae growth in coastal basins is intense farming of land. Estuarine farmlands with significant nutrient application (N, P and K) contribute to large blooms of macro algae which wash up on strands or beaches along the estuaries.

Areas in continental Europe which experience mass wash up of *U. l*actuca include Lannion Bay and St Brieuc (Brittany, France) and Seden Beach (Odense Fjord, Denmark). In the Lannion estuary 25,000 wet tonnes were washed up in one season (as compared to 10,000 wet tonnes in Argideen). Eleven sites have reported yields of between 1,500 m^3 and 20,000 m^3 of sea lettuce [25].

6.1.4 Research on digestion of *U. lactuca*

Authors in Japan and Brittany [26, 27] suggest anaerobic digestion as a more viable waste management option than composting or landfill. However research on biogas produced from *U. lactuca* is very limited. The literature is dominated by laboratory scale work and in particular biomethane potential (BMP) tests. Table 6.1 summarises relevant outputs. The values from fresh *U. l*actuca are in the range of 126 to 174 L CH_4 kg^{-1} VS. Pre-treatments, such as maceration and washing, lead to increased methane yields in the range of 180 to 271 L CH_4 kg^{-1} VS. Peu and Sassi [28] generated 330 L CH_4 kg^{-1} VS for juice extracted from *U. lactuca.*

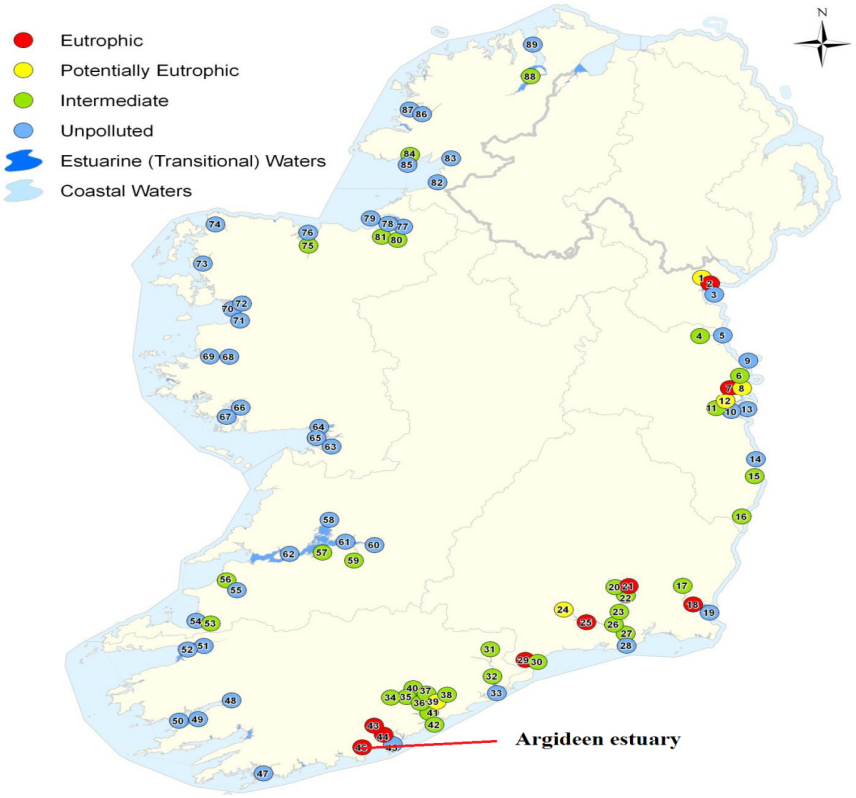


Figure 6.1 Estuary conditions and eutrophic status, (adapted from EPA, 2010; reproduced with permission).

Table 6.1 Bio-methane yields of *U. lactuca* from the literature

***U. lactuca* from:**	**Pre-treatment type**	**BMP yield** (CH_4 L kg^{-1} VS)
No pre-treatment		
Japan [a]	Fresh	126
Denmark [b]	Fresh	174
France [c]	Fresh	128
Pre-treatment		
Denmark [b]	Unwashed and macerated	271
Japan [a]	Washed grinded	180
Hydrolytic juices		
Brittany [d]	Juices	330

6.1.5 Objectives

The objectives of this paper are to:

- Collect and categorise *U lactuca* from Irish beaches.
- Assess the biomethane potential of treated and untreated *U. lactuca*.
- Assess any variation in *U lactuca* over consecutive years.
- Establish if synergistic effects occur in co-digestion with dairy slurry.

6.2 Materials and methods

6.2.1 Collection, pre-treatment and characterisation of *U. lactuca*

Samples of *U. lactuca* were collected initially in June 2011 from the Argideen estuary (Figure 6.1). Six, 1 m^3 bags were collected. Samples were processed in a warehouse on large drying tables with openings on the underside to allow any water to flow through. Wilted samples were left on the drying tables to air dry naturally. For dried samples, two kerosene space heaters were used. They were placed below a suspended floor and were operated for up to 36 hours. Treatments may be described as below:

- Fresh seaweed as sampled from the shoreline.
- Wilting or natural drying was effected by laying samples out on the perforated table. Samples were turned after a day and left to wilt for a further 24 hours and then frozen (-20^{o}C).
- Washed seaweed was rinsed with water to remove any sand and other impurities collected with the seaweed when removed from the beach.
- Dried seaweed involved laying the seaweed on top of wood pallets with a gas furnace placed underneath burning constantly for 2 weeks at a temperature of 80 ^{o}C.



Figure 6.2 Comparison between the fresh sample and dried, macerated pre-treated sample.

pH was measured daily on samples of digestate using a Jenway 3510 pH meter. The dry solids (DS) and volatile solids (VS) were determined gravimetrically using the methods described in [29]. To obtain percentage dry solids the samples were placed in triplicate into an oven for 24 hours at 105 degrees Celsius. The samples were then placed in a furnace at 550 degrees from between 6 to 12 hours to obtain the volatile solids content of the samples.

To obtain the percentages of Carbon, Hydrogen and Nitrogen (which allows generation of stoichiometric description of biomass) the samples were oven dried at 100 degrees Celsius, ground down and passed through a 600 µm sieve. Each waste stream was sampled and tested in triplicate for total carbon (C), hydrogen (H) and nitrogen (N) on a total solids basis and was attained by ultimate analysis using element analyser (CE 440 Model) at the Chemistry Department in University College Cork, Ireland. The samples analysed were given a range of +/- 0.5 percentage error factor.

Table 6.2 highlights 4 samples of *U. lactuca* subjected to the pre-treatment processes described above. The dried samples of *U. lactuca* had a DS content of 72% with a VS content of 40% (expressed as a percentage of weight of sample). The fresh and wilted samples the DS content was in the range 19% to 32%; while the VS content was in the range 11% to 16%. The day before the samples were digested they were defrosted in a cold storage room for 24 hours at 4^{o}C. Samples were passed through a bench top grinder to ensure a homogeneous sample was prepared. The dried sample was easily broken to a powder like consistency which ultimately would aid in its bio-degradability. All other samples were broken down to a sample size of between 10 and 15mm.

Table 6.2 Ultimate analysis of *U. lactuca* samples

U. lactuca	**C**	**H**	**N**	**C:N ratio**	**DS %**	**VS %**	**pH**	**Protein %**
1. Fresh	25.4	3.7	3.3	7.7	19	11	6.99	21.4
2. Wilted & unwashed	27.2	4.0	3.1	8.7	20	11	7.77	21.1
3. Washed & dried	22.3	3.3	2.3	9.6	72	40	7.35	23.7
4. Washed & wilted	23.3	3.2	2.6	8.8	32	16	7.60	20.8

6.2.2 Assessment of potential energy using Buswell equation

Using the Buswell equation [30] (Eqn. 6.1) a stoichiometric equation can be developed to obtain the potential Methane (CH_4) and Carbon Dioxide (CO_2) volumes produced when the substrate is broken down by the consortium of micro bacteria present in the digester.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 \quad \text{Eqn. 6.1}$$

6.2.3 Biomethane potential assessment of *U. lactuca*

Batch BMP tests were carried out in triplicate. The batch reactors consisted of 500 ml glass bottles with an outlet port for biogas to flow out and a purging port to allow Nitrogen to flush the head space of the reactors (Figure 6.3). The reactor bottles had a continuous stirring motor attached which was timed to come on for 60 seconds and off for 60 seconds at 60rpm, for the duration of the experiment. Three blank inoculum mixes were prepared; the BMP results for these blanks were subtracted from the biomethane production yield curve of the substrate to output a substrate only BMP. The ratio selected was 3:1 inoculum to substrate (I:S). This was chosen to ensure good destruction of volatile solids by

overcoming possible inhibitory results due to the low C:N ratio of the substrate. This is in line with recommendations of Angelidaki, Alves [31] and Raposo, Banks [32]. Inoculum was sourced from a large scale digester operating on a mix of food wastes, cattle slurry and grease trap waste located in Co. Kilkenny, Ireland operating at 2 kg VS m^3 d^{-1}.

The required amount of inoculum and substrate were evaluated for each reactor. This was adjusted to allow the same amount of inoculum in each reactor to facilitate accurate and easy calculation of the BMP. The sample with the lowest VS percentage required was chosen to generate the quantity of inoculum; this volume of inoculum was placed in each bottle. The substrate amounts were calculated at 3:1 (Inoculum: Substrate). After each sample was prepared and the adjusted weight calculated it was placed inside the reactor with the set volume of inoculum. The solution was made up to 400ml by de-ionised water. Reactor bottles and caps were screw tightened and sealed with silicon spray. The head space which accounted for 250 ml, including cleaning solution, connection pipe work and reactor head space was flushed immediately after sealing each reactor with 99.99% purity Nitrogen. The counter mechanism was a set of 15 electronic tipping buckets submerged in water, each tipper corresponding to a specific amount of gas from a specific reactor (Figure 6.3). The biogas produced was passed through an individual solution of NaOH 3M concentration to remove CO_2 and any other impurity. The outputted results were automatically adjusted for Standard Temperature and Pressure (STP).

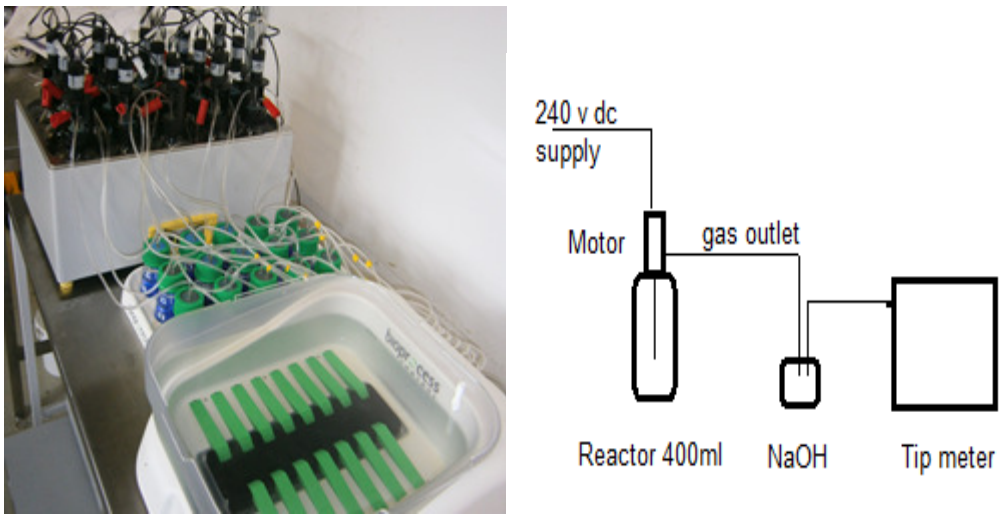


Figure 6.3 Biomethane potential test system.

6.2.4 Mono and co-digestion of *U. lactuca* and dairy slurry

U. lactuca was harvested for a second year (2012) with the purpose of identifying annual variation in biomethane production. A series of BMP assays were completed for mono-digestion of fresh and dried *U. lactuca* and co-digestion of the *U. lactuca* with dairy slurry. The dairy slurry was taken from 18 – 24 month year old cows, housed during the summer period. In co-digestion, ratios of both dried and fresh *U. lactuca* with dairy slurry were chosen at 25, 50 and 75% respectively.

6.2.5 Calculation of kinetic decay constant, half-life and lag phase

Using a combination of first order and second order kinetics for the degradation of organic material, a Matlab programme was developed to process all data from BMP assays to determine a set list of parameters. Equation 6.2, is used to develop decay constants, K. The modified Gompertz formula (Eqn. 6.3) [33] was used to determine the lag phase Δ, which was calculated for each BMP assay to establish the time taken to initiate biomethane production within the vessels. A regression coefficient R^2, was obtained for each BMP trial substrate to determine how well the curve fit expression worked on each graph of biomethane production. The half-life determined the time taken for half of the biomethane production to be produced.

$$\boldsymbol{G(t) = G_0\,(1 - e^{(-kt)})} \qquad \text{Eqn. 6.2}$$

$$\boldsymbol{G(t) = G_0\,.e[-e\left[\left(\frac{u}{G_0}\right).(\Delta - t) + 1\right]]} \qquad \text{Eqn. 6.3}$$

Where,

G (t) = the cumulative biomethane yield at time t (L CH_4 kg^{-1} VS).

G_o = Biomethane potential of the substrate recorded (L CH_4 kg^{-1} VS).

k = Biomethane production decay rate constant ($days^{-1}$).

t = time of BMP test in days.

Δ = lag phase (days).

6.3 Results

6.3.1 Characterisation of *U. lactuca*

Table 6.3 is used to outline the stoichiometric equation of fresh *U. lactuca*. The analysis in table 6.4 suggests that *U. lactuca* with 11% VS (1. Fresh), a theoretical maximum yield of 431 L CH_4 kg^{-1} VS may be achieved. Using this methodology the theoretical maximum production of methane, the portion of methane in the biogas and the maximum production of biogas may be generated from each sample (Table 6.5).

Table 6.3 Generation of stoichiometric equation of fresh *U. lactuca.*

Component	**Number of Atoms per mole**	**Atomic Weight**	**Weight Contribution** (kg t^{-1})	**Percentage of TS** (%)
Carbon	21.19 (8.98)	12.00	254.28	25.43
Hydrogen	37.01 (15.69)	1.00	37.01	3.7
Oxygen	17.23 (7.3)	16.00	275.69	27.5
Nitrogen	2.36 (1)	14.00	33.03	3.3

Table 6.4 Theoretical assessment of energy production from fresh *U. lactuca*

Theoretical assessment of energy production from fresh *U. l*actuca

Biogas production as assessed by Buswell equation:

$C_{8.98}\ H_{15.7}\ O_{7.3} + 1.41\ H_2O \rightarrow 4.63\ CH_4 + 4.35\ CO_2$

110kg VS + 11.6 kg water → 33.9kg CH_4 + 88kg CO_2 (Fresh *U. lactuca* is 11% VS)

Density of CH_4 = 0.714kg m^{-3}, Density of CO_2 = 1.96kg m^{-3}

Gas by volume → 47.45m^3 CH_4 + 44.76m^3 CO_2 = 92.2m^3 biogas @ 51.5% CH_4

Theoretical maximum methane production:

47.45 m^3 CH_4/ 110 kg VS: 431 L CH_4 kg^{-1} VS

Table 6.5 Theoretical methane yields of all pre-treated samples of *U. lactuca* collected.

U. lactuca	**Biomethane** (L CH_4 kg^{-1} VS)	**Biogas** (L kg^{-1} VS)	**Methane** (%)
Fresh	431	838	51.5
Wilted & unwashed	460	864	53.3
Washed & dried	394	793	50.4
Washed & wilted	402	816	49.6

The samples which showed the most *theoretical* methane yield were the unwashed samples (Table 6.5) which suggest there is biomethane potential in the juices which may be removed when washing the fresh material. However there are benefits from a practical perspective in washing the seaweed prior to treatment in an anaerobic process. Mechanical problems which may arise as a result of debris and sand collecting in the digester system are minimised through washing. Sand is also abrasive to moving parts

(mixers and pumps). Potentially salts may also be removed in this process which will lead to a more stable digestion process.

6.3.2 Mono-digestion of *U. lactuca* from year 1

The BMP results for mono-digestion of *U. lactuca* are illustrated in figure 6.4. Table 6.6 outlines a comparison of the BMP results with theoretical methane production. The yields are significantly lower than theoretical maximum potential suggested by use of the Buswell Equation. The unwashed samples (samples 1 and 2) the BMP achieved 42% and 36% of theoretical value respectively. The fresh sample generated a higher value than the wilted sample. The washed samples exhibited a higher methane yield with sample 3 (washed and dried) generating the highest yield at 250 L CH_4 kg^{-1} VS (64% of the theoretical value).

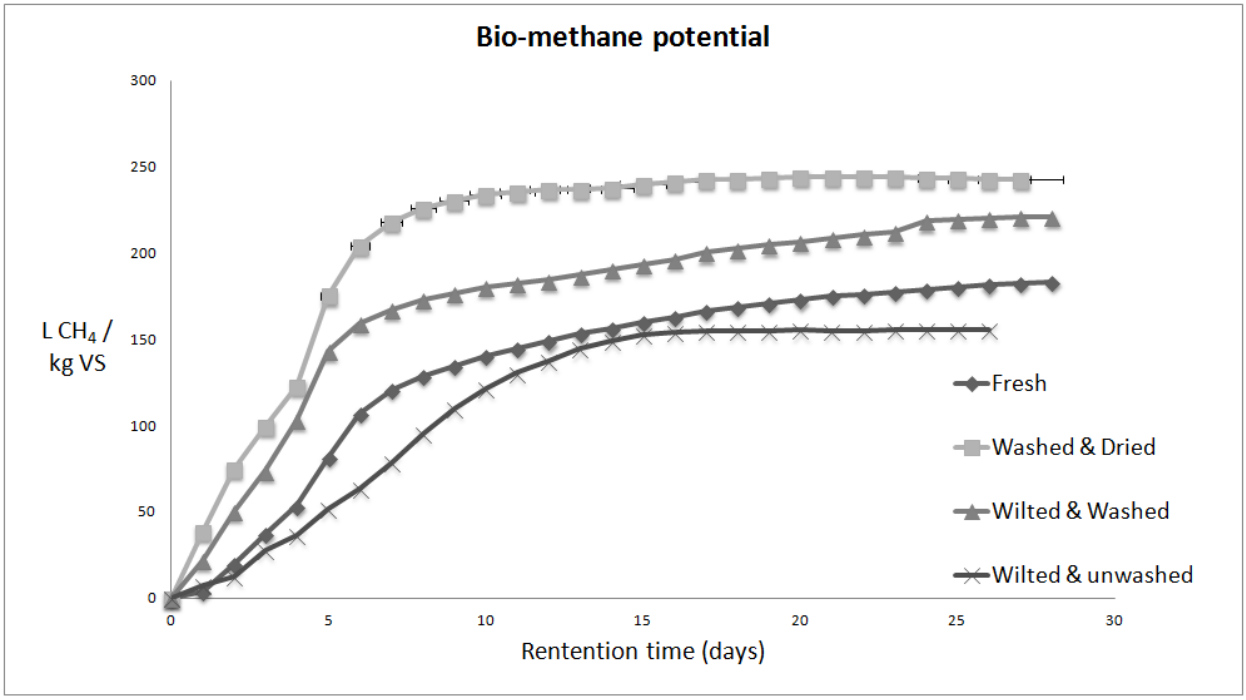


Figure 6.4 Cumulative BMP, fresh and pre-treated samples from Year 1.

Table 6.6 BMP results compared to theoretical yield.

Sample	BMP result (L CH_4 kg^{-1} VS)	Standard deviation (L CH_4 kg^{-1} VS)	Max potential from Buswell, (table 6.5) (L CH_4 kg^{-1} VS)	Specific yield (m^3 CH_4 t^{-1})	C:N ratio	Increased yield in co-digestion (%)
Year 1						
1 Fresh	183.2	5.83	431	20.2	7.7	
2 Wilted & unwashed	165.0	9.47	460	18.2	8.7	
3 Washed & dried	250.2	13.32	394	100.1	9.6	
4 Wilted & washed	221.1	22.74	402	35.4	8.8	
Year 2						
Slurry	136	2.99	382	7.00	19.8	
Dried *U. lactuca*	226	6.66	401	104.86	7.1	
Fresh *U. lactuca*	205	5.01	412	21.32	9.1	
Co-digestion Yield based on mono-digestion						
75% Fresh	220	4.91	188	20.11	11.77	+ 17.0
50% Fresh	200	11.2	171	15.88	14.45	+ 17.0
25% Fresh	183	7.85	153	12.30	17.12	+ 19.6
75% Dried	210	6.31	203	75.915	10.275	+ 3.4
50% Dried	193	5.42	181	50.064	13.45	+ 6.7
25% Dried	186	8.81	158	29.24	16.62	+ 17.7

6.3.3 Mono and co-digestion of *U. lactuca* from year 2 and dairy slurry

The BMP results for mono-digestion of *U. lactuca* from year 2 are illustrated in figure 6.5. Table 6.6 outlines a comparison of the BMP results with theoretical methane production. Again the yields are significantly lower than theoretical maximum potential suggested by use of the Buswell Equation. A comparison was drawn between the expected BMP results based on a pro-rata analysis of mono-digestion and the result in co-digestion. This approach was used to establish whether there was a positive symbiotic reaction between the two substrates when digested. This was expected due to the forecasted increase in the C:N ratio. Table 6.7 outlines the results of the kinetic analysis.

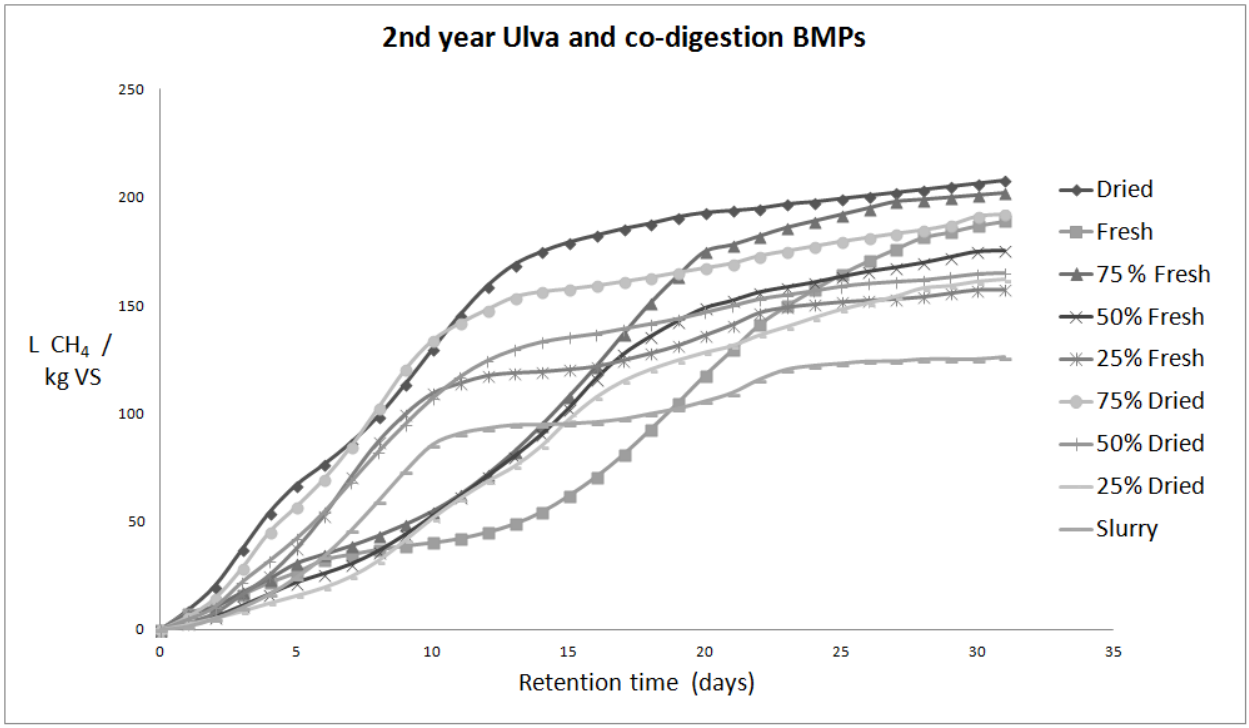


Figure 6.5 Cumulative BMP for *U. lactuca* from the second year in mono and co-digestion.

Table 6.7 Kinetic decay constants and related values for all BMP assays of *U. lactuca* and dairy slurry.

Sample	**BMP result** (L CH_4 kg^{-1} VS)	**Standard deviation** (L CH_4 kg^{-1} VS)	**Max potential from Buswell, (table 6.5)** (L CH_4 kg^{-1} VS)	**Specific yield** (m^3 CH_4 t^{-1})	**C:N ratio**	**Increased yield in co-digestion** (%)
Year 1						
1 Fresh	183.2	5.83	431	20.2	7.7	
2 Wilted & unwashed	165.0	9.47	460	18.2	8.7	
3 Washed & dried	250.2	13.32	394	100.1	9.6	
4 Wilted & washed	221.1	22.74	402	35.4	8.8	
Year 2						
Slurry	136	2.99	382	7.00	19.8	
Dried *U. lactuca*	226	6.66	401	104.86	7.1	
Fresh *U. lactuca*	205	5.01	412	21.32	9.1	
Co-digestion Yield based on mono-digestion						
75% Fresh	220	4.91	188	20.11	11.77	+ 17.0
50% Fresh	200	11.2	171	15.88	14.45	+ 17.0
25% Fresh	183	7.85	153	12.30	17.12	+ 19.6
75% Dried	210	6.31	203	75.915	10.275	+ 3.4
50% Dried	193	5.42	181	50.064	13.45	+ 6.7
25% Dried	186	8.81	158	29.24	16.62	+ 17.7

6.4 Discussion

6.4.1 Comparison of BMP results from fresh and pre-treated samples with literature

Both years dried *U. lactuca* produced the highest yield (Table 6.6) both in terms of L CH_4 kg^{-1} VS (226 to 250 L CH_4 kg^{-1} VS Year 2 and 1 respectively) and more obviously in terms of m^3 CH_4 t^{-1} volume (100 to 105 m^3 CH_4 t^{-1}). It is suggested that this is a feed stock that would have considerable interest for developers of biogas facilities. However a significant parasitic thermal demand can be associated with drying unless surplus heat is available from a biogas combined heat and power (CHP) facility.

Wilted and washed *U. lactuca* produced more methane than wilted and unwashed *U. lactuca* (Table 6.6). Fresh *U. lactuca* produced more methane than wilted and unwashed *U. lactuca*. In this experiment we may suggest that wilting of unwashed *U. lactuca* is the least desirable pre-treatment while drying is the most desirable pre-treatment.

Research undertaken in Brittany [27] suggested that juices pressed from sea lettuce can provide significant levels of biomethane; in their work they generated a methane yield from hydrolytic juices from *U. lactuca* of 330 L CH_4 kg^{-1} VS or 261 L CH_4 kg COD^{-1}. The liquor was shown to be easily degradable as a destruction rate of 93% was obtained. These results indicate the biomethane potential of juices associated with sea lettuce and suggests that pressing or collecting these liquors could lead to a significant biomethane potential.

Besides harvesting existing *U. lactuca* there is potential to cultivate *U. lactuca*. The maximum growth rate in a Danish bay was found to be 45 t DS $ha \cdot yr^{-1}$ [34]. This is similar to the required yield according to Bird and Benson [35], when harvested at a large scale from an off shore farm producing Laminaria (brown macro algae). Samples of *U. lactuca* were treated to a varying range of pre-treatments from drying, macerating and washing. The optimum methane yield of 271 L CH_4 kg^{-1} VS [34] came from algae which was unwashed and macerated; this may be compared with 250 L CH_4 kg^{-1} VS for washed and dried *U. lactuca* in this experiment. Fresh *U. lactuca* had a very similar BMP result to the fresh *U. lactuca* in this experiment (174 L CH_4 kg^{-1} VS versus 183 L CH_4 kg^{-1} VS respectively). Brown seaweed in particular kelps, are documented to produce higher biomethane yields: up to 280 L CH_4 kg^{-1} VS Laminaria and 410 L CH_4 kg^{-1} VS for a giant kelp variety Macroystis [36]. Similar results were obtained in studies carried out on sea lettuce samples in Japan where

just macerating the feedstock led to an increase in biomethane yields. Values of 180 L CH_4 kg^{-1} VS were obtained; values of 70% of this (ca. 126 L CH_4 kg^{-1} VS) were reached for untreated *U. lactuca* samples [26] The literature (Table 6.1) indicates how basic pre-treatment can aid in producing greater BMP yields.

6.4.2 Variation in algae from year 1 to year 2

The *U. lactuca* assessed in this paper varied from year 1 to year 2. The BMP yield of the dried sample decreased by 10%; this may be explained by the decrease in C:N ratio from 9.6 to 7.1. The BMP yield of the fresh sample increased by 12% in the second year, again corresponding to an increase in the C:N ratio. A small decrease was observed in the dry solids content of the *U. lactuca* (19.6 % to 17.8%). The volatile solids content also decreased from 11.2% to 10.4%. A larger variation was observed in the dried samples but this can be attributed to the level of completion of drying.

6.4.3 Process kinetics

Table 6.7 outlines the results of the kinetic modelling of the *U. lactuca* year 1 and 2 and for co-digestion of *U. lactuca* with dairy slurry. Higher K values, (indicating faster degradation rates) were recorded for *U. lactuca* collected in year 1 than year 2. The highest K value (0.23 d^{-1}) was associated with dried *U. lactuca* in year 1. With respect to figures 6.4 and 5 the K values are indicative of a steeper curve and as such a faster degradation rate, a shorter retention time and a smaller cheaper digester. This K value may be compared with values of 0.433 d^{-1} food waste and 0.239 d^{-1} grass silage from trials carried out by the authors (data not included in this paper).

R^2 values indicate the fit of the model to the result, with an R^2 value of 100 suggesting a perfect fit. Δ is the lag phase which represents the time taken for the process to produce significant amounts of methane. T50 is the half-life (when half of the methane has been produced). It may be noted that these values would suggest relatively small digesters with retention times typically less than 30 days. Typically substrates with large lag phases have longer half-lives and those with small lag phases have short half-lives. The ideal substrate

would have a high K value, a short lag phase and a short half-life. Dried *U. lactuca* year 1 would appear to be the best substrate.

6.4.4 Inhibition

There has been wide scale research into monodigestion of various bioenergy substrates which have, at short retention times proven successful with efficient biomethane yields. Overtime, however there can be a build-up of VFAs with increased organic loading rates, until there is inhibition of the methogenic bacteria and methane production ceases (Jiang et al., 2010). The inhibition can occur at increased rates depending on the Carbon to Nitrogen ratio (C:N). When C:N is lower than 20, there is an imbalance between carbon and nitrogen requirements for the anaerobic micro flora [37] leading to increased levels of ammonia in the bioreactor which can eventually lead to failure [2, 38]. Other factors relating to inhibition of biomethane production is high Sulphur and high Sodium concentrations of macro algae, which can disturb the anaerobic digestion process and ultimately methogenic activity [39]. Macro algae and specifically *U. l*actuca has a particularly low C:N ratio (in the range 7.7 to 9.6: table 6.2 and 6.6). Despite this low C:N ratio, elevated sulphur content, high sodium concentration, *U. lactuca* when either mono digested or co-digested with animal slurry produces a lower than expected level of H_2S without inhibition taking place [40]. An explanation suggested for the continuation of biomethane production is that the sludge or inoculum acclimatises to the substrate content and the inhibiting substances present. The ratio of inoculum to substrate and organic loading rates are also important in inoculum acclimatisation [41]. It should however be noted that there is little practical commercial experience of digestion of *U. lactuca*.

6.4.5 Mono-digestion and co-digestion of *U. l*actuca

The literature on mono-digestion of *U. l*actuca is dominated by lab scale work and even more so dominated by BMP analyses. The Tokyo Gas Company [20] however commissioned the development of a large scale biogas plant which ran for 150 days. It ran two trials digesting 2 different macro algae, brown algae (Laminaria) and green algae (*U. lactuca*). Trials for *U. lactuca* operated for 70 days with a biomethane yield of between 15 and 17 m^3 t^{-1} of seaweed added. The seaweed sampled had a DS of approximately 10% (lower than in this experiment). There was no volatile solids percentage given in these trials. The results

are similar to sample 1 (Fresh: 20 m^3 t^{-1}: table 6.6) and sample 2 (Wilted & unwashed: 18 m^3 t^{-1}: table 6.6) in this experiment.

However the conditions which are described fit more closely to sample 4 (washed & wilted) which in this experiment yielded 35 m^3 CH_4 per tonne wet weight at a dry solids content of 31%. At 10% dry solids content this would equate to approximately 12 m^3 t^{-1}. We can say that our results are not dissimilar to those of the Tokyo Gas Company.

However it is well documented that there tends to be a reduction in the biomethane yield when operating a continuous system when compared to batch BMPs. A recent paper which followed on from the work completed in the Brittany region on anaerobic digestion of *U. lactuca*, co-digested collected *U. lactuca* with pig slurry [28]. A total yield was not given between the combined substrates but a specific yield of 128 L CH_4 kg^{-1} VS was given for the *U. lactuca* by subtracting the yield from a reactor with pig slurry only from the total biomethane yield produced from a mix of *U. lactuca* and pig slurry (48% and 52% respectively). H_2S as predicted was present in large amounts (up to 35,000 ppm) but did not inhibit the process. This successful digestion of *U. lactuca* provides a future avenue of research where a co-digestion potential feedstock is dairy slurry with *U. lactuca*. The Argideen estuary is surrounded by an intensive dairy farming industry, which is partly to cause for the development of the macro algae bloom. With over 38 million tonnes of cattle slurry produced in Ireland each year, this provides a possible potential co-substrate to digest with *U. lactuca* [42].

6.4.6 Results of co-digestion of *U. lactuca* with slurry

In co-digestion with slurry of the order of 17% more biomethane yield was produced than in mono-digestion of *U. lactuca* and slurry separately (Table 6.6). The highest yield is between 75% fresh *U. lactuca* and 75% dried *U. lactuca* which have specific yields of 220 and 210 L CH_4 kg^{-1} VS respectively. However on a m^3 CH_4 t^{-1} basis 75% Dried *U. lactuca* and 25% slurry give the highest yield (203 m^3 CH_4 t^{-1})

Co-digestion with slurry should lead to a reduction in H_2S as a large array of minerals are present in dairy slurry which could help reduce sulphide inhibition of the digestion process [43]. An advantage of drying *U. lactuca* is that it can be stored and digested when dairy

slurry is of higher quality; dairy slurry taken from the same farm during the winter period, achieved a yield of 239 L CH_4 kg^{-1} VS.

6.4.7 Resource of biomethane produced from *U. l*actuca

U. lactuca is abundunt in shallow coastal estuaries in West Cork (Figure 6.1). In the Argideen Estuary approximately 10,000 wet tonnes are produced per annum. This equates to ca. 1900 t DS/a (Fresh at 19% dry solids) or 1100 t VS/a (Fresh at 11% VS). Digesting *U. lactuca* should produce at least 180 L CH_4 kg^{-1} VS. Thus a digester in West Cork should have the resource of 198,000 m^3 of CH_4 which has an energy equivalent of 198,000 L of diesel. An average car in Ireland travels approximately 15,000 km/a at a fuel efficiency of 5 l diesel /100 km using 750 L of diesel per annum. Thus this biomethane resource is equivalent to 264 cars. Obviously this may be increased using a co-digestion system with available slurries in the region.

6.5 Conclusion

U. lactuca is detrimental to the amenity of a bay. This paper indicates that it is a viable source of third generation gaseous biofuel. Three pre-treatments were undertaken to assess the best method of optimising digestion. A combination of washing and drying yielded the best BMP result. A yield of 250 L CH_4 kg^{-1} VS was achieved which is equivalent to 100 m^3 CH_4t^{-1} of substrate. This is significant. Digestion of sea lettuce can be problematic due to the low C:N ratio. Co-digestion of fresh and dried *U. lactuca* with dairy slurry was assessed at various ratios. The slurry had a C:N ratio of 20. In all cases synergistic effects were noted. For example co-digestion of fresh *U. lactuca* and dairy slurry (75% VS in *U. lactuca*: 25% VS in slurry) resulted in 17% more biomethane than the sum of mono-digestion of the substrates.

Acknowledgments

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7 Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel

Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel

Eoin Allen [ab], David M. Wall [ab], Christiane Herrmann [ab], Jerry D. Murphy, [ab]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract

U. lactuca lactuca, a green seaweed, accumulates on beaches and shallow estuaries subject to eutrophication. As a residue, and macro-algae, it is a source of sustainable third generation biofuel. Production of biomethane from mono-digestion of *U. lactuca*, however is problematic due to high levels of sulphur and low ratios of carbon to nitrogen. Fresh and dried *U. lactuca* were continuously co-digested with dairy slurry at ratios of 25%, 50% and 75% (by volatile solid content) in 6 number 5L reactors for 9 months. The reactors digesting a mix with 75% *U. lactuca* struggled to reach sustainable operating conditions. Failure was dominated by volatile fatty acid inhibition. The levels of ammonia increased with percentage *U. lactuca* in the mix. Optimum conditions were observed with a mix of 25% fresh *U. lactuca* and 75% slurry. A yield of 170 L CH_4 kg^{-1} VS was achieved at an organic loading rate of 2.5 kg VS m^{-3} d^{-1}.

Keywords: seaweed; *U. lactuca lactuca*; biomethane; biofuel

7.1 Introduction

7.1.1 The rationale for macro-algae as a source of biofuel

By 2020 according to The Renewable Energy Directive [1], 10% of energy use in transport should be renewable. In 2011 first generation biofuels provided for approximately 5% renewable energy supply in transport (RES-T) in the EU. In October 2012 an EC proposal [2] suggested limiting first generation food based biofuels to 5% RES-T. This limit was proposed to be raised to 6% in September 2013 [3] at which time it was also stipulated that advanced biofuels, such as sourced from seaweed, should represent at least 2.5% of RES-T by 2020.

Seaweed (or macro-algae) biofuels are deemed to be third-generation. They do not interfere with food production directly (they do not use food crops) or indirectly (they do not use agricultural land). From an energy perspective the differentiation between first, second and third generation biofuels can be noted with reference to potential gross energy yield per hectare. Per example rape seed biodiesel (first generation) generates approximately 1350 L (44 GJ) of biodiesel per hectare per annum [4], willow biomethane (second generation biofuel produced through gasification) generates a gross energy yield of ca. 130 GJ^{-1} ha^{-1} yr^{-1} [5]. The yields per hectare of algae are not fully documented; however Christiansen [6] stated that yields of 130 t of kelp may be achieved per hectare. Allowing for 15% volatile solids and 330 L CH_4 kg^{-1} VS [7] the gross energy per hectare would be in the order of: 230 GJ^{-1} ha^{-1} yr^{-1}.

Ireland, with over 7,500 miles of coastlines and direct access to the Atlantic Ocean offers itself as an ideal location to utilise macro-algae as a source of biofuel. Algae can be either cultivated in aquaculture farms or harvested from beaches or from the sea. *U. lactuca,* a green seaweed is a particular case in that it is a scourge to coastal environments that have long shallow bays which are subject to eutrophication. This has become more endemic in recent years in France, Denmark and Japan. It is seen as an algae bloom and can result in thousands of tonnes washing up on to beaches, forcing closures [8]. However the quantity of the bloom can lead to a cheap source of biofuel as it greatly reduces the harvesting costs.

7.1.2 Biomethane production from green seaweed

Anaerobic digestion (AD) is a relatively low energy input process which converts wet substrates to a gaseous biofuel. Germany has in excess of 7,000 digestion facilities with substrates dominated by crops such as maize, cereals and grass [9]. These systems which can have very good energy balances [10] are never the less classified as first and second generation biofuels, and directly or indirectly compete with land for food production [11]. Production of biogas from macro-algae has not occurred in a commercial setting. The scientific literature on biogas from macro-algae, in particular *U. lactuca,* is very recent and relatively sparse. Most of the work relates to laboratory batch studies that do not give any indication of the operating conditions in continuous commercial operation of a bio-digester. Continuous co-digestion of *Ulva sp*. with pig or dairy slurry, respectively was tested by Peu and Sassi [12] and Sarker [13], however these studies did not focus on optimising the mixtures of *U. lactuca* with co-substrates or the effect of varying the organic loading rates, which are essential parameters for continuous co-digestion

U. lactuca has been shown to have potential as an AD feedstock reaching yields of between 128 to 271 L CH_4 kg^{-1} VS [14]. *U. lactuca* has extremely low levels of lignin making it readily accessible for microbial digestion [15]. However a crucial aspect of anaerobic digestion is the carbon to nitrogen (C:N) ratio; optimum values range from 20 – 30. *U. lactuca* has a C:N ratio less than 10 [14]. This can lead to problematic digestion due to excess levels of total ammonia nitrogen (TAN) which may inhibit methanogenesis. Co-digestion benefits from increased C:N ratios which aid digestibility and have been found to increase the level of digestibility for specific substrates [16]. One method of increasing the C:N ratio is to co-digest with dairy slurry, which is characterised by a higher C:N ratio of above 20:1 and in addition has a rich base of trace minerals, increasing digester efficiency [17, 18].

U. lactuca also has a high sulphur content which can result in significant levels of hydrogen sulphide (H_2S) in the produced biogas [19]. H_2S is toxic and results in increased corrosion of equipment in biogas plants. High levels of dissolved H_2S can also act as an inhibitor to microorganisms of the AD process [12]. Potential H_2S concentrations in biogas produced from a particular substrate can be forecasted by examining the carbon to sulphur ratio (C:S). The minimum recommended ratio is 40. Sulphur available for reduction or fermentation to H_2S is proportional to the biodegradable content of carbon in the substrate

[20]. A substrate with a C:S below 40 will tend to have larger accumulations of H_2S gas as experienced by seaweed digestion trails [20].

7.1.3 Aims and Objectives

This paper builds upon previous work by the authors who evaluated co-digestion of *U. lactuca* and slurry in biomethane potential (BMP) assays operated in batch mode (Allen et al., 2013a). The aim of this paper is to assess the suitability of green seaweed in continuous long term co-digestion and to glean information on optimal operating parameters. The objectives are to establish:

(1) What is the optimum percentage of *U. lactuca* that may be co-digested with dairy slurry in a stable continuous anaerobic process?

(2) What are the ideal operating conditions including for organic loading rate (OLR)?

(3) What parameters are likely to lead to failure in digestion of green seaweed?

7.2 Materials and methods

7.2.1 Materials

Approximately 300 kg of *U. lactuca* was sampled from Harbour view beach, Timoleague, Cork, Ireland in August, 2012. This was the same *U. lactuca* used in previous trials by the authors (Allen et al., 2013a). The seaweed was not washed. Drying was effected by placing on airing tables with hot air passed up through the seaweed for 36 hours at 80^{o}C. The seaweed was separated into 20 kg bags, frozen and stored at -20^{o}C.

Dairy slurry was collected from a dairy farm of 200 milking cows, in Cork, Ireland. The sampled slurry came from cows housed indoors, at the end of the lactation period. A significant quantity of slurry was collected and frozen in separate containers. *U. Lactuca* and dairy slurry samples were defrosted prior to feeding of the digesters. The inoculum used for the trials, was taken from a pilot scale reactor, fed only dairy slurry, which was operating in the same research lab. The inoculum was sieved through a 1 mm sieve and placed in each reactor and left to run for 1 week to remove residual gas which may

contribute to biogas production. Biomethane potential (BMP) was previously assessed (Allen et al. 2013a) in a proprietary biomethane potential (BMP) system (Bioprocess AMPTS II system) as described in section 2.2.2.The substrates are described in table 7.1.

7.2.2 Methods

7.2.2.1 Analytical methods

Total solids (TS) and volatile solids (VS) were analysed and calculated by using standard methods [21]. The pH was measured by a Jenway 3510 pH metre. The ratio of organic acid concentration to alkalinity (referred to as Fos:Tac) was carried out according to the Nordmann titration method using 0.1 N sulphuric acid with pH 5.0 and 4.4 endpoints [22]. The ammonia concentration in each reactor was measured in terms of Total Ammonical Nitrogen (TAN) using Hach Lange CLK 303 cuvettes, with a 1:100 dilution of digestate using a Hach Lange DR3900 spectrometer to read samples. Free ammonia (NH3) was calculated from a standardised equation relating the ammonia content to pH and temperature of the liquor [23] total volatile fatty acid (tVFA) content was measured using gas chromatography (Agilent HP 6890 Series) equipped with a NukolTM fused silica capillary column (30m x 0.25mm x 0.25µm), argon as a carrier gas and flame ionisation detector. Samples were tested every second week for acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and enanthic acid. Ultimate analysis of each substrate and digestate was carried out using an EAC CE 4500 elemental analyser. Samples for ultimate analysis were oven dried to 105^{o}C for 24 hours and were ground to < 0.6 mm particle size. Trace element analysis was carried out by a commercial lab (Agrolab Labor GmbH) using standard methods [24, 25]. Salinity and conductivity were calculated using a VWR hand held C0310 monitor. These parameters were measured in conjunction with chloride concentrations to determine the extent of salt concentrations in the reactor and how it effects biogas production over time. Hach Lange CLK 303 cuvettes were used at a dilution of 1:100 to determine chloride levels. Biogas was analysed for methane, carbon dioxide, oxygen, and hydrogen sulphide using a handheld Ntron Mentor CombI-R biogas analyser.

Table 7.1 Characterisation of substrates and inoculum (adapted from Allen et al., 2013a).

Substrate	**TS**	**VS**	**C:N**	**C:S**	**sCOD**	**BMP.**		**Specific yield**
	%	%			mg l^{-1}	L CH_4 kg^{-1} VS	L kg^{-1} TS	m^3 kg^{-1} wwt
Fresh *U. lactuca*	17.75	10.35	7.1	10.5	-	205	120	21.2
Dried *U. lactuca*	77.94	46.36	9.1	9.9	-	226	134	104.7
Dairy slurry	8.65	5.75	19.8	70.9	67,900	136	90	7.8
Inoculum	2.43	1.40	18.4	120	30,860	53	30.5	0.7

TS: Total solids; VS: volatile solids; C:N: carbon to nitrogen ratio; C:S: carbon to sulphur ratio; sCOD: soluble chemical oxygen demand; SMY: specific methane yield; BMP: biomethane potential.

Table 7.2 Design mixes used in continuous experiments.

Reactor Number	**Dairy slurry**	**Dried *U. lactuca***	**Fresh *U. lactuca***	**C:N ratio**	**C:S ratio**	**BMP** (L CH_4 kg^{-1} VS)	**Biodegrad-ability Index**
	%	%	%				
R1	25	75	-	10.2	25.2	210 (6.3)	0.53
R2	50	50	-	13.4	40.4	193 (5.4)	0.49
R3	75	25	-	16.6	55.7	186 (8.8)	0.48
R4	25	-	75	11.8	27.4	220 (4.9)	0.54
R5	50	-	50	14.4	41.9	200 (11.2)	0.50
R6	75	-	25	17.1	56.4	183 (7.8)	0.47

Standard deviation in parenthesis; VS: volatile solids; C:N: carbon to nitrogen ratio; C:S: carbon to sulphur ratio; BMP: biochemical methane potential.

7.2.2.2 Biomethane potential and biodegradability index

Two Bioprocess AMPTS II systems were used to assess the BMP. 500 ml glass bottles were used as batch vessels with a semi continuous stirring system operating at 30 rpm. Biogas produced was passed through a 1M NaOH solution to remove non methane gases. Biomethane volume was measured using a gas tipping device. The BMP is calculated as the sum of the methane volume produced during a period of 30 days, referring to the VS of the sample added to the test. All samples were conducted in triplicate. A detailed description of the BMP assay is given by [26]. An ultimate analysis was carried out on each substrate to determine theoretical methane yields using the Buswell equation [27]. Theoretical yields obtained from the Buswell equation were used to determine a biodegradability index which is defined as the ratio of the BMP to the theoretical methane yield obtained using the Buswell equation.

7.2.2.3 Continuous system

A total of six, 5 litre PVC cylindrical reactors with a working volume of 4 litres were used in parallel. Each reactor had a 25 mm inlet feeding port which was sealed with a rubber bung. A gas outlet valve was mounted at the top of the reactor which was connected to a tipping bucket gas meter via polythene gas tight tubing. A centrally mounted vertical axis stirrer was used to continuously stir the reactor contents at 40 rpm. A 12 V dc motor was used to power the motors. The temperature was controlled by water (circulated continuously through a brass coil which sat around the reactor) at a constant temperature of $37^{o}C + 1^{o}C$. A cover with insulated panels was placed over the reactors and heating coil. A Labjack data-logger was used to count each tip from the gas tipping-buckets, each of which had a known volume. Volumes each reactor were corrected for standard temperature and pressure at $0^{o}C$ and 101.325 kPa. A 1 litre Tedlar gas bag was used to collect biogas which had passed through the tipping buckets daily for analyses of gas composition. Gas bags were typically connected 8 – 12 hours a day.

7.2.2.4 Operation of continuous system

Six reactors were operated with different mixes of dried or fresh *U. lactuca* (25% to 75% on a VS basis) and dairy slurry (R1 – R6) as outlined in table 7.2. Previous work (Allen et al., 2013a) assessed these mixes in a BMP system. The BMP values and the biodegradability

were similar whether the *U. lactuca* was dried or fresh. BMP values and biodegradability fell as the ratio of *U. lactuca* in the mix dropped. The BMP value obtained for each co-digestion mixture was used as a target yield for each continuous reactor. Biomethane efficiency ratios (Eff) were calculated by dividing the yield of each continuous reactor by its BMP yield. Each reactor was started at an OLR of 2 kg VS m^{-3} d^{-1} and operated for an initial period of one hydraulic retention time (HRT) to assess stability of the process and potential for an increase in OLR or the necessity to decrease OLR. Depending on the biomethane efficiency ratio and the Fos:Tac ratio, the OLR was decreased or increased. Reactors were fed 5 days a week. A calculated wet weight quantity was fed to each reactor to provide the required OLR; this wet weight quantity was also used to calculate an initial HRT. The amount of digestate removed equated to the amount of fresh feedstock fed into the reactor less the amount of organic matter converted to biogas. A specific amount of sieved digestate was then returned to each reactor to reduce the total solids content to less than 10%. This had the effect of reducing the actual HRT, operated within the reactor trials. H_2S concentrations within the biogas were found to be at high levels. In order to control the H_2S levels FerroSorp® DG solution was added at the levels recommended by the manufacturer which was 700 g m^{-3} feedstock added.

7.3 Results and discussion

7.3.1 Methane yields and the relationship to Fos:Tac, ammonia and chlorides

The TAN was recorded at 1,796 mg l^{-1}, chloride at 1,840 mg l^{-1}, total VFA (tVFA) at 1,312 mg l^{-1} and soluble chemical oxygen demand (sCOD) at 30,860 mg l^{-1}. Figure 7.1 outlines the variation in specific methane yield (SMY) and the Fos:Tac for the six mixes over the time period of the experiment. The process may be considered stable when the Fos:Tac is in the range 0.2 to 0.4 and the SMY is approaching the BMP value.

Figure 7.2 compares the six reactors in terms of VFA, TAN and chloride. R6 (25% fresh *U. lactuca*, 75% slurry) has one of the lowest continuous values of Fos:Tac, of TAN and of chloride. R1 (75% dried *U. lactuca*, 25% slurry) is highlighted as having the highest continuous values of these parameters.

Table 7.3 highlights the results of the operation of the reactors. The description of results and operating procedures are discussed in detail for the least stable reactors (R1) and most stable reactor (R6).

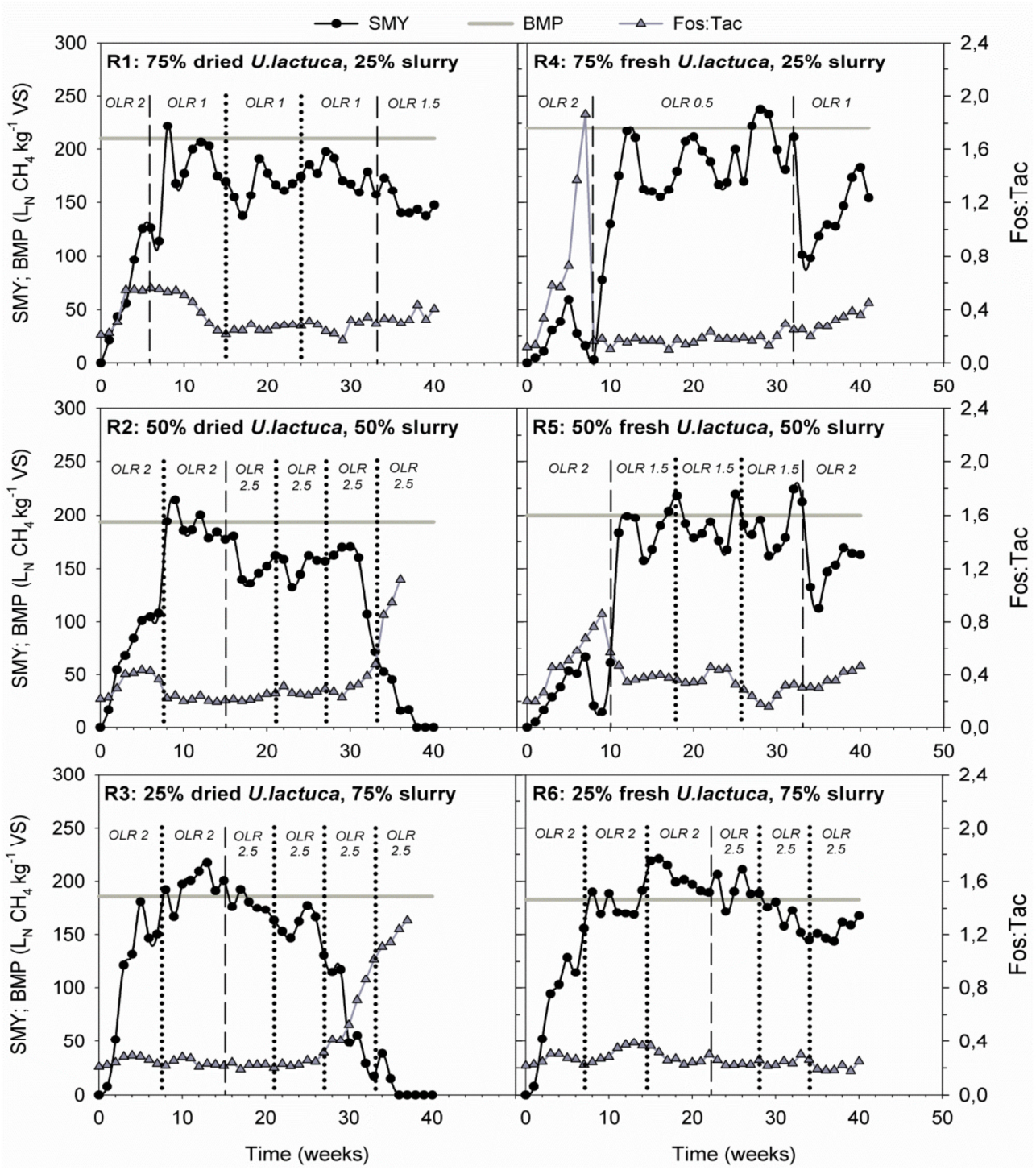


Figure 7.1 Performance and operational parameter curves R1 – R6.

Vertical dashed lines indicate changes in organic loading rate (OLR), vertical dashed and dotted lines indicate retention times. Horizontal lines indicated Biochemical methane potential (BMP) yield and organic acid to alkalinity ratio (Fos:Tac) of different mixes of dried or fresh *U. lactuca* with dairy slurry.

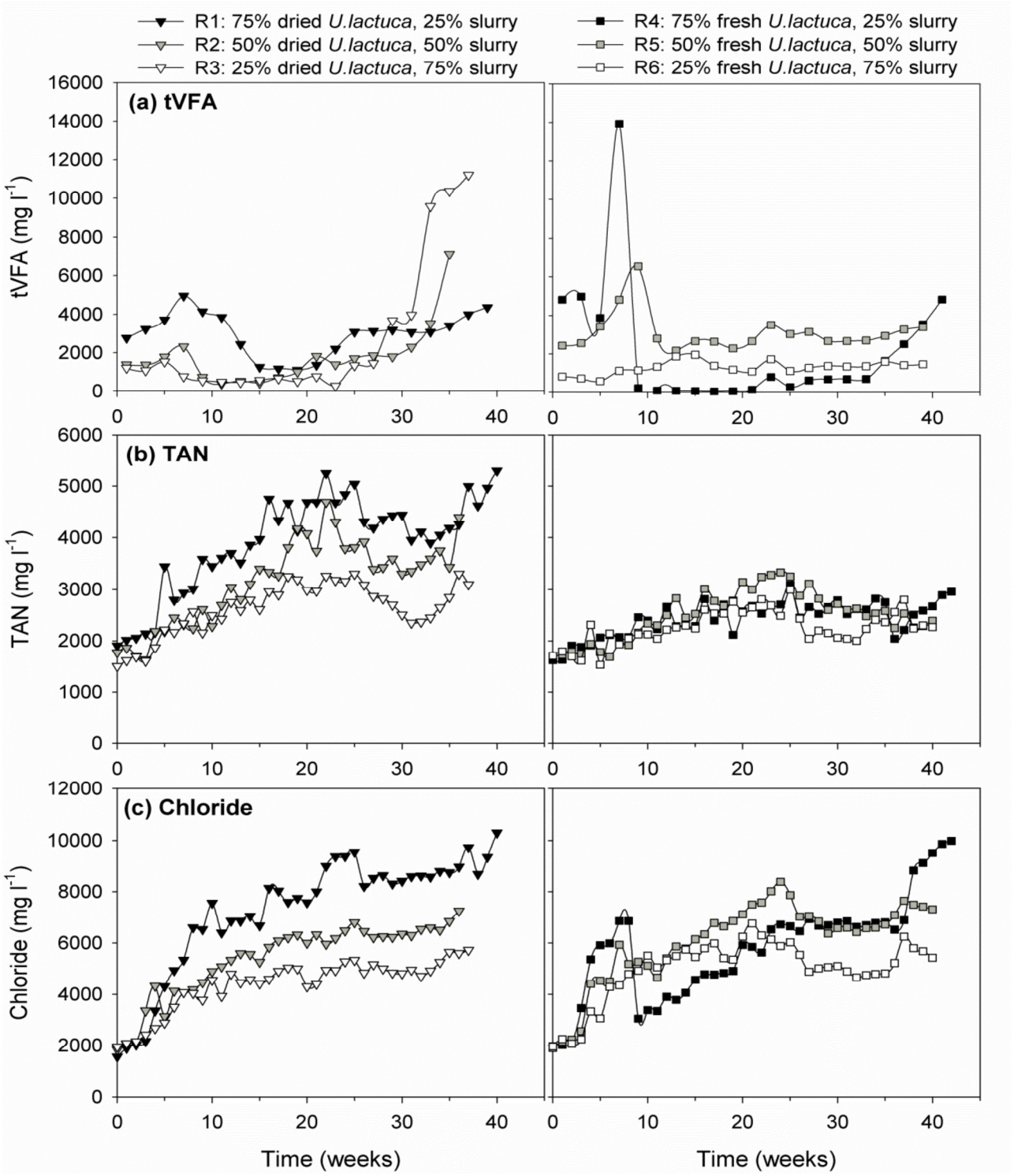


Figure 7.2 Environmental parameters R1 – R6.

(a) Total volatile fatty acid content (tVFA), (b) total ammonia nitrogen content (TAN) and (c) chloride content for R1 to R6.

7.3.2 Detailed assessment of R1 (75% Dried *U. lactuca*, 25% slurry)

From co-digestion batch trials a BMP of 210 L CH_4 kg^{-1} VS was recorded. During the first HRT the Fos:Tac ratio and tVFAs rose steeply from 0.21 to 0.56 (Figure 7.1a) and 1306 mg l^{-1} to 4954 mg l^{-1} respectively (Table 7.3). A maximum specific methane yield (SMY) of only 126 L CH_4 kg^{-1} VS was reached (week 7).

The OLR was reduced to 1 kg VS m^{-3} d^{-1} and operated for 3 HRTs (27 weeks). The Fos:Tac dropped to sustainable levels (<0.4). tVFAs also reduced to 1250 mg l^{-1}. A maximum SMY was reached of 222 L CH4 kg VS m^{-3} d^{-1}, with an average of 177 L CH_4 kg^{-1} VS (Eff 0.84, table 7.3). TAN levels rose up to 5250 mg l^{-1} but had reduced to 3900 mg l^{-1} by the end of the 3rd HRT (Figure 7.2b). The pH of the reactors remained between 7.81 and 8.12.

Finally the OLR was increased to 1.5 kg VS m^{-3} d^{-1}. The SMY however dropped to 145 L CH_4 kg^{-1} VS (Eff 0.69) and Fos:Tac increased to 0.43. TAN rose to 5,300 mg l^{-1}. The optimum OLR was decided upon as being 1 kg VS m^{-3} d^{-1}.

As with all reactors which were trialled, a steady increase in chloride and conductivity concentrations was observed (Figure 7.2c). The maximum level reached was 10,300 mg l^{-1} and 40.2 mS cm^{-1} respectively. There was no clear correlation between SMY and chloride or conductivity concentration. CH_4 levels slowly increased from 33% + 8% (average HRT1) to an average of 47% + 4% over the course of the second and third OLR of 1 and 1.5 kg VS m^3 d^{-1}. H_2S concentrations, as predicted by having a low C:S ratio (25.2 which is less than 40), were found to rise well above recommended levels of 250 ppm [28]. Addition of Ferrosorp® DG solution reduced the concentrations of H_2S to below 600 ppm and maintained this level in the biogas.

7.3.3 Detailed assessment of R6 (25% Fresh *U. lactuca*, 75% slurry)

This mix in R6 consists of the least amount of fresh *U. lactuca*, and the largest amount of dairy slurry. In the BMP trials (Allen et al., 2013a) it showed a 19% increase in biomethane yields in co-digestion (as opposed to mono-digestion). It has the largest C:N ratio of the 6 mixes (Table 7.2). In continuous trials it showed the highest biomethane efficiency ratio at 95% (Table 7.3). R6 was able to operate at an initial OLR of 2 kg VS m^{-3} d^{-1} but took 3 HRTs to achieve stable operating conditions (Fos:Tac between 0.2 and 0.4 with methane yield

approaching BMP value). This may be compared with R2 and R3 (the only other reactors that were deemed stable at 2 kg VS m^{-3} d^{-1}) which achieved stability after 2 HRTs. The Fos:Tac ratio of R6 was at 0.39 at the end of the 2nd HRT, and reduced to 0.25 at the end of the 3rd HRT. Biomethane yields averaged 178 kg VS m^{-3} d^{-1} (Eff 0.95) with TAN and pH levels reaching 2168 mg l^{-1} and 7.68. The maximum tVFA concentration recorded was 1955 mg l^{-1} for this OLR (Table 7.3).

After 22 weeks the OLR was increased to 2.5 kg VS m^{-3} d^{-1}. R6 showed biological stability but the specific methane yield began to drop in the second and third retention time even with Fos:Tac in the stable range (Figure 7.2a), and consistent TAN levels (Figure 7.2b). Fos:Tac and tVFA levels reduced gradually to 0.23 and 1,720 mg l^{-1}. Biomethane yields reduced slowly, rather than sharply or catastrophically as observed in R2 and R3. The maximum SMY recorded was 221 L CH_4 kg^{-1} VS and, on average for this OLR period was 170 L CH_4 kg^{-1} VS (Eff 0.93). Biogas composition was stable for both OLRs operated (51% + 3 and 52% + 2 respectively). H_2S concentrations in R6 were the lowest of all reactors partially predicted due to a higher C:S ratio. R6 still reached excessive levels of H_2S (>250 ppm), the reactor was treated with FerroSorp® DG which reduced levels to below 200 ppm. TAN concentrations marginally rose from the first OLR to 3,000 mg l^{-1}. TS and pH remained at low levels and within stable operating ranges at 6.48% and 7.73. Chloride and conductivity levels followed similar trends to the other reactors with a steady rise in concentrations reaching 6300 mg l^{-1} and 30.8 mS cm^{-1} respectively. Industry literature states that an optimum range of conductivity in a biogas reactor is within 20 - 25 mS cm^{-1} and higher conductivity levels may result in reduced biogas production [29].Thus increasing conductivity levels ins R6 which suggests an accumulation of salts within the reactor could have contributed to the slow decline in ethane yield in the second and third retention time at an OLR of 2.5 kg VS m^{-3} d^{-1}.

R6 showed stable signs operating at both OLRs. However an OLR of 2 kg VS m^{-3} d^{-1} is recommended as the operation was more stable. A reason for the reducing performance at the higher loading rate is elusive, but may be related to low levels of Selenium (discussed in section 3.7) as well as an elevated conductivity concentration. 17

7.3.4 Reactors R2 to R5

7.3.4.1 R2 and R3 (fed with 50% and 25% dried *U. lactuca* respectively)

Both R2 and R3 reached steady state biomethane production after 2 HRTs at an initial OLR of 2 kg VS m^{-3} d^{-1} (figure 7.1). However after a period of operation at an increased OLR of 2.5 kg VS m^{-3} d^{-1} extremely high levels of VFAs were experienced in R2 and R3 (7,138 mg l^{-1} and 11,208 mg l^{-1} respectively) and Fos:Tac reached 1.2 for R2 and 1.3 for R3 (Table 7.3). TAN, TS, pH all were within range for R2, and R3, before failure occurred. It is recommended that mixes with 50% and 25% dried *U. lactuca* operate at 2 kg VS m^{-3} d^{-1}.

7.3.4.2 R4 and R5 (fed with 75% and 50% fresh *U. lactuca* respectively)

Both R4 and R5 started at an OLR of 2 kg VS m^{-3} d^{-1}, and both showed signs of failure at an early stage reaching an average SMY of 24 L CH_4 kg^{-1} VS (Eff 0.11) for R4 and 36 L CH_4 kg^{-1} VS (Eff 0.18) for R4 (Table 7.3). Fos:Tac and tVFA were at elevated levels for both reactors at the end of the first HRT (1.86 and 13,929 mg l^{-1} for R4; 0.68 and 6538 mg l^{-1} in R5). The OLR in R4 was dropped to 0.5 kg VS m^{-3} d^{-1} and in R5 to 1.5 kg VS m^{-3} d^{-1}. Steady state biomethane production was achieved in both reactors at these OLR, with the SMY for R4 being 184 L CH_4 kg^{-1} VS (Eff 0.84) and 186 L CH_4 kg^{-1} VS (Eff 0.93) for R5. The OLR was further increased in both reactors but in both cases SMY decreased and Fos:Tac rose above 0.40. It is not recommended to digest a mix with 75% fresh *U. lactuca*. An OLR of 1.5 kg VS m^{-3} d^{-1} is recommended for a mix with 50% fresh *U. lactuca*

Table 7.3 Highlights of results from continuous digestion.

Continuous Results	BMP	SMY	Efficiency factor	CH_4	HRT	Fos:Tac (max)	tVFA (max)	TAN
	L CH_4 kg^{-1} VS	L CH_4 kg^{-1} VS		%	d		mg l^{-1}	mg l^{-1}
Dried *U. lactuca*								
R1 75% *Ulva*	210							
OLR 2		83	0.40	33	49	0.56	4,954	3,443
OLR 1		177	0.84	47	63	0.34	4,135	5,250
OLR 1.5		145	0.69	47	56	0.43	4,355	5,300
R2 50% *Ulva*	193							
OLR 2		137	0.71	45	49	0.44	2,311	3,106
OLR 2.5		127	0.72	48	35	1.20	7,138	4,690
R3 25% *Ulva*	186							
OLR 2		158	0.85	49	50	0.29	1,527	2,794
OLR 2.5		121	0.65	48	41	1.30	11,208	3,300
Fresh *U. lactuca*								
R4 75% *Ulva*	220							
OLR 2		24	0.11	25	40	1.86	13,929	2,113
OLR 0.5		184	0.84	46	160	0.29	801	3,090
OLR 1		137	0.61	50	80	0.45	4,825	2,955
R5 50% *Ulva*	200							
OLR 2		36	0.18	32	44	0.68	6,538	1,939
OLR 1.5		186	0.93	51	56	0.46	3,502	3,330
OLR 2		151	0.76	50	44	0.46	3,386	2,580
R6 25% *Ulva*	183							
OLR 2		178	0.95	51	49	0.39	1,955	2,168
OLR 2.5		170	0.93	52	42	0.30	1,720	3,000

VS: volatile solids; SMY: specific methane yield (weekly yields averaged over the HRT); BMP: biomethane potential; HRT: hydraulic retention time; Fos:Tac: organic acid concentration to alkalinity ratio; tVFA: total volatile fatty acid content; TAN: total ammonia nitrogen content. Units of OLR kg VS m^{-3} d^{-1}.

7.3.5 Comparison of SMY and OLRs with data in the literature

Peu, Sassi [12] co-digested *U. lactuca* with pig slurry on a 48% - 52% wet weight ratio at a constant OLR of 2.5 kg VS m^{-3} d^{-1}; a SMY of 126 L CH_4 kg^{-1} VS was achieved. Reactor R5 (50% fresh *U. lactuca* on a volatile solid basis) generated a SMY of 151 L CH_4 kg^{-1} VS at an OLR of 2.0 kg VS m^{-3} d^{-1}.

Sarker [13] co-digested *U. lactuca* in the ratio 24% *U. lactuca* : 76% dairy slurry (on a VS basis) at an OLR of 2.9 kg VS m^{-3} d^{-1} and produced a SMY of 133 L CH_4 kg^{-1} VS. This may be compared to R6 (25% *U. lactuca*) which generated 170 L CH_4 kg^{-1} VS at an OLR 2.5 kg VS m^{-3} d^{-1}.

These two papers operated for shorter periods and did not vary the organic loading rates to the extent of this work. Long term build-up of inhibitors cannot be forecasted with short experimental times. Per example R2 (50% dried *U. lactuca*) experienced three hydraulic retention periods at an OLR of 2.5 kg VS m^{-3} d^{-1} in a steady state before failure occurred in the 4^{th} retention period.

7.3.6 Composition of biogas

Methane content of the biogas remained in a steady range between 49% CH_4 $\pm$ 3% for all rectors when operated at a steady state at their optimised OLR. This was in agreement with other co-digestion trails with dairy slurry [16, 18]. When not steady, reduced SMY was experienced in reactors. R1 (75% dried *U. lactuca*), R4 (75% fresh *U. lactuca*) and R5 (50% fresh *U. lactuca*) experienced very low methane percentages of 35%, 25% and 32% respectively. H_2S concentrations reached very high levels, + 19,000 ppm (inhibitory levels in excess of 200 ppm). This was forecasted by a selection of the reactors having a C:S ratio below 40 and the *U. lactuca* having a C:S ratio itself of below 13:1. The levels of H_2S were found to decrease as this ratio increased from 25.2 (R1) to 56.4 (R6) for the worse and best case scenario reactors. Treatment with FerroSorp® DG (using maximum recommended rates of 700 g m^3) led to levels below 600 ppm.

7.3.7 Operational parameters

7.3.7.1 Sodium chloride

Sodium chloride has been identified as an AD process inhibitor but is still required in small concentrations to enable biomethane production [30]. There was no direct correlation

between chloride levels and SMY. A gradual increase in chloride concentration was observed in all 6 reactors. This steady increase was mimicked by conductivity and salinity levels recorded. It was reported that when ammonia levels are low that the tolerance for salts can be higher [31]. In these trials TAN levels remained low.

7.3.7.2 Total ammonical nitrogen concentrations

Values of TAN in excess of 5000 mg l^{-1} are deemed to be in an inhibitory range [32]. TAN remained in a relatively low range (especially for the fresh *U. lactuca* mixes; Figure 7.2) despite the low level of C:N ratio which can result in reduced organic conversion [16]. Higher levels of TAN can also lead to reduced SMY [33]. However it is postulated that low OLRs and increased C:N ratio of the co-digestion mixtures helped to keep TAN relatively low.

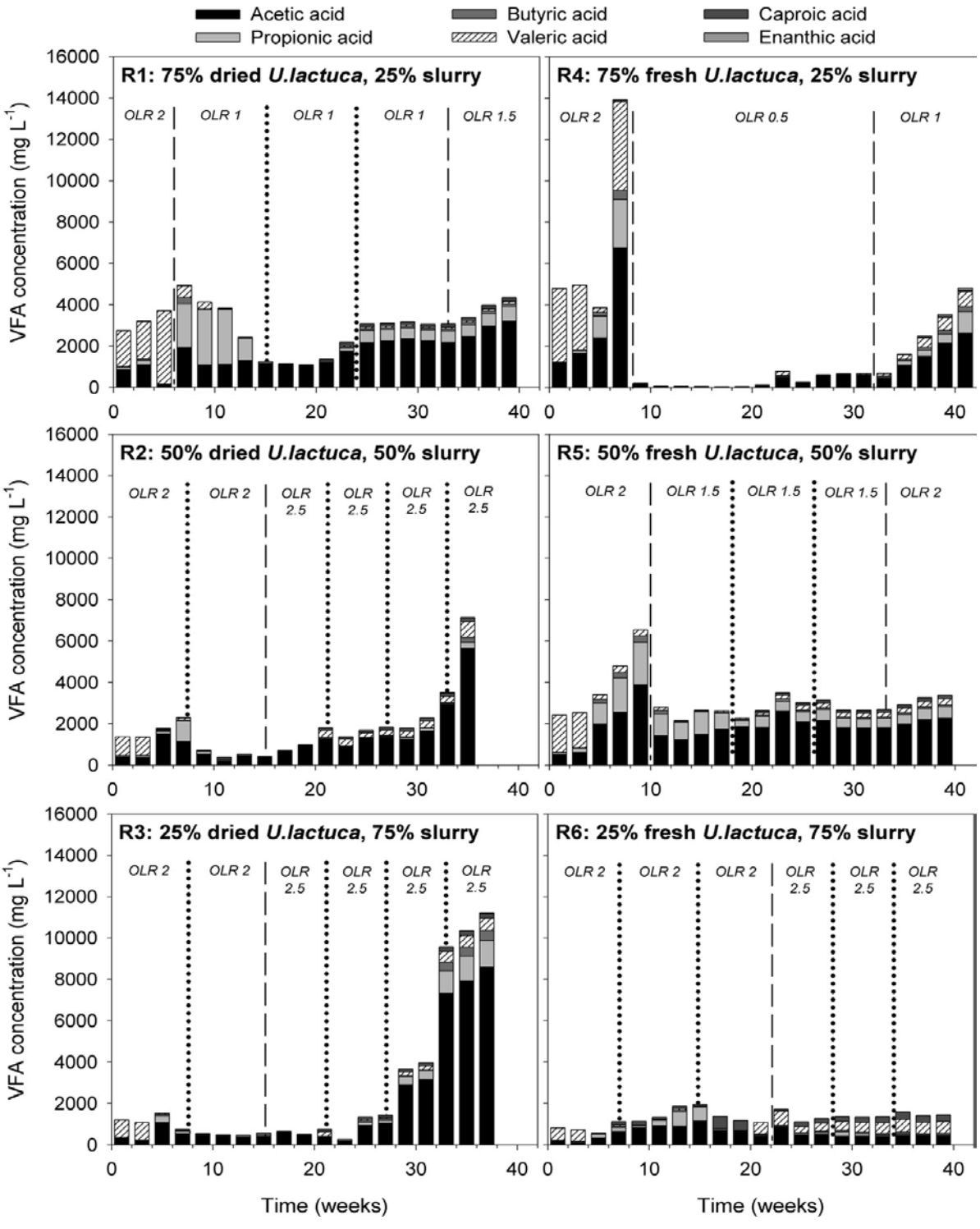


Figure 7.3 Breakdown of VFA concentrations for reactors R1-R6

Butyric acid is the sum of iso-butyric acid and n-butyric acid; Valeric acid is the sum of isovaleric acid and n-valeric acid; Caproic acid is the sum of iso-caproic acid and n-caproic acid.

7.3.7.3 Volatile fatty acid inhibition

Volatile Fatty Acid inhibition is suggested as the prime reason for failure or stress in reactors R1 – R5. Initially higher tVFA concentrations were observed in the reactors fed with fresh *U. lactuca* (R4-R6) leading to lower initial OLR in these reactors as opposed to reactors fed with dried *U. lactuca* (R1 – R3). However the reactors fed dried *U. lactuca* (R2 and R3) accumulated large concentrations of VFAs towards the end of the digestion trials, leading to their failure over time.

This suggests that the VFA content in the fresh *U. lactuca* is more concentrated and can lead to quick VFA accumulation whilst the drying pre-treatment reduces this initial VFA accumulation.

Typically acetic acid comprised between 49% and 66% of the tVFA concentration for all reactors; propionic acid ranged between 15% and 30%; iso-valeric acid ranged between 9% and 18%. One case of an outlying concentration was iso-valeric acid (38%) in R4 when tVFA reached a level close to 14,000 mg l^{-1}. Previous studies with *U. lactuca* as a co-digestion substrate showed that with increased percentage of *U. lactuca* in the co-digestion mix there was a large increase of VFAs [13]. Safe operating ranges of tVFA lie below 4,000 mg l^{-1}. Over this range, unstable biomethane production occurs as well as process failure [34].

Table 7.4 Trace element analysis of substrates and digestate from R1 to R6.

Substrate	Mg	S	Na	Ca	Mn	Mo	Co	Se	Cd
	g l^{-1}	g l^{-1}	g l^{-1}	g l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
Dried *U. lactuca*	24.24	22.99	11.38	32.52	109.12	<0.10	1.57	<0.04	<0.02
Fresh *U. lactuca*	5.22	4.63	5.31	9.95	19.53	<0.10	0.34	<0.04	<0.02
Dairy Slurry	0.65	0.53	0.15	0.12	21.63	0.18	0.21	0.14	<0.02
R1	2.95	0.92	1.43	4.65	22.97	3.29	0.79	<0.04	0.04
R2	2.21	0.76	1.34	5.54	19.63	0.94	0.37	<0.04	0.02
R3	1.77	0.63	0.85	3.45	22.51	0.68	0.36	<0.04	0.03
R4	2.59	0.78	2.49	5.32	17.09	1.06	0.40	<0.04	<0.02
R5	2.68	0.76	2.35	2.80	21.59	0.93	0.40	0.07	0.05
R6	2.41	0.67	1.66	2.42	25.54	0.91	0.49	0.06	<0.02

Table 7.5 Heavy metal analysis of substrates and digestate from R1 to R6.

Substrate	Ni	Fe	Cu	Zn
	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
Dried U*lva*	17.07	3094.22	5.64	30.47
Fresh *Ulva*	1.25	706.45	0.65	3.62
Dairy Slurry	0.45	279.22	5.18	26.30
R1	27.24	673.20	3.75	9.82
R2	7.63	517.93	2.69	10.34
R3	5.22	473.35	7.62	17.21
R4	9.63	508.94	9.95	10.96
R5	6.97	431.76	9.04	17.89
R6	7.19	498.46	15.48	22.83

7.3.7.4 Trace elements and heavy metals

The concentration of each of the substrates and final reactor contents were analysed for trace elements and heavy metals (Table 7.4 and 7.5). Sodium was the one trace element which differed significantly between reactors fed with fresh and dried *U. lactuca*. Levels of Na were significantly higher in dried *U. lactuca*; this however did not carry over to the operation of the digesters and neither were at critical levels (6 - 30 g l^{-1}) reached.

Levels of Selenium were low in all reactors. The recommended range of Se for stable growth of methane producing bacteria varies from author to author with no definitive critical limit established. The upper range of critical values suggested for Se lies between 0.1 – 0.35 mg l^{-1} [35]. Low levels of Selenium were recorded in all the reactors with values below the measuring range (<0.04 mg l^{-1}) recorded in 4 reactors and initial *U. lactuca* substrates. These low concentrations may have caused poor methane efficiency rates and inhibition to occur. This is possibly a reason for the declining SMY at the OLR of 2.5 kg VS m^{-3} d^{-1} in R6. However, lower trace element recommendations of 0.008 to 0.79 mg l^{-1} are also found in literature and values in commercial biogas plants showed ranges comparable to Selenium concentrations found in the present study [35].

Sulphur was found to be at high concentrations in the *U. lactuca* substrate with drying seeming to concentrate the levels. However concentrations of S (though highest in the reactors with higher portions of *U. lactuca*), were lower in all the reactors due to the co-digestion with slurry and the binding of H_2S and formation of iron sulphide through addition of FerroSorp® DG. The addition of FerroSorp® DG probably led to the elevated levels of Iron (Table 7.5).

High Calcium concentrations were analysed in *U. lactuca* substrates which resulted in elevated amounts of Ca especially in reactors R1 to R4. Ca levels of 2.5 to 4 g l^{-1} which are reported to be moderately inhibitory [28] were found within these reactors, but Ca levels with strong inhibitory effects of 8 g l^{-1} (Chen et al., 2008) were not reached. Copper and Zinc were at higher levels in the slurry than *U. lactuca* (Table 7.5). This led to higher levels of Cu and Zn in the reactors with more slurry (R3 and R6). It is not considered that these levels were at high enough levels to inhibit the process [35].

7.4. Conclusions

The optimum mix of *U. lactuca* and dairy slurry in a biogas facility is suggested as 25% fresh *U. lactuca* with 75% dairy slurry. This reached 95% of the BMP value at an OLR of 2 kg VS m^{-3} d^{-1}. Initially it operated well at an OLR of 2.5 kg VS m^{-3} d^{-1} but it experienced a decreasing trend in methane yield with time. It is postulated that addition of trace elements could allow satisfactory operation at higher OLR. Mixes in excess of 75% *U. lactuca* are not recommended. Critical parameters include high levels of VFA, Calcium, chloride and low levels of Selenium.

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8 Conclusions and recommendations

8.1 Conclusion of thesis

A number of conclusions and recommendations are presented:

- The best performing substrates available to an emerging biogas industry in Ireland are not the high yielding substrates such as used cooking oil (804.61 $\pm$ 57.0 L CH_4 kg^{-1} VS) or cheese processing waste (DAF) (787.36 $\pm$ 59.51 L CH_4 kg^{-1} VS). These substrates do not have the desired C:N ratio or they have excess lipids or other inhibitory elements. Preferential substrates include substrates such as energy beet (375.13 $\pm$ 6.93 L CH_4 kg^{-1} VS) or grass silage (399.56 $\pm$ 4.12 L CH_4 kg^{-1} VS) which are abundant and are suitable for co-digestion with slurries.
- Eleven macro algae substrates were sampled, of which the best performing species in terms of biomethane yield was *S. latissima* (341.46 $\pm$ 36.40 L CH_4 kg^{-1} VS or 34.41 m^3 t^{-1} wwt). This seaweed had a C:N ratio of 24:1. Also the potential biomethane yield per hectare of *S. latissima* (10,244 CH_4 m^3 ha^{-1} yr^{-1}) may surpass that of terrestrial first and second generation biofuel crops.
- The use of *U. lactuca* as a primary or mono digestion feedstock is not recommended in large scale AD at levels in excess of 25% by VS content. The combination of a low C:N ratio and high sulphur concentration leads to rapid VFA production within a continuous biogas reactor.
- The optimum co-digestion of *U. lactuca* is 25% with 75% dairy slurry at an OLR of between 2 and 2.5 kg m^3 d^{-1}. Even at this low OLR stability issues arose over the period of 40 weeks. This was due to microbial population depletion over time due to chloride or salinity associated issues.
- Anaerobic digestion of *U. lactuca* indirectly inhibits acetogenic and methanogenic processes, with ammonia showing the strongest causative correlation. Through the use of 16S rDNA analysis, it is shown that for high *U. lactuca* volumes, decreasing the OLR was not sufficient to recover the acetoclastic methanogens required to remove acetic acid and prevent over-loading. Neither could the system retain hydrogenotrophic methanogens. At low *U. lactuca* volumes, inhibition of acetogenesis caused *Methanosarcina* populations to shrink, affecting biogas yield. Chloride accumulated but did not clearly correlate with inhibition. Effects of *U.*

lactuca loading rates significantly affected community makeup, with higher *U. lactuca* loading rates characterised by diverse, facultatively anaerobic, marine and halotolerant taxa, lack of methanogens, and a predicted reliance on alternative carbon metabolism

- In light of resulting data from trials with *U. lactuca*, it is recommended that further trials be conducted with lower percentages of *U. lactuca* and dairy slurry (< 25%). Also that possibly a third substrate be co-digested with *U. lactuca* and dairy slurry in a bid to increase biogas yields, possibly using grass silage.
- Potentially, Ireland can establish a strong AD industry with a combination of specific feedstocks particularly from diverting wastes streams from landfill to biogas production. The opportunity of converting industrial organic wastes to biomethane could be very effective. A potential output of over 100 PJ of energy potential could be captured if milk processing and agricultural wastes were streamed to an energy recovery system like AD.
- Milk processing wastes in particular offer a great potential in terms of reaching the RES-T of 10%. Over 6 times the 10% RES-T target of 18.8 PJ could be met if dairy processers and creameries combined and sent their waste to biogas facilities. Macro algae can also pay a part in meeting these targets as *S. latissima* showed greatest potential as a biofuel, yielding 10,244 CH_4 m^3 ha^{-1} yr^{-1}.
- A final recommendation is that biogas research is added to future mandates and provisions in governmental poloicies. Where Ireland has now began to fall behind in its initial RES-T targets which are set for the year 2020. It is necessary that as a nation Ireland achieve it's initial targets to finally achieve the greater goals of 2050 which see a net reduction of 80% of $C0_2$ emissons based on 1990 levels. Policy makers need to be aware and where possible, implement biogas systems into future strategies to increase penetration of renewables into the energy markets.

Future work

- Co-digestion of seaweed species with slurries
- Greater levels of detail of the yields associated with seaweeds which are cultivated or beach cast. The natural harvest tends to be older and accumulate heavy metals. Cultivated seaweeds are newer and do not accumulate the same levels of heavy metals. There is a lack of literature available on seaweed biogas. An economic assessment is essential to determine the commercial viability of second and third generation biofuels and the establishment of an AD industry in Ireland.

Final remarks

Ireland has a myriad of potential substrates for anaerobic digestion. There is potential to meet RES-T targets through bio-methanation by the year 2020 from a combination of substrates from any of the 6 groups or 83 substrates investigated. The low hanging fruits are the waste feedstocks. Larger resources will necessitate crops, particularly marine crops such as seaweed. An economic analysis is required to determine which feedstocks are viable in terms of their characteristics and/or their transport distance to a biogas plant.

Appendix A: Evaluation of the biomethane potential from multiple waste streams for a proposed community scale anaerobic digester

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Evaluation of the biomethane potential from multiple waste streams for a proposed community scale anaerobic digester

James D. Browne[a,b], Eoin Allen[a,b] and Jerry D. Murphy[a,b]*

[a]*Department of Civil and Environmental Engineering, University College Cork, Cork, Ireland;* [b]*Environmental Research Institute, University College Cork, Cork, Ireland*

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This paper examines the biomethane potential from organic waste for a proposed community scale anaerobic digester in a rural town. The biomethane potential test is used to assess the suitability of waste streams for biomethane production and to examine the variation in biomethane potential between waste sub-streams. A methodology for accurately estimating the biomethane potential from multiple heterogeneous organic waste substrates is sought. Five main waste streams were identified as possible substrates for biogas production, namely Abattoir waste (consisting of paunch and de-watered activated sludge); cheese factory effluent; commercial and domestic food waste; pig slurry and waste water treatment sludge. The biomethane potential of these waste streams ranged from as low as 99 L CH_4 kg VS^{-1} for pig slurry to as high as 787 L CH_4 kg VS^{-1} for dissolved air floatation (DAF) sludge from a cheese effluent treatment plant. The kinetic behaviour of the biomethane production in the batch test is also examined. The objective of the paper is to suggest an optimum substrate mix in terms of biomethane yield per unit substrate for the proposed anaerobic digester. This should maximize the yield of biomethane per capital investment. Food waste displayed the highest biomethane yield (128 m_n^3 t^{-1}) followed by cheese waste (38 m_n^3 t^{-1}) and abattoir waste (36 m_n^3 t^{-1}). It was suggested that waste water sludge (16 m_n^3 t^{-1}) and pig slurry (4 m_n^3 t^{-1}) should not be digested. However, the biomethane potential test does not give information on the continuous operation of an anaerobic digester.

Keywords: anaerobic digestion; biogas; biomethane potential test; waste to energy

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1. Introduction

1.1. Benefits of anaerobic digestion in waste treatment and energy recovery

Anaerobic digestion uses a large variety of organic substrates to produce biogas. Germany has over 6000 anaerobic digesters with energy crops as the dominant feedstock.[1] However with world food prices continuing to rise into the foreseeable future, there is much international concern over the use of energy crops for fuel production. This concern can be seen in the latest amendment to the European Union renewable energy directive [2] as Europe seeks to introduce a limit to the contribution made from liquid biofuels produced from food crops, such as those based on cereals and other starch rich crops, sugars and oil crops. Attention has been redirected to utilizing waste and residues for energy recovery. The renewable energy directive [3] states that biofuels produced from wastes, residues, non-food cellulosic material and ligno-cellulosic material shall be considered at twice their energy value for assessment of compliance with the 2020 target of 10% renewable energy supply in transport. This suggests that compressed biomethane produced from food waste and residues are more sustainable transport biofuels than first generation food crop-based biofuels such as ethanol produced from wheat. The typical greenhouse gas savings (as compared with the displaced fossil fuel) for compressed biomethane from municipal solid waste is quoted as 80%. This may be compared with 32% for wheat ethanol and 45% for rapeseed biodiesel.[4] One of the advantages of anaerobic digestion is the flexibility in substrates that may be used to produce biogas and the flexibility in the use of the biogas. Biogas (which is between 50% and 70% methane) may be used as renewable heat, renewable electricity or if upgraded to biomethane (∼98% methane) it may be used as a renewable gaseous transport biofuel in compressed natural gas vehicles.

1.2. Anaerobic co-digestion of organic wastes

There have been many previous studies on the co-digestion of agricultural slurries and energy crops or organic wastes, however, there are limited publications on the assessment of multiple waste streams for a single anaerobic digestion process. As the spectrum of potential substrates for biogas production broadens to include more organic wastes

*Corresponding author. Email: jerry.murphy@ucc.ie

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Evaluation of the biomethane potential from multiple waste streams for a proposed community scale anaerobic digester

James Browne[a] Eoin Allen[a],. Jerry D. Murphy[a,b]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract

This paper examines the biomethane potential from organic waste for a proposed community scale anaerobic digester in a rural town. The biomethane potential test is used to assess the suitability of waste streams for biomethane production and to examine the variation in biomethane potential between waste sub streams. A methodology for accurately estimating the biomethane potential from multiple heterogeneous organic waste substrates is sought. Five main waste streams were identified as possible substrates for biogas production, namely: Abattoir waste, (consisting of paunch and dewatered activated sludge); cheese factory effluent; commercial and domestic food waste; pig slurry; and waste water treatment sludge. The biomethane potential of these waste streams ranged from as low as 99 L CH_4 kg^{-1} VS for pig slurry to as high as 787 L CH_4 kg^{-1} VS for dissolved air floatation sludge from a cheese effluent treatment plant. The kinetic behaviour of the biomethane production in the batch test is also examined. The objective of the paper is to suggest an optimum substrate mix in terms of biomethane yield per unit substrate for the proposed anaerobic digester. This should maximise the yield of biomethane per capital investment. Food waste displayed the highest biomethane yield (128 m^3 t^{-1}) followed by cheese waste (38 m^3 t^{-1}) and abattoir waste (36 m^3 t^{-1}). It was suggested that waste water sludge (16 m^3 t^{-1}) and pig slurry (4 m^3 t^{-1}) should not be digested. However the biomethane potential test does not give information on the continuous operation of an anaerobic digester.

Keywords: anaerobic digestion; biogas; biomethane potential test; waste to energy;

Introduction

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Anaerobic digestion uses a large variety of organic substrates to produce biogas. Germany has over 6,000 anaerobic digesters with energy crops as the dominant feedstock [1]. However with world food prices continuing to rise into the foreseeable future, there is much international concern over the use of energy crops for fuel production. This concern can be seen in the latest amendment to the EU renewable energy directive [2] as Europe seeks to introduce a limit to the contribution made from liquid biofuels produced from food crops, such as those based on cereals and other starch rich crops, sugars and oil crops. Attention has been redirected to utilising waste and residues for energy recovery. The Renewable Energy Directive [3] states that biofuels produced from wastes, residues, non-food cellulosic material and ligno-cellulosic material shall be considered at twice their energy value for assessment of compliance with the 2020 target of 10% renewable energy supply in transport. This suggests that compressed biomethane produced from food waste and residues are more sustainable transport biofuels than first generation food crop based biofuels such as ethanol produced from wheat. The typical greenhouse gas savings (as compared to the displaced fossil fuel) for compressed biomethane from municipal solid waste (MSW) is quoted as 80%. This may be compared with 32% for wheat ethanol and 45% for rapeseed biodiesel [4]. One of the advantages of anaerobic digestion is the flexibility in substrates that may be used to produce biogas and the flexibility in the use of the biogas. Biogas (which is between 50 and 70% methane) may be used as renewable heat, renewable electricity or if upgraded to biomethane (~98% methane) it may be used as a renewable gaseous transport biofuel in compressed natural gas vehicles.

1.2 Anaerobic co-digestion of organic wastes

There have been many previous studies on the co-digestion of agricultural slurries and energy crops or organic wastes, however there are limited publications on the assessment of multiple waste streams for a single anaerobic digestion process. As the spectrum of potential substrates for biogas production broadens to include more organic wastes and residues, a suitable method to determine the methane potential of a potential substrate is the biochemical methane potential (BMP) test. Such tests can provide information such as

the rate of material degradation and the expected methane yield per gram of material added, which is known as the specific methane yield.

1.3 Feedstock sampling and screening for biomethane potential

As the demand for selecting and pricing biomass substrate for anaerobic digestion continues to increase the biomethane potential test is an increasingly recognised tool for screening potential feedstocks for biomethane potential. Many anaerobic digesters treat a variety of organic wastes that may change throughout the year. Representative sampling can often be difficult to achieve in practise due to heterogeneity of certain waste streams, fluctuations can occur in daily waste production and in sampling location. Within certain processes there may be several sub streams of waste production which can have widely different characteristics and therefore will affect widely different biomethane yields. The importance of accurate feedstock sampling and analysis for biogas production cannot be under estimated [5]. The biomethane potential (BMP) test is arguably the most significant part of an initial substrate analysis for biogas production and has a major impact on the design of an anaerobic digester. The methodology used in the BMP test is extremely important. Various authors have indicated a potential for different results depending on the methodology chosen [6-9]. The BMP test aims to assess the biomethane yield per unit of mass of feedstock under favourable anaerobic conditions. The BMP result is usually seen as the maximum methane potential for a particular feedstock, however, the BMP does not exactly replicate conditions in a continuously feed AD system and therefore the BMP result should not be viewed as an absolute value. Thamsiriroj and Murphy [10] suggest that some reactor configurations and process parameters, such as a high solid retention time, may result in higher methane yields than the BMP test.

1.4 Aims and Objectives

This paper sets out a methodology to assess and screen potential substrates from five major waste streams for a proposed anaerobic co-digestion facility using the biochemical methane potential (BMP) test as a selection tool. The BMP test is also used to assess the level of variability of biomethane potential within the waste streams. The objective is to select substrates with a high methane production per unit mass which will lead to an economic digester design. This paper is part I of two papers in this issue. The second paper [11] examines the biomethane production and bioreactor performance from continuously

fed laboratory trials over a period of 8 months based on the selection strategy of this paper.

2 Materials and Methods

2.1 Methodology for assessing potential substrates for biomethane production

The methodology used by the authors to assess the suitability of substrates for biogas production is as follows;

- Carry out an ultimate and proximate analysis on samples of all potential substrates
- Using values from ultimate analysis (CHN) use the Buswell equation to get a theoretical biomethane yield
- Carry out a BMP test on each sample in triplicate
- Compare the BMP yield to the theoretical yield to get a biodegradability index
- Use BMP test data to select substrates for a preliminary design on expected biomethane yield
- Carry out continuous lab scale AD trials to determine parameters such as organic loading rate and any inhibitory effects from the substrates (paper 2/chapter 4).

The results of the ultimate analysis were used to calculate the theoretical methane yield using the Buswell equation [12] (Equation 1) and the carbon to nitrogen ratio for each waste stream.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \qquad \text{Eq. 1}$$

The biodegradability index is defined as the ratio of the biomethane yield from the BMP test expressed as a percentage of the maximum theoretical value based on the Buswell equation. This parameter can be used to assess the associated methane conversion efficiency of the waste material.

2.2 Experimental Outline

Two rounds of BMP tests were conducted. The first round of tests was conducted to assess the BMP yield from composite waste samples collected by the waste producers. These samples were deemed to be representative samples for each of the five major waste streams (as shown in Figure 1) according to the waste producers. The second round of BMP tests was carried out to check for variation in BMP yield within waste streams and to check the difference between the BMP result of the composite samples and the weighted average BMP sub samples from the second round. Nine sub samples were tested for BMP in round 2. All samples were tested in triplicate to get a mean and standard deviation. By sampling each individual sub stream a more accurate estimation of the variation of biogas yield within each main waste stream can be achieved. In total there were 14 samples tested in triplicate for BMP between round 1 and 2.

The five major waste streams which were investigated in round 1 were as follows:

- Abattoir waste mix (paunch grass, green sludge and dewatered activated sludge at equal ratios based on mass of fresh matter)
- Cheese process waste
- Food waste mix (domestic and commercial food waste at equal ratios based on mass of fresh matter)
- Pig slurry mix (slurry from weaners and fatteners at equal ratios based on mass of fresh matter)
- Waste water treatment sludge – final sedimentation sludge

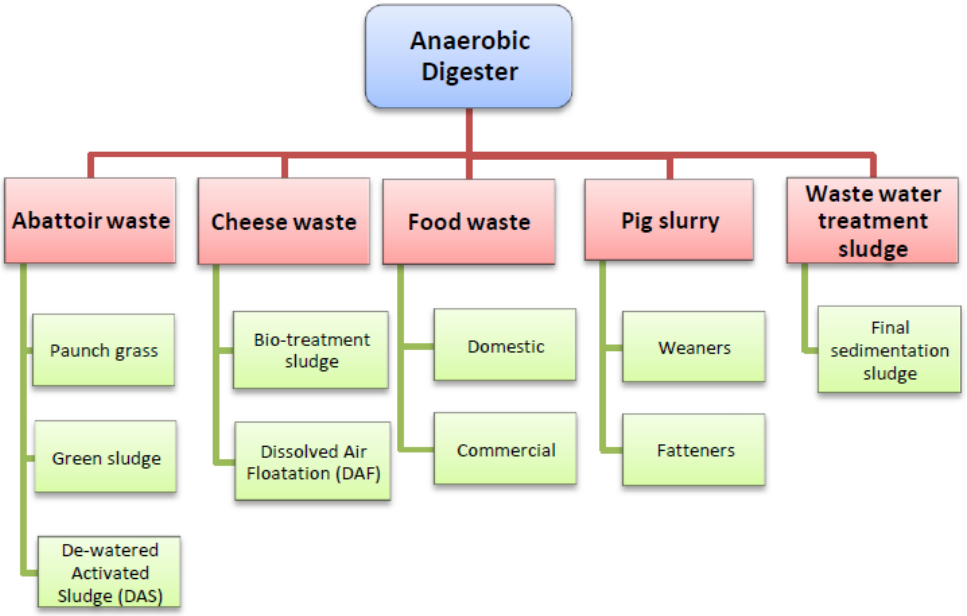


Figure 1 Major waste streams and sub streams considered for the anaerobic co-digestion facility

2.3 Waste Materials

The five major waste streams and associated sub streams to be investigated for BMP for the proposed AD plant are shown in Figure 1. These five waste streams were identified as possible substrate sources for the proposed anaerobic digester as they are the most available locally produced wastes within a 20 km radius of the proposed site.

1. Abattoir Waste: A local abattoir produces about 4700 tonnes per annum (t yr^{-1}) of paunch content from the slaughter of cattle. Three categories of paunch and process sludge material were produced: paunch grass; green sludge; and dewatered activated sludge (DAS).
2. Cheese processing waste: A cheese factory produces approximately 6000 (t yr^{-1}) of treated sludge which includes biologically treated effluent (5000 t yr^{-1}) and dissolved air floatation (DAF) sludge (1000 t yr^{-1}).
3. Food waste: A local waste collector operates a collection service for 1000 t yr^{-1} of source segregated domestic and commercial food waste. The quantity of domestic household source separated food waste is expected to significantly increase over

the next two years due to the implementation of national organic waste separation policy. It is estimated that approximately 5000 tonnes per annum of source separated domestic food waste will be collected in the area once the waste separation policy is implemented.

4. Pig slurry: a local pig farm produces 20,500 t yr^{-1} of pig slurry. The pigs are housed on concrete slats which allow slurry to flow to under-floor pits. Slurry samples were collected from weaners (young pigs from 3 months) and from fatteners (maturing pigs for market).
5. Waste water treatment sludge: the local waste water treatment plant is licensed to treat a maximum of 6,500 population equivalent. The characteristics of the waste samples are shown in table 1.

Table 1 Characterisation of potential substrates for biogas production

Source	TS (%)	VS (%)	VS/TS	C (% TS)	H (% TS)	N (% TS)	C:N ratio
Abattoir waste							
Paunch grass	17.0(0.07)	15.6(0.08)	0.92	46.5(0.09)	6.3(0.03)	2.8(0.2)	16.6
Green sludge	19.6(0.6)	18.1(0.6)	0.93	57.3(0.3)	8.4(0.07)	3.0(0.2)	19.1
DAS	13.3(0.08)	10.7(0.06)	0.81	41.0(0.1)	5.8(0.02)	6.5(0.1)	6.3
Cheese processing effluent							
Bio-treatment sludge	9.4(0.3)	7.6(0.3)	0.81	43.9(0.1)	6.8(0.04)	5.6(0.3)	7.8
DAF	7.8(0.3)	6.8(0.3)	0.88	65.1(0.5)	10.3(0.03)	1.3(0.3)	50
Food waste							
Domestic	21.9(0.7)	19.9(0.7)	0.91	46.8(0.2)	6.3(0.07)	2.7(0.1)	17.3
Commercial	35.4(0.7)	30.1(0.3)	0.85	49.0(0.5)	7.0(0.06)	3.4(0.2)	14.4
Pig slurry							
weaners	4.7(0.01)	3.3(0.02)	0.70	38.3(0.4)	5.2(0.07)	3.1(0.3)	12.4
fatteners	6.5(0.03)	4.8(0.01)	0.74	40.3(0.5)	5.3(0.07)	2.5(0.2)	16.1
Waste water treatment sludge	8.6(0.08)	6.7(0.05)	0.77	43.3(0.2)	5.8(0.06)	2.2(0.4)	19.7

Values are presented as mean with (standard deviation)

2.4 Sampling Technique

The standards VDI [13] and ISO 5667 [14] were followed for sampling methodology. For solid material such as brown bin waste, a representative sample was obtained by taking a large sample from different locations in the bulk material. This large sample was spread on a clean surface and then mixed well. A cross was drawn through the middle of the spread sample, and two opposite quarters were removed. The remaining two quarters were spread and mixed again, and again a cross was drawn and two quarters removed. This process was repeated until the required amount of sample was obtained [5]. Liquid feedstocks were sampled at different frequencies, liquid levels and process streams to ensure good representation. Solid waste materials such as abattoir waste, food waste and waste water treatment sludge were processed through a food mincer (Buffalo 800W) to a particle size of less than 5mm. Liquid waste such as pig slurry and cheese processing effluent were homogenised in a blender. All samples were stored in a freezer at - 20°C until required.

2.5 Source of Inoculum

The inoculum for round 1 of the BMP tests was obtained from a farm scale anaerobic digester (farm A) operating at mesophilic temperatures, treating mostly cattle slurry and a small portion of grease trap waste from a local catering premises. The approximate feed ratio of cattle slurry to grease trap waste was 9:1 on a volumetric basis. Inoculum from farm A was incubated at 35 °C for 3 weeks prior to the BMP round 1. The inoculum had a pH of 7.9, total solids (TS) of 33.0 gTS kg^{-1} and volatile solids (VS) content of 17.1 gVS kg^{-1} after passing through a 2mm sieve. Inoculum used in round 2 BMP tests was sourced from another farm scale anaerobic digester (farm B) operating at mesophilic temperatures treating a mixture of cattle slurry, poultry litter and a small quantity of grease trap waste at an approximate ratio of 5:4:1 respectively, on a volumetric basis. The inoculum from farm B was incubated at 35 °C for 1 week prior to BMP round 2. The inoculum from round 2 had a pH of 7.95, TS of 59.4 gTS kg^{-1} and VS content of 42.9 gVS kg^{-1} after passing through a 2 mm sieve. The higher VS content in the inoculum from round 2 is due to the operation of higher total solids digestion process on the second farm which included finely macerated straw associated with the poultry litter as part of the substrate.

Both samples of inoculum were taken from stable anaerobic digesters operating on substrate mixes dominated by cattle slurry and at similar temperatures. Inoculum from both rounds was tested using cellulose as a standard control substrate ($C_{12}H_{20}O_{10}$). The maximum theoretical methane yield from cellulose according to the Buswell equation is 415 L CH_4 $kgVS^{-1}$. Inoculum from source A gave a specific methane yield of 354 ± 6 L CH_4 kg^{-1} VS while inoculum from source B gave 371 ± 4 L CH_4 $kgVS^{-1}$. As both sources of inoculum gave over 85% of the theoretical max, this proves that a healthy consortium of anaerobic microbes were present in both rounds. Indeed some recent research carried out by Holliger and colleagues (2012) indicate that there is little or no influence on the source of inoculum or its adaptation on the BMP result for the tested substrates, provided the inoculum contains sufficiently diverse microbial communities to cope with the degradation of complex substrates [15]. In the study by Holliger and colleagues (2012) no significant difference was found between BMP results using four different sources of inoculum. In another inter-laboratory study by Raposo and colleagues (2011), related to BMP testing four substrates (starch, cellulose, gelatine and mung bean) the influence of inocula source was insignificant with respect to the extent of anaerobic biodegradation [16]. However the source of inoculum can have an effect on the kinetic rate of degradation.

2.6 BMP Apparatus

The apparatus used to conduct the BMP tests was the Automatic Methane Potential Test System II (Bioprocess Control Sweden AB). This laboratory instrument is specially designed for determination of the BMP of a substrate. The AMPTS II system consists of three major parts as can be seen in Figure 2.

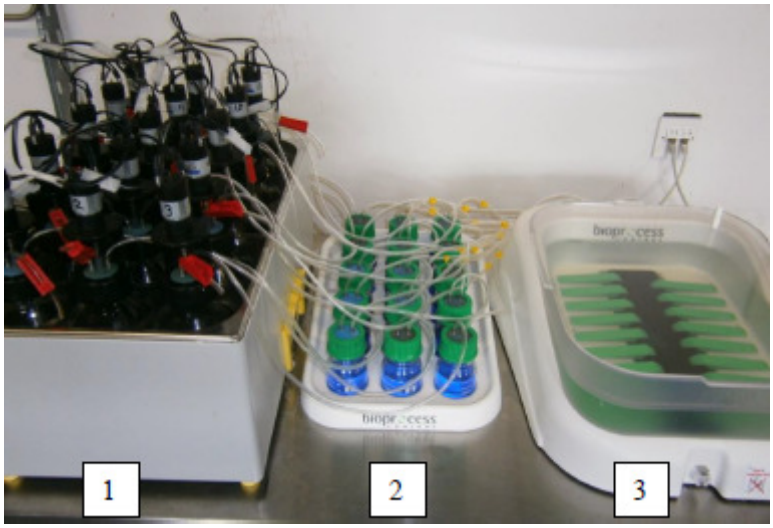


Figure 2 BMP apparatus used in the paper: Automatic Methane Potential Test System II (Bioprocess Control Sweden AB). 1. Water bath with 15 No. 500 ml bottle reactors: 2. Carbon dioxide fixing unit: 3. Gas flow measuring unit

1. A temperature controlled water bath with 15 bottle reactors of 500 ml volume, each equipped with a mixer that can be run in either continuous or intermittent mode
2. A carbon dioxide fixing unit with an alkaline solution (3N sodium hydroxide) that absorbs the carbon dioxide and hydrogen sulphide produced during the anaerobic digestion process
3. A gas measuring unit consisting of 15 parallel operating cells, where the gas is measured through water displacement. When approximately 10 ml of gas has been accumulated each cell opens and releases the gas. For each opening, the time, temperature and pressure are registered and stored locally in an embedded Central Processing Unit (CPU). Based on these measurements, normalised (0°C, 1 atm and dry gas) accumulated gas production and gas flow rate are calculated.

The BMP tests were performed with a working volume of 400 ml. The ratio of inoculum to substrate was chosen to be 2:1 on a volatile solids (VS) basis. The inoculum to substrate ratio is a critical parameter in conducting a BMP test according to the Anaerobic Digestion Specialist Group of the International Water Association [6]. A ratio of 2:1 or greater of inoculum to substrate on a VS basis is recommended for BMP trials to limit any inhibitory effects due to the chemical composition of the substrate such as inhibition associated with accumulation of ammonia and volatile fatty acids (VFA) [6, 7, 16].

If several substrates are to be tested with the same inoculum, the amount of inoculum used in each bottle is calculated in accordance with the substrate with the lowest VS content. This amount of inoculum is then subsequently used for the other substrates and the amount of substrate is then adjusted so that the desired VS ratio is achieved. In this way only one set of blanks (corresponding to the amount of inoculum added in each reactor) is used. If some reactors contain a total volume less than 400 ml then these reactors are topped up to 400 ml with de-ionized water. The amount of inoculum to be used is calculated in accordance with equation 5.4 which is derived from equations 5.2 and 5.3. The mass of substrate on a VS basis is calculated based on equation 5.5. The adjusted mass for the other test substrates is calculated using equation 5.6.

$$\frac{M_{in} \cdot VS_{in}}{M_{out} \cdot VS_{out}} = 2 \qquad \text{(Eqn. 2)}$$

$$M_{sub} + M_{in} = 400 \qquad \text{(Eqn. 3)}$$

$$M_{in} = \frac{800 \cdot VS_{sub}}{VS_{in} + 2 \cdot VS_{sub}} \qquad \text{(Eqn. 4)}$$

$$M_{sub} = 400 - M_{in} \qquad \text{(Eqn. 5)}$$

$$M_{sub} = \frac{M_{in} \cdot VS_{in}}{2 \cdot VS_{sub}} \qquad \text{(Eqn. 6)}$$

Where M_{in} is the mass of inoculum, M_{sub} is the mass of substrate, VS_{in} is the volatile solids content of the inoculum and VS_{sub} is the volatile solids content of the substrate. The headspace volume (260ml) in each of the reactor bottles was flushed with nitrogen for 3-4 minutes at a rate of 500ml per minute to eliminate oxygen and create a fully anaerobic environment.

The BMP tests were run for a period of 30 days or until biogas production was less than 5ml day $^{-1}$. The reactor bottles were maintained at a constant temperature 37^{o}C (± 0.5 °C) by means of a water bath. The biogas is passed through a solution of sodium hydroxide (3 N NaOH) to remove carbon dioxide and other non methane gases. The methane is then

passed through individual gas tippers which automatically count and record gas flow. This is a well established method for removing carbon dioxide from the biogas in order to get an accurate measurement of methane flow rate [5]. Removing carbon dioxide using an alkaline solution prior to measuring the gas flow is desirable for volumetric methods based on water displacement since a certain amount of carbon dioxide will always dissolve in water leading to inaccurate measurements [17, 18].

2.7 Analytical methods

The total solids (TS) and volatile solids (VS) were determined gravimetrically using the methods described in APHA 2005 [19]. Each waste stream was sampled and tested in triplicate for total carbon (C), hydrogen (H) and nitrogen (N) on a total solids basis and was attained by ultimate analysis using element analyser (CE 440 Model) at the Chemistry Department in University College Cork, Ireland.

3 Results and Discussion

3.1 Results from BMP trials

The cumulative biomethane potential yields for round 1 and 2 of BMP tests are shown in Table 5.2. Methane yields are reported as the average of triplicate samples with standard deviations. Table 5.2 Biomethane potential and biodegradability of composite samples and sub Streams.

Table 2 Biomethane potential and biodegradability of composite samples and sub streams

Waste Source	Sub stream	Round	BMP ($L\ CH_4\ kgVS^{-1}$)	Theoretical BMP (Buswell eq)	Bio-degradability (BMP/BMP_T)
Abattoir	Composite sample	1	336 ± 15.0	481	71
	Paunch grass	2	238 ± 15.9	469	51
	Green sludge	2	403 ± 15.1	683	59
	DAS	2	165 ± 7.7	408	40
Cheese	Composite sample	1	454 ± 19.3	508	89
	Bio-effluent	2	461 ± 30.8	492	94
	DAF	2	787 ± 46.7	826	95
Food waste	Composite sample	1	508 ± 21.5	537	95
	Domestic	2	419 ± 45.3	471	86
	Commercial	2	535 ± 20.0	550	97
Pig slurry	Composite sample	1	99 ± 8.4	340	34
	Weaners	2	38 ± 2.0	352	11
	Fatteners	2	70 ± 12.8	328	21
WWTS	Final sedimentation	1	247 ± 10	406	61

3.2 Abattoir Waste

In the first round of BMP trials the mixed abattoir waste sample gave an average specific methane yield of 336 L CH_4 kg^{-1} VSwith a standard deviation (SD) of 4.5% (Table 5.2). The biodegradability of the abattoir waste from round one was 71% of the theoretical methane yield calculated by the Buswell equation. The composite abattoir waste sample tested in the first round of BMP tests consisted of equal parts paunch grass, green sludge and dewatered activated sludge on a fresh matter basis. Based on the volatile solids content of each abattoir sub stream, the ratio of the mixture on a volatile solids basis is 0.35 grass paunch, 0.41 green sludge and 0.24 DAS.

The BMP results from round 2 shows that there is large variation in specific methane yield between the three sub streams of abattoir waste. The grass paunch sub stream gave an average methane yield of 238 L CH_4 kg^{-1} VSwith a SD of 6.7%; the green sludge gave an average specific methane yield of 403 L CH_4 kg^{-1} VS with a SD of 3.7%, while the de-watered activated sludge yielded the lowest average methane potential at 165 L CH_4 kg^{-1} VSwith SD of 4.6%. As shown in Table 5.2 all three abattoir sub streams had lower biodegradability than the composite sample in round 1. It is expected that the de-watered activated sludge (DAS) which has undergone a biological treatment process would have a reduced biomethane potential due to biological aerobic degradation of the waste stream. To compare the BMP results from round 1 and 2 the weighted average BMP of the 3 sub streams from round 2 is compared to that of round 1. The weighted BMP mix is calculated based on the specific methane yield of each abattoir sub stream (in round 2) multiplied by the respective ratio of sub stream used in round 1 on a volatile solids basis.

Weighted BMP mix = (0.35 * Paunch grass) + (0.41 * Green sludge) + (0.24 * DAS) The mean weighted BMP result is 288 ± 11 L CH_4 kg^{-1} VS. Based on this calculation the average specific methane yield from round 2 is approximately 14% less than was achieved in round 1. Using the two-sample t-test for equal variances ($t = 4.51$, $p < 0.0107$), there is a statistically significant difference between the weighted BMP from round 2 and the actual BMP result of the composite mixed sample in round 1 with 95% confidence. This indicates a positive synergistic effect in co-digestion of the three sub streams as opposed to mono digestion of each sub stream. This can be attributed to improvement of the C:N ratio. For example the C:N ratio of the DAS is 6.3, while the C:N ratio of the composite is 15.2. Although there

have been many studies done on anaerobic digestion of mixed slaughter house waste, few studies assess only the paunch content from ruminants. Palatsi and colleagues (2011) achieved methane potentials of 270-300 L CH_4kg COD^{-1} (208-230 L CH_4 kg^{-1} VS) from mixed pig and cattle slaughterhouse wastes [20]. Wang and Banks (2003) previously achieved 210 L CH_4per kg TS added using a two-stage anaerobic digestion system for treating mixed abattoir wastes [21]. Abattoir waste such as paunch content from ruminants is desirable as a co-substrate for AD due to the presence of ruminant bacteria which produce enzymes that help hydrolyse complex carbohydrates such as cellulose [22].

The abattoir waste stream as produced at the cattle slaughtering facility in this study produces different quantities of waste sub streams. The abattoir waste is dominated by the paunch grass sub stream at the approximate ratio of 2.5 paunch grass to 1.5 de-watered activated sludge (DAS) to 0.7 green sludge on a fresh matter basis. The weighted average biomethane potential from the abattoir waste is calculated based on the BMP results from round 2 multiplied by the proportions at which each material occurs. Weighted average methane yield from abattoir waste as it occurs in the processing plant is estimated as follows; [(2.5 * 238) + (1.5 * 165) + (0.7 * 403)] / 4.7 = 239 L CH_4kgVS^{-1}.

This can be viewed as a conservative estimate as the BMP results from the composite sample in round 1 indicate that there may be positive synergetic effects from the co-digestion of the three sub-streams which may increase the actual BMP yield of the mix.

3.3 Cheese Processing Waste

The cheese process effluent from round 1 gave a maximum BMP of 454 L CH_4 kg^{-1} VSwith a SD of 4.3%; this was 89% of the theoretical methane yield calculated by the Buswell equation (Table 5.2). In round 2 the dissolved air floatation (DAF) sludge yielded a BMP of 787 L CH_4 kg^{-1} VS. DAF is the highest methane yielding substrate of all sub streams tested; this can be attributed to the high carbon to nitrogen ratio of 50 which is also the highest of all substrates tested. DAF is known to contain dissolved fats from the cheese process effluent. The bio-treatment sludge gave an average methane yield of 461 L CH_4 kg^{-1} VS which is 1.5% higher than the previous BMP result for cheese waste in round 1 and is within the standard variation of 4.3% of the previous BMP result from round 1. It was noted that the composite cheese process sample from round 1 was very similar to the bio-

effluent sample in round 2. After further enquiries from the waste producers it was discovered that on the day of sampling the composite sample of cheese process waste from round 1 contained only the biologically treated effluent as the Dissolved Air Floatation (DAF) tank was not connected. Therefore the BMP results for cheese process waste in round 1 are representative of the bio-effluent sub stream.

The two sub streams of cheese waste effluent tested in round 2 showed a relatively large difference in BMP with the DAF yielding 71% extra specific methane yield than the bio-effluent sludge. Typically there are 5 parts biologically treated effluent to 1 part DAF at the cheese processing plant. This gives a weighted average of 515 L CH_4 kg^{-1} VSfor the combined sub streams. This value is higher than other reported values for cheese processing waste. Erguder and colleagues (2001) achieved a maximum of 424mL CH_4 $gCOD^{-1}$ using cheese whey [23] while Labatut et al. (2011) reported a BMP yield of 423.6 L CH_4 kg^{-1} VS for cheese whey [24]. It can be concluded that the higher methane yields from cheese effluent in this study is attributed to the DAF portion of the waste stream which consists mostly of dissolved fats which contribute to higher methane yields.

Harvest 2020- A vision for Irish agri-food and fisheries [25] has projected an increase of 50% in milk production to supply increasing world demand for dairy products. This will result in more cheese processing effluent requiring further treatment and additional waste management options. To this effect the inclusion of cheese processing sludge as a substrate for biogas production is an attractive waste to energy concept.

3.4 Food Waste

The composite mixed sample of domestic and commercial source separated food waste gave the highest specific methane yield of round one with an average BMP of 508 L CH_4 kg^{-1} VSwith a standard deviation of 4.2%, giving approximately 95% of the theoretical methane as per the Buswell equation. Previously reported BMP yields from canteen food waste are similar to these results (480-530 L CH_4 $kgVS^{-1}$) [9].

A large variation in biomethane potential was also noted between the two main substreams of source segregated food waste. Commercial food waste which is typically collected from canteens, restaurants, hotels and catering premises gives a relatively high methane potential of 535 L CH_4 g VS^{-1} with a SD of 3.7%. The biodegradability of the

commercial food waste is 97% of the theoretical which indicates very good degradation under anaerobic conditions. This BMP result for commercial food waste is similar to the maximum BMP yield from university canteen waste of 527 L CH_4 g VS^{-1} previous observed by Browne and Murphy (2013) for canteen food waste [9]. The domestic food waste samples gave an average methane potential of 419 L CH_4 kg^{-1} VS with a larger SD of 11% and biodegradability of 85%. Zhang and colleagues (2012) achieved between 445-456 L CH_4 kg^{-1} VS from domestic source segregated food waste [26]. The BMP result for domestic food waste is approximately 22% lower than the BMP for commercial food waste and is due to the physiochemical differences between the two food waste sub streams as shown in Table 5.1. The variability in BMP yield from the domestic food waste stream is larger than that of the commercial waste stream; however the variability in BMP yield between waste streams was not found to be statistically significant using the Ftest two sample for variances in Excel ($p<=0.183$, $F=4.47$). The weighted average BMP from food waste in round 2 is 477 L CH_4 kg^{-1} VS.

This is 6.1% less than the BMP from round 1 (composite mixed sample). Using the two-sample t-test for equal variances $t = 1.87$, $p < 0.135$ (two tail), there is no significant difference between the weighted BMP from round 2 and the actual BMP result of the composite mixed sample in round 1 with 95% confidence. This indicates that the difference in BMP result between round 1 and 2 for food waste is attributed to the level of variability of BMP yields within the food waste streams. The BMP results for food waste samples in this study are higher than other reported methane yields for similar food waste substrates, Davidsson and colleagues (2007) reported methane yields of between 300-400 L CH_4 kg^{-1} VS for a large number of source sorted OFMSW samples which had all been through different pretreatment processes [27]. In the current study, food waste samples were collected as produced therefore ensuring the samples had not undergone degradation and produced relatively higher methane yields.

3.5 Pig Slurry

The pig slurry gave much lower BMP results than expected, with an average methane yield of 99.3 L CH_4 kg^{-1} VSin round 1. This is about half the expected BMP yield reported in the scientific literature for pig slurry [28], typically in the range of 200-250 L CH_4 $kgVS^{-1}$. Astals and co-workers (2011) achieved methane yields of 188 L CH_4 kg^{-1} VS from batch mono-

digestion of pig slurry [29]. Kafle and Kim (2013) reported BMP yields for pig slurry in the range of 259-268 ml CH_4 g COD $_{added}$ [30].

In round 2 pig slurry sub stream samples from weaners (young pigs about 28 days old) and fatteners (older pigs being prepared for market) were tested to confirm if the initial BMP results for mixed slurry were accurate. Similar to the pig slurry mix in round 1, slurry taken from the fattener's sub stream of pig slurry, with a volatile solids concentration of 4.76% of total mass, gave a reduced average methane yield of 70 L CH_4 kg^{-1} VS. The slurry collected from the weaner's slurry tank which had a lower volatile solids content of 3.28% of the total mass, yielded an extremely low specific methane yield of 38 L CH_4 kg^{-1} VS. The cumulative methane curve started to decrease after day 7 (at 38 L CH_4 kg^{-1} VS) and dropped to only 18 L CH_4 kg^{-1} VS by day 30. The rate of methane production from the inoculum alone (blank) was greater than that of the weaner pig slurry (with the same amount of inoculum as the blank). This indicates that the weaner pig slurry sampled may have contained toxins or inhibitors to the anaerobic inoculum. The first sample of mixed pig slurry used in round 1 BMP trials had a volatile solids concentration of only 1.35% of the total mass. It was initially thought that the low methane yield from pig slurry in round 1 was possibly due to an error in sampling. However after additional sampling and testing of individual sub streams of pig slurry in round 2, even lower BMP yields were achieved.

The reason as to the low methane yields from pig slurry is unclear; great effort was taken in the second round of BMP trials to attain slurry samples from different depths in the slurry tank and samples were well mixed and homogenised prior to the BMP tests. One hypothesis is that the weaner slurry may have been contaminated with a bio-toxic substance such as an anti-biotic. However a bio-toxicology test is beyond the scope of this paper. In this particular case study it is recommended that pig slurry would not be included as part of the substrate mix due to its low methane yield and low solids content.

3.6 Biomethane kinetics

The characteristics of biomethane production were examined by using the modified Gompertz equation to predict cumulative biomethane production in batch mode [30- 32]. Biomethane production can be predicted as follows;

$$M(t) = P \cdot \exp\{-\exp[\frac{R_{max} \cdot e}{P}(\Delta - t)] + 1\} \qquad \text{Eq. 7.1}$$

Where,

M is the cumulative methane yield a given time (L CH_4 g VS^{-1}),

P is the max methane potential (L CH_4 kg^{-1} VS) from BMP test,

R_{max} is the maximum methane production rate (L CH_4 kg^{-1} VS d^{-1}),

e is the mathematical constant = 2.7183,

λ is the lag phase for methane production to begin (days),

t is the time (days).

R_{max} and λ are can be determined by non linear regression using the SOLVER function in Microsoft Excel, which employs an iterative least squares fitting routine to produce the optimal goodness of fit between data and function. The statistical indicators, correlation coefficient (R_2) and root mean square error were calculated [33].

The cumulative methane production curves from round 1 BMP tests shown in figure 5.3 have a slightly S-shape or sigmoid shaped cumulative curves which indicate a delay in methane production. This time lag is most noticeable for samples tested using inoculum from farm A during round 1 BMP trials. This inoculum was incubated for a longer period of time (3 weeks) prior to the commencement of BMP tests than inoculum from farm B (1 week). The lag time in the first round of BMP tests is between 6.4 and 7.3 days for abattoir, cheese and food waste but is 0.5 day for wastewater treatment sludge and 0 for slurry. All substrates have a BMP half life (BMP $T_{0.5}$) of less than 13 days which indicates that these waste materials are readily biodegradable under favourable anaerobic conditions. The model curve fitting generated using the modified Gompertz equation, gave a coefficient of determination of greater than 0.95 for the all waste streams in round 1, which indicates a very close fit.

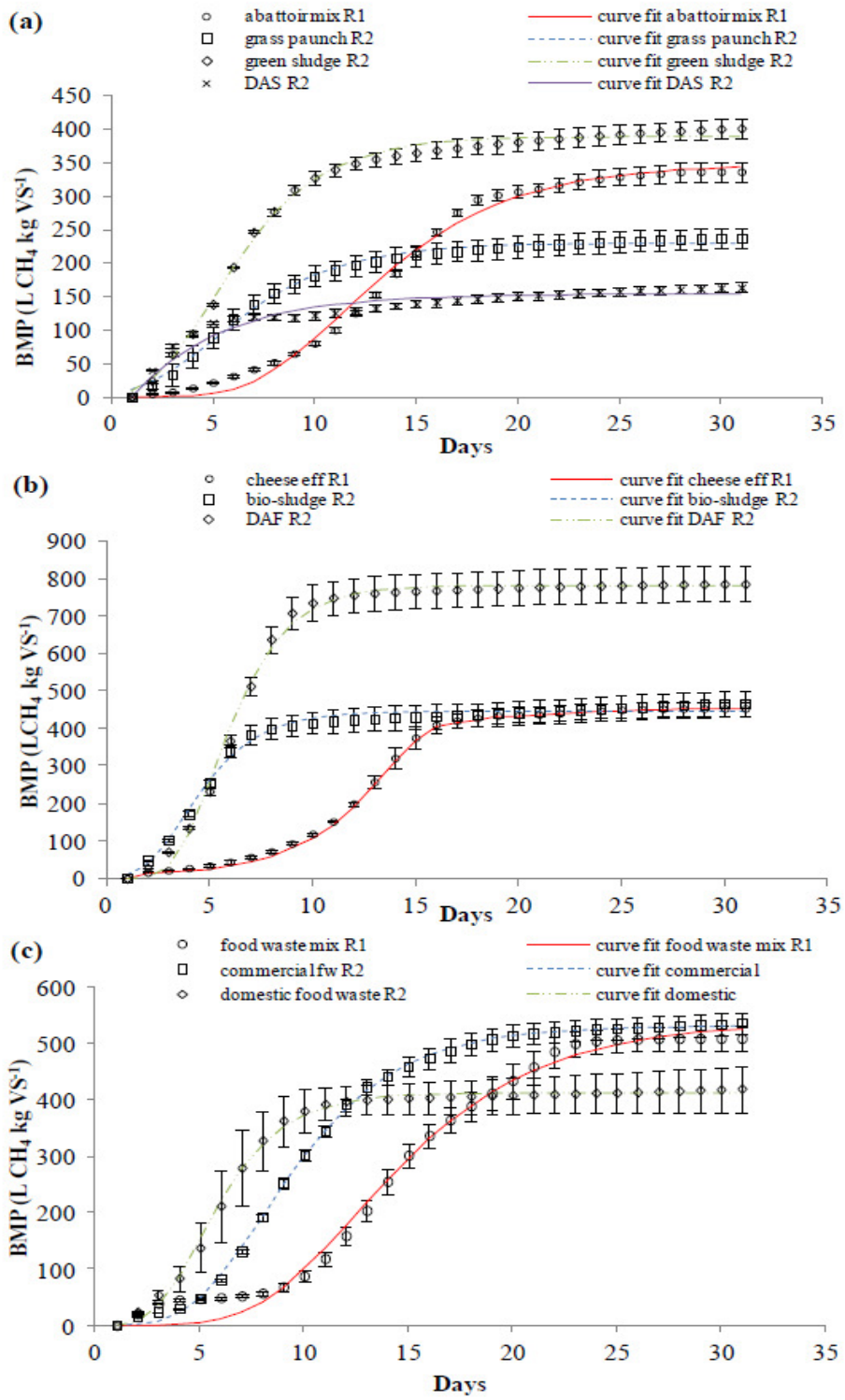


Figure 3 (a) BMP of abattoir waste round 1 & 2 (b) BMP of cheese processing waste round 1 & 2 (c) BMP of food waste round 1 & 2

The time lag for methane production in the second BMP trial was found to range from 0 to 3 days. All substrate samples tested in round two have a BMP half life (BMP $T_{0.5}$) of less than 9 days which indicates that these waste materials are readily biodegradable under favourable anaerobic conditions. Except for the weaner's pig slurry sample, the curve fits for all the waste streams in round 2 have a coefficient of determination of greater than 0.95 which indicates a very close fit. The kinetic parameters of the modified Gompertz equation are shown in Table 5.3.

Table 3 Biomethane kinetics using the modified Gompertz equation

Waste Source	Sub stream	Round	BMP (LCH_4 $kgVS^{-1}$)	R_{max} (LCH_4 $kgVS^{-1}$ day^{-1})	BMP $T_{0.5}$ (days)	Time lag λ (days)
Abattoir	composite sample	1	336 ± 15.0	28.5	5.5	6
	Paunch grass	2	238 ± 15.9	24.3	5.6	0.5
	Green sludge	2	403 ± 15.1	47.9	5.3	0.9
	DAS	2	165 ± 7.7	28.4	4.2	0
Cheese	composite sample	1	454 ± 19.3	55	11.2	7
	Bio-effluent	2	468 ± 30.8	82.8	3.7	0.8
	DAF	2	787 ± 46.7	149.6	5.0	2.3
Food waste	composite sample	1	508 ± 21.5	40.9	12.8	6.7
	Domestic	2	419 ± 45.3	69.4	4.9	1.8
	Commercial	2	535 ± 20.0	55.5	8.4	3.5
Pig slurry	composite sample	1	99 ± 8.4	13	5	0
	Weaners	2	38 ± 2.0	-	-	-
	Fatteners	2	70 ± 12.8	-	-	-
WWTS	Final sedimentation	1	247 ± 10	60.8	2.6	0.5

3.7 Methane potential per mass of substrate

Typically for an operator of an anaerobic digester the input substrate is best described in terms of wwt or actual weight arriving at the facility. Methane production is best understood in terms of $m^3$of methane per tonne of substrate delivered to the facility. The methane potential per tonne of substrate is outlined in Table 5.4. The food waste is the

highest yielding substrate per tonne of wet weight (128 m^3 CH_4 t^{-1} wwt) followed by the cheese waste and the abattoir waste (38 and 36 m^3 CH_4 t^{-1} wwt respectively). The wastewater sludge and the pig slurry are the weakest substrates (17 and 4.2 m^3 CH_4 t^{-1} wwt respectively). The significance of volatile solids content may be noted immediately. The weighted average BMP result of cheese waste yielded 515 L CH_4 kg^{-1} VS with an average VS content of 7.5% VS, this equates to 38 m^3 CH_4 t^{-1} wwt. This may be compared to the food wastes samples which yielded a similar, weighted average BMP of 512 L CH_4 kg^{-1} VS at a VS content of 25% of total weight equating to 128 m^3 CH_4 t^{-1} wwt.

Table 4 Weighted average methane potential per tonne of feedstock from round 2

Source	Quantity available (tonne annum^{-1})	BMP (L CH_4kg VS^{-1})	Volatile Solids (% total weight)	Methane Yield	
				m^3 CH_4tww^{-1}	$m_n^3 a^{-1}$
Abattoir					
DAS	1500	165	10.7	17.7	
Green sludge	700	403	18.1	72.9	
Paunch grass	2,500	238	15.6	37.1	
Sub total	4,700				
Weighted average		239	14.4	36.2	170,140
Cheese					
Bio-treatment	5,000	461	7.6	35.0	
DAF	1,000	787	6.8	53.8	
Sub-total	6,000				
Weighted average		515	7.46	38.1	228,600
Food waste					
Commercial	800	535	30.1	164.3	
Domestic	200	419	19.9	84.4	
Sub-total	1,000				
Weighted average		512	25.0	128.0	128,000
Pig slurry					
Fatteners		38	5.0	1.9	
Weaners		70	3.3	2.3	
Mix [a]		99	4.2	4.2	85,920
Sub-total	20,457				
WWTS	1,000	247	6.7	16.5	16,500
Total Feedstock	33,157	250	6.9	19.0	629,160

[a] The pig slurry mix taken from round 1

3.8 Suitability of waste substrates for commercial biogas production

The total quantity of substrate available is approximately 33,000 tonnes per annum with an average methane yield of 19 $m^3 t^{-1}$ wwt which would be expected to generate

approximately 629,000 m^3 of methane annually. This is a low average methane yield per tonne of feedstock and would not be deemed conducive to a financially viable biogas system. The Danish model typically is built upon a minimum average methane yield of 30 m^3 CH_4 t^{-1}[34]. With reference to Table 5.4 it may be noted that pig slurry accounts for approximately 67% of the total available feedstock. However it would only contribute approximately 14% of the total estimated methane yield. The value for the maximum BMP of pig slurry is particularly low (maximum of 99 L CH_4 kg^{-1} VS). The authors recommend that pig slurry should not be part of the feedstock for this digester system. The waste water treatment sludge (WWTS) only provides 2% of the total estimated methane yield from 3% of the total available waste. Final sludge from a typical extended aeration basin, as is used in this location, is considered a low methane yielding feedstock for anaerobic digestion. It is not recommended that the WWTS is part of this digester system based on the relatively low BMP yield of 19 m^3 t^{-1} of feedstock. Conversely in the case of food waste, 21% of the total estimated methane yield

energy comes from only 3% of the total available feedstock. The objective of this proposed waste to biogas anaerobic digester is to have a cost effective design with an optimum specific volumetric methane yield. Therefore it is recommended that pig slurry and waste water treatment sludge be removed from the proposed AD substrates. The authors recommend that the design of the biogas plant should be based on three substrates: abattoir waste, cheese waste and food waste.

3.9 Recommended waste substrates for proposed digester

The scenarios include for substrates with a high specific methane capacity. Information from waste collectors in the region would suggest that the availability of source segregated food waste will increase (from a low base) over the next few years as landfill levies rise. An assumption is made that 4300 t yr^{-1} of source separated food waste may be sourced. From Table 5.5 it may be noted that 947,600 m^3 of CH_4 may be produced for 15,000 t of substrate. This equates to 63 m^3CH_4 per tonne of substrate. This is more than a three-fold increase in the specific methane capacity than from digestion of all substrates (see Table 5.4: 19 m^3 CH_4 t^{-1}). By eliminating the lower methane yielding feedstocks such as pig slurry and WWTS a smaller digester volume with a higher volumetric methane yield will be achieved. This will improve the biomethane yield per unit of capital cost.

Table 5 Suggested input feedstock for proposed digester

Source	Quantity ($t\ a^{-1}$)	% of total substrate	Methane Yield (m^3CH_4/t ww)	% CH4	Methane yield ($m^3\ CH_4$)	C:N
Abattoir	4700	31%	36	169, 200	18%	14
Cheese	6000	40%	38	228, 000	24%	15
Food waste	4300	29%	128	550, 400	58%	15
Total	15,000	100%	63	947,600	100%	14.7

3.10 Limitation of designing anaerobic facilities based on BMP results

The results of BMP tests yield data on the potential biomethane yield from substrates digested in an anaerobic digester. The test is however limited in its ability to model a continuous AD process. A continuously fed AD trial is required to assess important parameters such as determining the optimum organic loading rate and hydrolytic retention time. The monitoring of inhibitory compounds such as the accumulation of volatile fatty acids and ammonia is required to assess the long term suitability of substrates at a specific organic loading rate.

4 Conclusions

The BMP test can be used to find the variability of biomethane potential between waste sub streams, identify any potential toxic substrates and can also be used to examine the kinetics of biomethane production. The BMP results as presented in this paper suggest that abattoir waste, source separated food waste and cheese process effluent sludge are all potentially high methane yielding feedstocks. However waste water treatment sludge and pig slurry in particular were deemed to be unsuitable for commercial scale digestion due to their low solids content and low specific methane yield. Of the potential waste substrates the best estimated methane yields range from 128 $m^3\ t^{-1}$ wwt for source separated food waste to 36 $m^3\ t^{-1}$ wwt for abattoir waste. However there are limitations to the test as it is essentially a batch reactor with optimum conditions for biomethane production. A small scale continuously feed AD trial is necessary to more accurately assess the long term digestion stability of the nitrogen rich substrates outlined in this paper. This is dealt with in a following paper in this journal [11].

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Appendix B: Microbial community analysis of anaerobic bacteria and the effect on biogas production stability on the digestion of macro algae and dairy slurry

Microbial community analysis of anaerobic bacteria and the effect on biogas production stability on the digestion of macro algae and dairy slurry

Jamie Fitzgerald[a,b], Eoin Allen[a,c], David M. Wall[a,c], Stephen Jackson[a,b], Alan Dobson[a,b] Jerry D. Murphy[a,c]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] Department of microbiology, University College Cork, Lee Road, Cork, Ireland

[c] School of Engineering, University College Cork, Cork, Ireland

Abstract

Algae represent ideal resources as next generation biofuels, but necessitate a refinement of the anaerobic digestion process. In a previous study, contrasting mixes of dairy slurry and the macroalga *U. lactuca* were anaerobically digested in continuously stirred tank reactors for 40 weeks. Higher proportions of *U. lactuca* in the feedstock led to inhibited digestion, rapid accumulation of volatile fatty acids, and required reductions in organic loading rate. Building on this work, the worst (R1) and best (R6) reactors were investigated via 16S pyrosequencing, allowing the microbial communities of both reactors to be characterised as they developed over a 39 and 27-week period, respectively. Comparison of the different communities revealed clear differences in taxonomy, metabolic orientation and mechanisms of inhibition, while constrained canonical ordination showed ammonia and biogas yield to be the strongest factors differentiating the two reactor communities. Significant biomarker taxa and predicted metabolic activities were found for successful and failing anaerobic digestion of *U. lactuca*. Acetoclastic methanogens were inhibited early in R1 operation, followed by gradual decline of hydrogenotrophs. Near total loss of methanogens led to an accumulation of acetic acid that crippled R1, while a slow reduction in biogas yield late in R6 operation is attributed to inhibition of acetogenic rather than methanogenic activity.. The improved performance of R6 was attributed to a large *Methanosarcina* population which enabled rapid removal of acetic acid, providing favourable conditions for substrate degradation.

Keywords: Biogas, algae, co-digestion, ammonia, inhibition, 16S, biomarker, *U. lactuca*, *Methanosarcina, Hydrogenispora, Psychrobacter*

1. Introduction

As the application of anaerobic digestion (AD) and biogas production garners support with both national energy suppliers and public interest, the need to fully optimise all process parameters involved with the bioconversion of feedstocks to bio-methane. The biogas production process has seen advances in industrial application in recent times courtesy of increased laboratory research. Initial biogas research was initiated in the 1930's where, [1] developed a greater understanding of the stages and steps involved in the biochemical conversion of organic matter in AD. The constant advances and uptake of biogas use and industrial installation has driven the need to optimise yields of specific substrates and the process itself. Such parameter guidelines exist in terms of inhibitory elements such as, volatile fatty acids (VFAs), ammonia (total ammonical nitrogen) inhibition and pH ranges within biogas reactors [2]. However these environmental factors only exist as values or concentrations in terms of their presence with a reactor.

These parameters mentioned give little insight as to the actual micro bacteria or taxonomy of the AD process. There is a need to identify which phylum of bacteria and sub-phylum exist within a biogas reactor to accurately identify what is happening within the biogas reactor. It took until 2007 for the first study to be undertaken on a conventional biogas reactor system digesting plant biomass in a continuously stirred tank reactor (CSTR) and the analysis of the microbial population or phylum of bacteria which exist within CSTR biogas reactor [3]. From then there has been an increase in the publications in this newly researched area of biogas analysis. A constraint however of this research is that with their being millions of various AD bacterial species, coupled with the fact there are 4 key stages of the AD process with all having specific bacteria communities [4]. Compiling a database of all bacteria phyla and their correlation to environmental conditions is difficult for these reasons. This issue is further complicated with the fact that each biogas reactor is different from the next, due to the various alternative feedstocks it may be fed. A method to optimise biogas reactors is to acclimatise an inoculum and over time and the reactor contents over time to a specific substrate or multiple substrates, which can increase yield and reactor efficiency [5]. This acclimatisation is a development of distinct bacterial communities developing which thrive particularly on a specific substrate and accounting for a greater population of the taxonomy present in the reactor which are greater suited to digest such feedstocks, hence increasing biogas yields [6]. Specific issues which cannot be

described exactly by existing parameters such as the formation of foaming in a biogas reactor can be more closely examined by identification of a reactors taxonomy and analysis of the consortium of bacteria present [7]

“Next generation” sequencing technologies (e.g. Pyrosequencing, Illumina) have become crucial to resolving biogas communities through generation of a microbial “census”, based on a population's signature 16S ribosomal subunit genes. Characterisation of whole environments based on 16S gene fragment sequences has been made feasible by the huge volume of sequences processed, relative ease of application to any sample type, and the swelling databases of annotated taxonomy against which to draw comparison. The utility of 16S metagenomics is bolstered by precursor methods of 16S analysis - notably Denaturing Gradient Gel Electrophoresis (DGGE). By addressing the same genetic variation as metagenomics, DGGE allows robust preliminary exploration of community structure/diversity to be carried out in efficient compliment. The emphasis placed on biogas communities has also produced considerable advantages for molecular methods: in particular, a wealth of genomic sequence data on which to base analysis and further work.

Furthermore as biogas research starts moves to new substrates such as macro-algae and third generation biofuels which can pose difficulties to the AD with low C:N ratios [8] and high concentrations of inhibitory constituents such as sulphur and sodium [9, 10]. The need to collectively identify microbial communities present or absent in a biogas reactor treating such substrates is necessary to tackle inhibitory pathways encountered. The work in this paper sets out an objective of identifying the causes of microbial de-population of 2 biogas reactors which were operated for 9 months. The digesters treated a macro algae *U. lactuca* commonly known as “*sea lettuce*” and dairy slurry. *U. lactuca* is a green macro algae which has significantly troublesome physical characteristics associated to it such as a low C:N ratio, below 9:1 and high sulphur concentrations up to 3% [9]. Optimum C:N ratios are between 25 - 30:1 [11]. The need to determine the present methanogens was greatly required as failure occurred in various stages and rates with biogas reactors treating 6 various ratios of u. lactuca. One reactor in particular showed no clear signs of conventional failure from increased VFA, TAN or trace element concentrations. The use of a molecular biology approach of genetic fingerprinting to establish microbial communities present in the digester at the time of performance drop off, may find an answer to these reactor inefficiencies.

2. Methods

2.1 Biogas reactor configuration.

A total of 6, 5 l one step CSTR were operated in parallel digesting mixes of *U. lactuca* and dairy slurry. 3 reactors treated dried *U. lactuca* in co-digestion mixes of 25, 50 and 75% with dairy slurry. This process was repeated for a further 3 reactors, substituting dried *U. lactuca* with a fresh sample of the algae substrate. The working volume of each of the biogas reactors was 4l. A specific volume of feed was inputted to each reactor each day which with an exact amount of digestate removed from the reactor to keep the volume constant at 4l. Initially each reactor was started with an OLR of 2 kg VS m^3 d^{-1}. Failure to obtain steady state biogas production was observed in 3 out of the 6 reactors with two further reactors reaching steady state production profiles but incurred heavy VFA inhibition with only one reactor achieving satisfactory yields. The 6 reactors which were trialled for a period of up to 42 weeks experienced a range of results in terms of VFA, biogas yields and trace element concentrations, which overall illustrated failure to establish high rates of substrate input. A clear observation from these trails to optimise the production of biogas was that, high percentages of *U. lactuca* in co-digestion mixes were overall a poor performing substrate and reduced reactor stability. The reactor digesting 25% fresh U. lactuca (R6) was the best performing reactor, which showed signs of stability in terms of VFA concentrations and yields at an OLR of 2.5 kg VS m^3 d^{-1}. R6 was one of the chosen reactors to be selected for further fingerprint rDNA to determine the microbial communities present within the reactor. The second reactor chosen was R1, which digested 75% dried *U. lactuca* and 25% dairy slurry. R1 was chosen on account that, out of the 5 reactors (R1 – R5) which experienced greater difficulties in biogas production it treated dried *U. lactuca* opposed to R6 (fresh *U. lactuca*)and it lasted for a longer digestion period than the alternative 2 reactors (R2 and R3) treating dried *U. lactuca*.

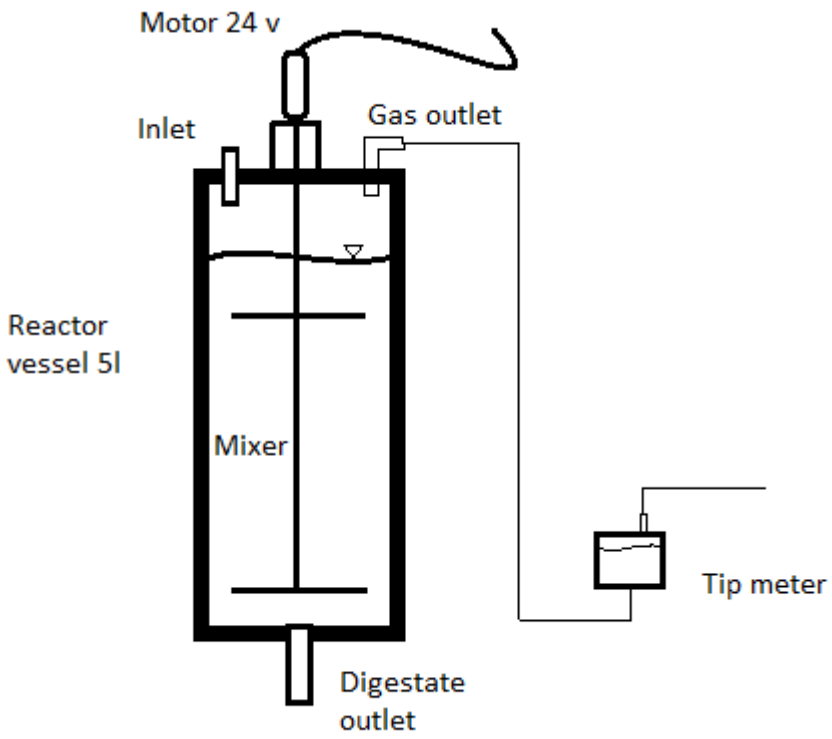


Figure 1. Schematic of Reactor set up for R1 and R6.

A sample from the top, middle and bottom of the reactor was removed from R1 and R6 on a weekly basis to determine homogeneity between these three zones and ensure an adequate bacterial population was present with the reactor

Reactor R1 was operated for a total 40 weeks. Initially an OLR of 2 kg VS m^3 d^{-1} was used for R1. However failure to reach the designated yields after the first hydraulic retention time (HRT) and the increase in VFA concentration saw this OLR reduced to 1 kg VS m^3 d^{-1}. Steady state biogas production was achieved throughout this period. Samples were taken at weeks 1, 5 , 13, 20, 30 and 39, spanning five retention times. R6 was also operated for 40 weeks. An OLR of 2 kg VS m^3 d^{-1} was successfully maintained for R6 after a period of 3 HRTs. The OLR was increased to 2.5 kg VS m^3 d^{-1}. Steady state biogas production was achieved throughout this period. Samples were taken at weeks 1, 5, 13, 21 and 27, spanning four retention times. A gradual decline was observed in the final HRT for R6 without a corresponding increase in VFA or ammonia concentrations to account for this reduction. A stable temperature of 37^{o}C was maintained throughout the course of the experimental trial. The decision to increase OLR was determined by two factors; VFA concentrations and

their effect on reactor performance in terms of biogas yields. This was determined exactly by the Nordmann [12] method, commonly known as the FOS:TAC ratio, which is a ratio of the volatile organic acids and the total inorganic carbonate. The operational ranges set out by this method dictates, as to whether the reactor is being over, under or fed satisfactorily. The second limitation was the biodegradability index (B_{ix}), this index is simply the biochemical methane potential (BMP) yield obtained from a 30 day batch test on that exact substrate(s) divided by the specific methane yield (SMY) of that reactor. The closer a B_{ix} is to 1 the better and even >1 is acceptable as residual feedstock may be retained in a reactor after a weekly period. A comprehensive detailing of the laboratory methods used to analysis all environmental factors of R1 and R6 is described by[9].

2.2 Molecular Methods

DNA extraction was carried out using the PowerSoil DNA extraction kit (MoBio, CA, USA), with a modified protocol: 1) wet-spin (30 seconds at 10,000 g) to remove an excess liquid fraction prior to cell lysis; 2) 3x cycles of 10 minute bead-beating followed by 5 minutes chilling at -20_C; 3) 2 washes of elution buffer. Extractions were performed in triplicate before combining equimolar quantities for each sample. Extractions were quantified by spectrophotometer (ND-1000, Thermo-Fisher, DE, USA) and viewed on 1% agarose gel with ethidium bromide (10mg:1ml agarose gel).

Denaturing Gradient Gel Electrophoresis (DGGE) [13] was used to validate that the CSTR design provided a homogeneously mixed environment. The Reactor 3, week 18 timepoint was taken as an example of a stable methanogenic community (OLR=2.5kg VS m^{-3} d^{-1}, avg. 64% of BMP). Sample material for this time point consisted of digestate from the top, middle and bottom portion of Reactor 3 at week 18; following the above protocol, DNA was extracted from each of the three digestate samples and diluted to concentrations of 6 ng l^{-1}.

For each sample (top, middle, bottom), primer pairs a340F/a1000R [14] and b27F/u1492R [15] were used to carry out an initial PCR covering the V3 to V5 and V1 to V3 16S regions for Archaea and Bacteria respectively. For Archaea, *Phu* Polymerase (New England Biolabs, MA, USA) was used with an initial denaturing step of 30 seconds at 98ºC, followed by 35 cycles of 98ºC for 7 seconds, 63.6ºC for 30 seconds, 72ºC for 1 minute, and a final extension of 2 minutes at 72ºC. For Bacteria, DreamTaq Polymerase (Thermo-Fisher

Scientific Inc., MA, USA) was used with the program was used with an initial denaturing step of 3 minutes at 95ºC, followed by 30 cycles of 95ºC for 1 minute, 50ºC for 1 minute, 72ºC for 1 minute, and a final extension of 5 minutes at 72ºC. To improve specificity [Boon *et al.*, 2002], Nested amplicons were then generated from these products in a subsequent PCR using archaeal primers a344FGC/a915R [16] and bacterial primers b63FGC/u518R [17], incorporating a 40bp GC-clamp. PCR programs with initial denaturing steps of 5 minutes at 94ºC were followed by 35 cycles of 94ºC, 65ºC, and 72ºC for 1 minute, and 92ºC, 59ºC, and 72ºC for 1 minute for Archaea and Bacteria respecitvely, with final extension steps of 5 minutes at 72ºC. Nested-PCR products were cleaned using a PCR Purification Kit (QIAGEN, Manchester, UK).

DGGE was carried out on the D-CODE (BioRad, CA, USA) platform using polyacrylamide gel with a 20%-80% denaturing gradient of formamide and urea. Two wells were run per sample, each containing 25 µl PCR product. Gels were run at 75V for 17 hours at 60ºC. Once run, gels were incubated at 32ºC in 1x SYBR Gold (Thermo-Fisher Scientific Inc., MA, USA) for 20 minutes, then exposed to UV light and recorded on digital camera.

Each sample was amplified in triplicate via polymerase chain reaction (PCR) using pyrosequencing primers with the following motif: adapter sequence (Roche-454 Lib-A and Lib-B chemistry); key sequence (TCAG); Roche-454 pyrosequencing MIDs 1-10 and 12 inclusive; and 16S universal primers U-789F (5' TAGATACCCSSGTAGTCC 3') and U-1053R (5' CTGACGRCRGCCATGC 3') [18]. PCR used a program of initial denaturation at 94ºC for 5 minutes, followed by 26 cycles of 30 seconds denaturing at 95ºC, 30 seconds annealing at 53ºC, and 45 seconds of extension at 72ºC, with a final extension step of 72ºC held for 6 minutes. Products in the expected range were extracted using a gel extraction kit (QIAGEN, Manchester, UK) which required subsequent use of a PCR purification kit (QIAGEN, Manchester, UK). Replicates were combined in equimolar quantities, and final pyrosequencing of samples was carried out by MACROGEN (Seoul, Republic of Korea).

2.3 Bioinformatic Analysis

Denoising was performed in Acacia [19] before import into the Quantitative Insights Into Microbial Ecology (QIIME) software pipeline [20] for demulitplexing, chimera-slaying, aligning, taxonomic assignment and exploratory analyses: Sequences were split into sample libraries with a maximum forward primer mismatch of 8bp; Chimera filtering was carried

out using USEARCH v6.1 [21]. Alignments and taxonomic assignments were carried out with reference to the Silva [22] 111 Database release at 97% similarity using PyNast [20]and the RDP Classifier 2.2 [23]. Tree building was carried out using FastTree [24]. Beta diversity was calculated using UniFrac [25]. 3D PCoA plots generated by Emperor [25].

Sequence data was combined with environmental data from Allen et al., 2014 inside the *R* statistics program [26]. *R* packages *vegan* and *phyloseq* [27] were used to subset OTUs by sample, reactor environment and perform statistical analysis.

Greengenes release 13.5 [28] was necessary to perform closed-reference OTU picking in QIIME prior to generating metabolic predictions with the HMP Unified Metabolic Analysis Network (HUMAnN) [29] package. Significant differences between the two reactors were then calculated using the LDA Effect Size (LEfSe) resource [30] on the Huttenhower Lab Galaxy server (Galaxy Ref 123) to analyse taxonomic and metabolic prediction data.

3.0 Results and discussion

The previous study trialled the anaerobic digestion of varying ratios of *U. lactuca* and dairy slurry, demonstrating severe inhibition at higher *U. lactuca laoding rates*. To determine the cause of inhibition, this study set out to characterize and contrast microbial communities within two reactors digesting opposite ratios of *U. lactuca* and dairy slurry. The significant composition of communities was determined, allowing characterisation of biomarker species and predicted metabolic activities for both reactors. Relationships with reactor process were then defined, illustrating a likely mechanism of reactor failure and the influence of *U. lactuca* on the microbiology of an anaerobic digester.

3.1 Process results of biogas reactors, R1 and R6

At steady state operation, the methane (CH_4) yield per kilogram of volatile solids ($kgVS^{-1}$) was similar between the two reactors: R1 produced an average 176.75 l CH_4 kg^{-1} VS; R6 produced an average of 176.38 l CH_4 kg^{-1} VS. However, R1 could only maintain steady-state operation for 26 weeks, with a fluctuating biodegradability index (B_{ix}) (0.40, 0.84 and 0.69 for the total trial period), and low OLR of 1 kg VS m^3 d^{-1}. These fluctuations correlated with toxic concentrations of VFAs [31], and unfavourable Fos:Tac ratios (outside

the recommended range of 0.2 – 0.4). In comparison, R6 showed steady-state biogas production for all 40 weeks of trialling: B_{ix} values of 0.95 and 0.90, Fos:Tac values consistently between 0.2 and 0.4, and low VFA levels even at the increased OLR of 2.5 kg VS m^3 d^{-1}. Comparison of reactor parameters clearly shows that R1 struggled at the higher *U. lactuca* composition, while R6 successfully digested the algae, at a higher OLR.

	R1			R6	
Parameters	HRT 1	HRT 2	HRT3	HRT 1	HRT 2
Reactor type	CSTR	-	-	CSTR	-
% *U. lactuca*	75 (dried)	-	-	25 (dried)	-
TS (%)	29.61	-	-	10.55	-
VS (%)	18.42	-	-	7.22	-
Temperature (^oC)	37	37	37	37	37
OLR (kg VS m^3 d^{-1})	2	1	1.5	2	2.5
BMP (CH_4 kg^{-1} VS)	210 $\pm$ 6.3	-	-	183 $\pm$ 7.8	-
SMY (CH_4 kg^{-1} VS)	83.31	176.77	145.21	178.11	170.46
B_{ix}	0.40	0.84	0.69	0.95	0.90
Methane content (%)	33	47	47	51	52
VFA (mg l^{-1})	4954	4135	4355	1955	1720
Fos:Tac	0.56	0.34	0.43	0.39	0.30
TAN (mg l^{-1})	3443	5250	5300	2168	3000

Table 1. Highlights of results of semi continuous digestion trials.

3.2 Characterizing Community Makeup

A full break down of community abundances is given in Table 1 of Supplementary Data. Rarefaction curves and UNIFRAC Community distances are given as Figures 1 and 2 of Supplementary Data.

3.2.1 Sequencing Results and Diversity Measures

Pyrosequencing returned 270,111 raw sequences: after denoising in Acacia and processing in QIIME, 89,251 (average length: 244bp) sequence reads were produced with an average of 8,114 reads per trial time-point.

To ensure representative samples from both reactors, diversity metrics were calculated to estimate coverage of species diversity (Chao1 index) and species abundances (Simpson's Index). Rarefaction curves of these indices indicate that the most abundant species were thoroughly characterised in this study. However, the curves suggest that a wealth of low-abundance *Archaea*, *Bacteria* and unidentified taxa remain undetected due to sparse abundance.

Both diversity indices (Chao1, Simpson's) were seen to decrease in later retention times and showed slight negative correlations with most process conditions; in particular CH_4 yield (Chao1: R=-0.64, Simpson's: R=-0.52) and biogas (Chao1: R=-0.62, Simpson's: R=-0.63). Rather than some direct interaction between biogas and diversity, this suggests the maturation of trophic systems in either reactor, where 'surplus' diversity is marginalized beyond the sequencing threshold**.** [32, 33] that species diversity forms an important reservoir of metabolic capability, invoked at establishment of reactor communities or during disruptions, being otherwise obscured by more abundant species.

3.2.2 Community Structure

Archaea		Proportional Abundance in Sample										
		Reactor 1: 25% dairy slurry : 75% *Ulva*						Reactor 6 – 75% dairy slurry : 25% Ulva				
	Genus:	Week 1	Week 5	Week 13	Week 21	Week 30	Week 39	Week 1	Week 6	Week 13	Week 20	Week 27
Acetoclasts	Methanosarcina	1.10%	1.20%	0.10%	0.00%	0.00%	0.00%	9.70%	20.50%	24.10%	17.70%	18.30%
	Methanosaeta	0.50%	0.20%	0.00%	0.00%	0.00%	0.00%	0.40%	0.20%	0.30%	0.10%	0.00%
Hydrogenotrophs	Methanobrevibacter	0.10%	0.00%	0.30%	0.30%	0.30%	0.10%	0.30%	0.70%	0.70%	0.30%	0.40%
	Methanoculleus	0.10%	0.20%	0.60%	0.60%	0.40%	0.20%	0.20%	0.40%	0.10%	0.10%	0.20%
	Methanobacterium	0.00%	0.00%	0.00%	0.00%	0.10%	0.00%	0.00%	0.10%	0.20%	1.10%	0.80%
	Methanosphaera	0.00%	0.00%	0.00%	0.00%	0.10%	0.00%	0.00%	0.00%	0.00%	0.10%	0.00%
	Methanocorpusculum	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	Methanospirillum	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.20%	0.00%	0.00%	0.00%	0.00%
	Other Archaea (non-methanogenic)	0.1%	0.0%	0.1%	0.0%	0.1%	0.0%	0.00%	0.10%	0.20%	1.20%	0.80%
	Sum Archaea	**2.00%**	**1.90%**	**0.90%**	**1.20%**	**1.10%**	**0.50%**	**11.00%**	**21.80%**	**25.30%**	**19.30%**	**19.60%**

Bacteria		Proportional Abundance in Sample										
		Reactor 1: 25% dairy slurry : 75% fresh *Ulva*						Reactor 6 – 75% dairy slurry : 25% Ulva				
	Phylum:	Week 1	Week 5	Week 13	Week 21	Week 30	Week 39	Week 1	Week 6	Week 13	Week 20	Week 27
Hydrolysers fermentaters acidogens	Firmicutes	15.30%	38.30%	26.70%	38.50%	50.80%	56.80%	36.80%	29.20%	37.80%	40.50%	31.60%
fermenters Acidogens	Bacteroidetes	12.70%	26.10%	8.70%	22.70%	16.20%	17.00%	14.80%	15.20%	15.20%	15.20%	10.30%
diverse, Acidogens	Proteobacteria	34.70%	10.60%	31.40%	12.90%	7.90%	7.00%	12.30%	5.80%	2.40%	8.80%	12.50%
-	Spirochaetes	12.60%	9.90%	15.10%	4.00%	4.40%	2.00%	5.50%	7.30%	2.60%	1.60%	4.30%
-	Synergistetes	6.00%	2.00%	6.40%	12.80%	7.30%	2.60%	8.10%	3.80%	2.60%	3.00%	4.40%
-	Chloroflexi	1.00%	1.10%	3.00%	0.30%	0.20%	0.00%	0.90%	3.10%	3.80%	2.20%	5.30%
-	Tenericutes	0.00%	0.50%	0.20%	2.60%	4.50%	9.20%	0.00%	0.50%	1.10%	1.00%	0.60%
-	Unidentified	0.70%	3.20%	1.00%	1.80%	2.20%	2.90%	1.20%	1.10%	0.90%	0.80%	0.50%

Table 2: Proportional abundances of the main community components.

The QIIME pipeline identified 2,824 Operational Taxonomic Units (OTUs, each representing a presumed species of microbe) in the 89,251 sequence reads (where read counts act as proxy for population size, i.e. relative abundance). Singleton and doubleton OTUs (abundances < 3 reads) were discarded to reduce statistical noise, giving 1,320 OTUs (82,914 sequence reads). Of the 1,320 OTUs, 1,057 were present in R1, 955 in R6. Taxonomic alignments provided by Silva [release 111] identified 2 phyla, at least 4 classes, 5 orders, 7 families and 8 genera of *Archaea* (20 OTUs, 9,010 sequences), and at least 34 phyla/candidate phyla, 44 classes, 86 orders, 124 families and 190 unique genera of

Bacteria (1,206 OTUs, 73,185 sequence reads). Lower taxonomic classifications could not be assigned to 16% of *Bacteria* families and 53% of *Bacteria* genera.

3.2.3 Unassigned Operational Taxonomic Units

A final 94 OTUs remained unidentified and were not assigned to *Bacteria* or *Archaea*. Unassigned taxa comprised 1% of sequence reads (72 OTUs) from R1, and <1% of reads (42 OTUs) from R6. Of these, only 6 OTUs exceeded an abundance of 11 sequence reads: 1 OTU persisted at these levels in late R1, showing 92% coverage at 84% identity with the Firmicute *Desulfomataculum* BLASTn[34], known oxidisers of VFAs and reducers of sulfur compounds.

3.2.4 Archaeal Components

Large *Methanosarcina* populations effectively buffer against fluctuations in substrate availability, preventing accumulation or shock loading of acetic acid [35, 36] *Methanosarcina* has a documented tolerance for acetic acid up to 15,000 mg/l, and a higher tolerance for changes in pH and salt (see review in [37]) than hydrogenotrophic counterparts. *Methanothrix,* an obligate acetoclast [Huser et al., 1982], was scarce or absent in this study, likely out-competed by the higher growth rate of *Methanosarcina* at non-limiting acetate concentrations [35, 38, 39], or inhibited by salt [40] or ammonia [41-44]

Hydrogenotrophic methanogens (*Methanoculleus*, *Methanobrevibacter*, *Methanobacterium*, *Methanocorpusculum, Methanospirillum* and *Methanosphaera* in this study) are commonly found in anoxic sediments [45], as gut flora [46-48], and in AD reactors where they sometimes dominate [49, 50] Most archaeal OTUs remained present at uninformatively low frequencies, often disappearing below the sequencing coverage threshold.

3.2.5 Bacterial Components

Bacterial components of these reactors show good agreement with documented biogas communities, while some key and accessory species are associated with marine or salt

environments. The most abundant phylum was *Firmicutes* (565 OTUs, 36% of all sequence reads), containing many groups known to hydrolyse polymers (e.g. cellulose, lignin, polysaccharides, proteins: *Lachnospiraceae, Peptostreptococcaceae, Ruminococcaceae*), ferment carbohydrates (e.g. saccharides, amino acids, organic molecles: *Hydrogenispora, Gelria, Christensenellaceae*), and produce organic acids as metabolic endpoints (i.e.: acidogens: *Sedimentibacter Tissierella, Syntrophomondaceae*). *Firmicutes* are major components of anaerobic environments such as digesters [49-51] and alimentary tracts [52, 53], in this study accounting for over a third of sequences in both reactors: in short, they are highly diverse, widely distributed, and understood as essential components of anaerobic digestion.

The second-most abundant phylum, *Bacteroidetes* (126 OTUs, 16% of all sequence reads), is also frequently detected in anaerobic reactors, with important roles as fermenters and acidogens. In particular, species from the family *Porphyromonadaceae* (9% of all reads) are known for degradation of proteins and amino acids, eschewing saccahrides (genera *Petrimonas* [54] and *Proteiniphilum* [55].

Phylum *Proteobacteria* (203 Otus, 13% of sequence reads) comprises the most diverse known taxonomic group of the *Bacteria* to date. The sub-ordinate classes *Alpha-* and *Gamma-Proteobacteria* contributed 3% and 7% of reads in this study respectively, with remaining proteobacteial classes totaling 3%. *Proteobacteria* are typical residents of anaerobic digesters [49, 50, 56] known to incorporate nitrogen and/or sulphur as electron acceptors in metabolism of varied carbohydrates (e.g.: *Nitrosimonas*, *Nitrobacter*). However, some species observed here are unexpected inclusions, with described preferences for aerobic metabolism (in some cases obligate: *Rhodobacteraceae*, *Granulosiococcaceae*, *Nannocytineae;*) and a high propensity for saline and marine environments (water: *Rhizobacteraceae*; sediments: *Desulfomicrobium*; seaweeds and plants: *Alteromonadaceae*, *Nannocystinaceae*, *Granulosiococcaceae*). As such, their presence in this study reflects persistent contributions from the *U. lactuca* feedstock alongisde species typical of a biogas digester habitat.

Phylum *Spirochaetes* (47 OTUs and 6% of sequence reads in this study) are diverse, highly motile, frequently anaerobic bacteria, but metabolic information is limited in anaerobic digesters despite being frequently encountered in low or medium abdundances. They have

been characterised both as acetogens [57, 58] and acetoclasts involved in methanogenic activity (as Syntrophic Acetate-Oxidising Bacteria) [59].

Phylum *Synergistetes* composed 6% of all sequence reads and 34 OTUs. *Synergistetes* are typically seen at lower abundances in a wide variety of environments [60], in syntrophic associations with hydrogenotrophic species. A possible role in this reactor was oxidising amino acids as a substrate in the presence of methanogens [61, 62].

Most phyla were present at much lower levels (< 2% of reads): Phylum *Chloroflexi* contains fermentative, acido- and acetogenic, obligate and facultative anaerobes seen in anaerobic digesters and hot springs respectively, and requires removal of hydrogen which suggests syntrophic roles[63] Phylum *Tenericutes* is represented by *Acholeplasma* spcs.- poorly characterised sugar fermenters [64]. Species from Phylum *Actinobacteria* contain many heterotrophic fermenters including lipidophiles, and obligate marine-associated species[65]. Phylum *Acidobacteria* species are uncharacterised but similar to sequences recovered from remedial petrochemical aquifers[isolate BPC102, NCBI accession AF154083.1]: Taxa from Phylum *Armatimonadetes* are expected to be chemo-heterotrophs, and are suggested to associate with degradation of photosynthetic biomass [66].

Although the eleven phyla outlined above describe over 94% of all sequence reads, the remaining *Bacteria* (6% of reads, 135 OTUs) correspond to at least a further 26 phyla, again reflecting the huge diversity in anaerobic reactor communities.

3.3 Relation between Process and Community Structure

3.3.1 Changes in Archaeal Abundances

R1 Archaea declined from an initial 2% of all Week 1 sequence reads to 0.3% by Week 39 – from 8 OTUs to 3. Abdundances of acetoclastic *Methanosarcina* increased in Weeks 1 and 5 to a maximum of half of all archaeal reads, but were negligible from Week 13 and not detected by Week 39. Despite reactor stabilising at a lower OLR, abundance of aceticlastic methanogens remained extremely low and high acetate levels accumulated in the following weeks. Hydrogenotrophic *Methanobrevibacter* and *Methanoculleus* became the dominant

Archaea from Week 13 onwards, contributing <0.3% and <0.6% of sequence reads for any time-point, with sustained low abundances corresponding to a poor methane yield and eventually decline seen from Week 30; Archaea contributed 0.3% of sequence reads for Week 39.

Despite the short lead-time, R6 Week1 showed methanogen sequences to be comparatively enriched: *Archaea* comprised 11% of sequence reads in R6 Week1, compared to 2% of reads from Week1 of R1. This may reflect a rapid acclimatization to feedstock (uncharacteristic of methanogens), or a greater methanogen contribution from the slurry portion of the feedstock, which was a three-fold larger volume in R6 than R1. R6 *Archaea* represented all methanogen taxa found in R1 in addition to *Methanospirillum*, *Methanocorpusculum* and *Methanomassiliicoccus*. After Week1 (16 OTUs), R6 *Archaea* peaked at Week13 (25% of reads, 17 OTUs), and reduced towards the end of the trial with 20% of reads (14 OTUs) by Week27. However when the most numerous genus (*Methanosarcina,* 90% of all *Archaea* sequence reads) is discounted, population levels remain stable: other *Archaea* contribute 1.4% of reads (6 OTUs) across Week13 to Week27, revealing a relatively stable (hydrogenotrophic) methanogen sub-community, with *Methanobacterium* and *Methanobrevibacter* as the largest sub-populations. Reduction in R6 *Methanosarcina* populations coincides with a slow decline in biogas yield observed towards the end of the study, despite optimal process conditions: FOS:TAC 0.22-0.24; free ammonia and chloride below inhibitory levels; VFA concentrations below inhibitory levels despite an increased OLR [37, 42]

Given the similar sensitivity of samples (as per rarefaction observation rates, see Supplementary Materials), attrition in R1 archaeal sequence reads reflects a real decrease in methanogen abundance (presumably due to inhibition), while in R6 a specific decline is seen in *Methanosarcina* abundance as the reactor matures and acetate becomes limiting.

3.3.2 Changes in Bacterial Abundances

Communities were considered at Order level (93% of OTUs assigned taxa). R1 showed several changes in composition. Week1 abundances appeared initially balanced between hydrolysers (*Clostridiales, Bacteroidales, Synergistales*), fermenters (*Clostridiales*,

Bacteroidales, *Desulfovibrionales, Synergistales), and* acido/acetogens (*Desulfovibrionales, Pseudomonadales, Clostridiales, Bacteroidales*), as well as species considered environmental inclusions (*Rhizobiales, Rhodobacterales*, *Myxococcales)* associated with slurry, *U. lactuca*, or marine sources.

At R1 Week5, *Clostridiales* and *Bacteroidales* orders (hydrolysers/fermenters) increased to 36% and 16% of the total sequence reads respectively: of note, increases in populations involved in cellulose degradation (*Ruminococcaceae*, *Lachnospiraceae*), protein degradation (*Proteiniphilum*), and an unknown *Sphingobacteriales* taxon from Group WCHB1-69. Increases were also seen in acido- & acetogenic fermenters of amino acids (*Spirochaetales* and *Sedimentibacter*).

In R1 Week13, hydrolysing and fermenting *Clostridiales* (24%) and *Bacteroidales* (6%) were displaced by a sharp rise in the abundance of a *Psychrobacter* OTU (from *Pseudomonadales)* to 25% of sequence reads: *Psychrobacter*, which is often associated with cold marine environments, is likely to reduce amino and organic acids to acetate [67]. This proliferation at Week 13 co-incides with the metabolism of large quantities of valeric acid accumulated from Week 6 coinciding with a drop in methanogen populations, suggesting an important role for *Psychrobacter* in continuous digestion of *U. lactuca* and slurry.

At R1 Week21 the reactor was dominated again by hydrolysers and fermenters: 33% of sequence reads for *Clostridiales* (*Hydrogenispora* (previously OPB54) *at 13% of reads*, with *Ruminococcaceae*, *Peptostreptococcaceae*, and *Gelria*); 21% for *Bacteroidales (*largely *Proteiniphilum* (16%), *some Petrimonas);* 13% for *Synergistales* (*Aminobacterium* and an unidentified OTU). It is worth noting most of these taxa are acetogens, degraders of proteins & amino acids, or both. *Psychrobacter* abundance is reduced to 6%, possibly reflecting low levels of propionic acid. Several taxa (e.g.: *Synergistaceae, Petrimonas, Gelria*) are also known or suspected methanogen syntrophs [54, 60, 68].

In later weeks, a relatively stable topography at order-level disguised underlying proliferation of single-genus populations, likely related to increased OLR and a gradual accumulation of VFAs (particularly acetic acid) to high levels (> 3,000 mg/l). R1 Week 30 produced small but suggestive changes in the community makeup: hydrolysers and fermenters (*Clostridiales, Bacteroidales*) remained at the fore; cellulytic and saccharolytic

species (*Acholplasmataceae, Ruminococcus, Gelria*) increased as did *Hydrogenispora*; while protein and amino acid metabolisers declined (*Aminobacterium, Proteiniphilum, Psychrobacter, Peptostreptococcaceae*). This may indicate a shift from protein to polysaccharide metabolism as higher OLR provides fresh substrate.

As acetic acid continued to accumulate (FOS:TAC in excess of 0.4), R1 Week 39 saw further increases in acetotrophic *Acholeplasmataceae* (9% of reads) and *Hydrogenispora* (36% of reads, saccharide-fermenter & acetogen), as well as *Proteiniphilum* (13% of reads, protein and amino acid fermenter & acetogen). These abundances displaced *Synergistales* (syntrophic heterotrophic acetogens)*, Ruminococcaceae* (cellulolytic acetogens) and *Clostridiaceae* (likely heterotrophic fermenters and acetogens) populations. Many populations persisted, albeit at very low levels: larger populations included Family XI (from *Clostridiales) Rhodobacterales, Rhizobiales* (possible enviromental inclusions)*,* and *Peptostreptococcaceae* (saccharide and amino acid fermenters).

R6 showed a more pronounced continuity between samples than R1 when considered at Order-level (inferred taxa for 84% of OTUs; archaeal order *Methanosarcinales* included for comparison). R6 Week 1 showed a community already typical of a biogas consortium: large populations of hydrolysers (e.g. *Clostridiales (*32% of sequence reads)*, Bacteroidales (*10%)), fermenters and acidogens (e.g. *Clostridiales, Bacteroidales, Synergistales* (8%)), with relatively abundant methanogens (i.e. *Methanosarcinales*: 10%) present at levels which persisted for the duration of the trial.

Shifts were apparent as the reactor matured: R6 Week 5 saw methanogen abundance double to 22% of sequence reads, with only 1 to 3% increases on Week 1 abundances in probable fermenters and acetogens (*Bacteroidales, Christensenellaceae, Rikenellaceae, Spirochaetaceae*), syntrophs (*Syntrophomonadaceae, Cloacamonas*), and uncharacterised *Acidobacteria*. Proteolytic, non-saccharide fermenting populations of *Synergistaceae* were replaced by smaller stable *Synergistaceae* populations of unknown activity. *Clostridiales* broadly involved in hydrolysis, fermentation and acidogenesis (incl. *Hydrogenispora*) declined to 27%.

R6 Week 13 represented the high-point in R6 acetic acid, biogas production, and methanogen abundance (25% of sequence reads), again consisting almost entirely of *Methanosarcina* (24%). Contrasting with other time-points from either reactor, there were

no large homogeneous bacterial populations: instead, heterogeneous hydrolytic/fermentative/acidogenic communities (*Clostridiales*: 33%; *Bacteroidales*: 14%; others: 19%) comprised of numerous smaller subpopulations were seen. The largest subpopulations (*Christensenellaceae*: 6%, *Rikensellanaceae*: 4%) are acetogenic saccharide fermenters associated with the gut; several protein fermenting populations are also evident (*Peptostreptococcaceae*: 5%, *Proteiniphilum*: 3%), as are diverse sub-populations of cellulytic *Ruminococcaceae* (5%) and *Lachnospiraceae* (4%). This combination of high-diversity, low-abundance *Bacteria* populations with low-diversity, high-abundance *Archaea* populations may typify a balanced *U. lactuca*-digesting reactor, operating at or within capacity. Wittebolle [Wittebolle et al., 2009] and Werner [Werner et al., 2011] suggest more balanced ('even') microbial populations exhibit greater metabolic capability and therefore long-term stability.

Reduced output at R6 Week 20 saw slight changes in the dominant fermenting, acidogenic and acetogenic populations. Such changes may be stochastic, or could reflect altered substrate availability: hydrolysing (*Peptostreptococcaceae*: protein and amino acid fermentation; *Lachnospiracceae*: polysaccharide lysis) and saccharide fermenting (*Christensenellaceae*, *Rikensellaceae*, *Lachnospiraceae, Clostridiales* Family XI) populations were displaced by populations with similar activities (*Ruminococcaceae*, *Proteiniphilum*, *Psychrobacter*, *Thermoanaerobacteraceae*, *Hydrogenispora*). Importantly, *Methanosarcina* abundance decreased to 18%. This decrease in *Methanosarcina* and increase in *Psychrobacter* could reflect the decreasing availabiltiy of acetic acid and increase in higher VFAs (particularly valeric acid and its products), again suggesting a role for *Psychrobacter* in metabolism of higher VFAs.

R6 Week 27 represented an OLR of 2.5, with abundances further concentrated within subpopulations. *Methanosarcina* levels remained stable at 18%. Saccharide and amino acid (i.e. monomer) fermenters, acidogens and syntroph populations (*Synergistaceae*, *Cloacamonas*, *Psychrobacter*, *Thermoanaerobacteraceae*, *Caldilineaceae*) displaced metabolic equivalents (e.g. *Christensenellaceae)* and degraders of polymers like cellulose (*Ruminococcaceae*, *Lachnospiraceae)* or proteins (*Syntrophomonadaceae*, *Peptostreptococcaceae*, *Proteiniphilum*, *Petrimonas*), likely reflecting increased availability of simple substrates with increased OLR. Although total VFA levels remained relatively low, valeric and caproic acids were accumulating while acetic acid was limited. Reduced

acetotroph (e.g. *Methanosarcina*) activity could inhibit catabolism of higher VFAs through unfavourable equilibria, but would present with an increased concentration of acetic acid. Instead, declines in acetic acid, *Methanosarcina*, and biogas output along with accumulation of higher VFAs suggests inhibition prior to methanogenesis, perhaps at the acetogenesis stage: as such, acetate may have been limiting for *Methanosarcina* in R6, with substrate inhibition affecting some acetogenic process.

3.4 Possible Mechanisms of Inhibition

3.4.1 Volatile Fatty Acids:

VFA levels for R1 show a gradual accumulation of acetic and iso-valeric acid until Week 3 (1,099 & 1,776 mg/l respectively), followed at Week 5 by a peak in iso-valeric acid (3,501mg/l) while acetic acid was depleted (155mg/l). *Methanosarcina* abundance increased in line with acetic acid metabolism while acetogenesis via iso-valeric acid was inhibited. After reducing the OLR to 1 kg VS m^3 d^{-1} at Week 7, iso-valeric acid was metabolised to equal portions of acetic acid - which the reactor was absorbing by Week 7 - and propionic acid, which persisted past Week 13. *Psychrobacter* abundances increased with availability of propionic acid, implying a role in metabolism. By Week 13 acetoclastic *Methanosarcina* abundances dropped (1.2% to <0.1% of sequence reads) despite stable reactor conditions until Week 26 (FOS:TAC 0.21 – 0.31), a lack of inhibitory VFAs (<4,000 mg/l, [37] and favourable levels of acetic acid for that genus (1100 – 1300mg/l [37]; also evidenced by similar concentrations in R6, Week 13). Small hydrogenotrophic methanogen populations continued with meagre biogas output, while a bacterial community with a focus on alternative carbon metabolisms developed. Later accumulation of acetic and propionic acid corresponded with abundance increases in an acetogenic *Hydrogenispora* sp., but no increase in *Psychrobacter* abundance. From Week 23 onwards (and particularly after OLR was increased at Week 33), apparent lack of acetoclasts led to acetic acid and propionate accumulation (3200mg/l and 700mg/l respectively).

Initial accumulation of iso-valeric acid (600mg/l) and acetic acid (200mg/l) was also seen in R6: however, iso-valeric acid was quickly metabolized, with acetic acid formed as the chief by-product until Week 9. Later peaks were seen in propionic acid (707mg/l, Week 13), and iso-capronic acid (606 mg/l, Week 17) which was converted to valeric acid (551mg/l, Week

21). Spikes in valeric and propionic acid levels were shadowed by increases in acetic acid, suggesting degradation via acetogenesis. Later R6 increases in VFAs levels (valeric and iso-capronic acids), and abundances of *Psychrobacter* and *Hydrogenispora* from Week 20 were similar to R1 when perturbed, partially attributed to an OLR increase to 2.5 kg VS m^3 d^{-1}. Although biogas output and *Methanosarcina* abundance decrease slightly from Week 25, overall stability of the methanogenic populations and scarce acetic acid levels again suggest constriction of the biogas process at some preceding point in acetogenesis, as occurred in R1, Week 5.

VFA accumulation can occur as a product of instability [69], can be transitionary [38, 70, 71] and can even have little to no effect on biogas output [72] Initial accumulation of iso-valeric and acetic acids was seen in both reactors: the relative difference between build-ups (initially three-fold higher in R1; higher thereafter) suggests this is due to hydrolysis and fermentation of the most accessible fractions of *U. lactuca*. Immediate availability of organic material should allow rapid acidogenesis, providing good biogas yield. However, inhibition of bacterial acetogens made acetate a rate-limiting substrate for biogas production, while inhibition of methanogenic acetoclasts lead to accumulation of acetic acid. This bimodal 'flood or famine' effect on levels of acetic acid is attributed to inhibition at different points in the biogas community.

3.4.2 NH3

The recommended ratio of carbon to nitrogen (C:N ratio) for anaerobic digestion is 20-30: C:N ratios for *U. lactuca* range between 7 [Allen et al., 2014] and 14.5 [73]. C:N ratios for feedstocks in this study were 10.2 for R1 and 17.1 for R6, with improved values reflecting addition of slurry (C:N ratio often >20: [74]). Proteins contribute nearly all of the nitrogen in *U. lactuca* [75], entering solution as free ammonia or the ammonium ion. Elevated pH, temperature, and headspace partial pressure increase concentration of the uncharged free ammonia (NH3) state. At sufficiently high concentrations NH3 diffusion across cell membranes can inhibit biogas process by causing loss of cellular potassium, de-potentiating the cell membrane, and accumulating in the cytoplasm [76]. Ammonia inhibition is well documented in methanogens [41, 43, 76, 77], affecting other taxa to a greater or lesser extent. Pure cultures of methanogens remain viable at TAN levels up to 10,000mg/l but have been documented declining at a range of TAN levels between 1,700 to 6,000 mg/l

when part of a reactor community. Differential responses between hydrogenotrophic and acetoclastic methanogens are documented but contradictory**.**

Initial levels of total ammoniacal nitrogen (TAN) were similar for R1 and R6: 2,000 and 1,800 mg/l respectively. R1 levels increased to 3,000 mg/l at Week 5, fluctuated between 4,000 and 5,000 mg/l from Weeks 15 to 25 before stabilising and returning to values closer to 4,000mg/l after Week 26. Increase of R1 OLR from 1 to 1.5 kg VS m^3 d^{-1} at Week 34 saw TAN increase again, exceeding 5,000mg/l by trial completion. Higher levels of TAN in R1 are associated with a demonstrated loss of methanogens and lower methane yield. Of note, the acetoclastic Methanosarcina declined first, at a TAN concentration of ~3500mg/l: hydrogenotrophic methanogens persisted but declined between Weeks 21 and 30 (peak TAN of 5,300mg/l Week 22). No recovery in methanogens was seen when TAN receded. R6 TAN levels were lower but behaved similarly: TAN exceeded 2,000mg/l at Week 5, peaked at 3,000 mg/l at Week 25 before stabilising to ~2,000 mg/l. OLR was raised to 2.5 kg VS m^3 d^{-1} at Week 23; TAN levels rose again at Week 33, before stabilising below 3,000mg/l by trial completion. A similar trend of TAN accumulation followed by metabolism may represent acclimatisation in both reactors, despite crucial differences in 'peak' (5,300mg/l in R1; 3,000mg/l in R6) and 'acclimatised' (4-5,000 mg/l in R1; 2-3,000 mg/l in R6) levels.

3.4.3 Mineral salts:

An inhibitory role for salts has long been recognised in anaerobic digestion [69]. Cations (e.g. Na^+, Ca^{2+}, Mg^{2+}, K^+) affect biogas production in a charge-dependent manner, possibly by inhibiting a Na^+ export channel necessary for the final methanogenic reaction [78]. However, complex and proportionate mixes of cations can offset the inhibitory effects of one another [69, 79], as well as ameliorating ammonia [Sprott et al., 1986] and VFA inhibition [Rinzema et al., 1988]. Pre-trial characterisations showed slurry to have low (< 2,000mg/l) total mineral content, while fresh *U. lactuca* provided 5,220, 5,310 and 9,950mg/l of Mg^{2+}, Na^+ and Ca^{2+} respectively. Cations were not monitored directly, but through Cl^- levels it can be inferred that salt-loading was significanlty higher in R1: Cl^- concentrations passed 5,000mg/l at Weeks 7 (R1) and 10 (R6), with R1 levels peaking at 10,300 mg/l at close of trial, while R6 peaked on Week 21 with 6,760 mg/l and decreased thereafter, completing trial at 5,400mg/l. Reported inhibitory levels of Na^+ and Ca^{2+} vary, with lower estimates registering from 5,000 mg/l [40]. As *U. lactuca* contributed a variety

of salts which accumulated gradually, acclimatisation of communities in R1 and R6 is expected, with performances in this trial displaying partial inhibition and/or later onset.

3.5 Statistical Resolution and Constrained Analysis

3.5.1 Taxonomic Characteristics

Anaerobic digestion is characterised by diverse communities degrading feedstocks in a step-wise manner. In the current study, digestion of *U. lactuca* and slurry in opposing ratios encourages subsets of hydrolysers, fermenters and chemo-organo-heterotrophs, including notable halotolerant and marine taxa. To improve characterisation of the microbe communities digesting *U. lactuca* mixes, the LDA (Linear Discriminant Analysis) Effect Size package (LEfSe,[30]) was used to detect taxa characteristic of digestion at high (R6) or low rates (R1), acting as 'biomarkers' for either setup.

Taxonomic LDA statistical ouptut is presented in Supplementary Table 2.

Taxa characteristic of the R1 environment show a strong affinity for marine environments and/or halotolerance. Additionally, most were originally isolated from marine sources; three from *U. lactuca* or other seaweeds (*Maritalea, Arenibacter, Alteromonadaceae*). Several are aerobes or facultative aerobes (*Nitratireductor, Altermonadaceae*) and many show degrees of fermentative and/or acidogenic activity. The most significantly associated taxa (LDA effect ≥4**,** $\alpha \leq 0.05$) are from the *Actinobacteria* (*Micrococcales*), *Alpha-Proteobacteria* (*Devosia, Nitratireductor, Rhizobium and Rhodobacteraceae sp.*), *Beta-Proteobacteria* (*Hydrogenophaga* and *Limnohabitans*), *Bacteroidetes* (*Proteiniphilum*) and *Firmicutes* (*Alkaliphilus*, *Bacillales, Lutispora, Syntrophomonadaceae, Tepidanaerobacterales, Tissierella*) phyla. As well as known fermenters, acidogens (*Proteiniphilum, Firmicutes*) and syntrophs (*Firmicutes*), these taxa suggest diverse saccharide use, and use of alternate electron acceptors (nitrogen, sulfur) detrimental to biogas production (*Alpha-* and *Betaproteobacteria*).

Indicators of the R6 environment were more closely linked to anaerobic digestion, but retained some associations with marine and haline habitats. The most significantly associated taxa (LDA effect ≥4, $\alpha \leq 0.05$) are more commonly anaerobic and documented as hydrolysers (*Alkaliflexus, Caldilineae, Lachnospiraceae, Proteiniphilum, Ruminococcaceae*), fermenters (*Caldilineae, Desulfomicrobia*) and acetogens (*Alkaliflexus, BPC102, Caldilinea,*

Christensenellaceae, Syntrophomonas, etc.), as well as including three methanogens: the acetoclastic *Methanosarcina* and hydrogenotrophic *Methanobacterium* and *Methanobrevibacterium*. Most methanogens were not significant indicators, as abundances were similar between reactors.

3.5.2 Predicted Metabolic Characteristics

A full table of inferred metabolism along with LDA statistical outrput is available in Supplementary Table 3.

Species abundances were combined with data from the Kyoto Encyclopedia of Genes and Genomes (KEGG; release 73.1[80]) using the HUMAnN package [29] to infer metabolic capabilities for the two communities. Again, using LefSe, significantly abundant metabolic processes were then identified, providing metabolic features expected to characterise the two reactors.

Diverse carbohydrate metabolism is likely to differentiate R1, with the highest LDA effect scores (4.1 – 3.9, α=0.006) for central carbohydrate metabolism and saccharide transport. Although carbohydrates are fundamental to all metabolism, the variety of metabolic pathways represented in these categories suggest that the R1 community utilises a more opportunistic and varied range of carbon sources, with significantly elevated predictions for the Entner Doudoroff Pathway, Pentose Phosphate Pathyway and Citrate Cycle (LDA effects: 3.18 – 3.42, α<0.05). Predicted markers for R1 also include transport of putrescine and spermidine, key components[81] in the formation and regulation of biofilms (LDA effect: 3.47 – 3.71, α= 0.006 – 0.011); and Type VI secretion systems used in competition for resources (LDA effect: 3.7, α=0.034).

Metabolism of methane is a strong recurring prediction for R6 (LDA effect: 3.53 – 3.98, p=0.006) with the emphasis on methanogenesis via methanol and acetate (LDA effect: 3.64 and 3.58 respectively, α = 0.006). However, the strongest predicted characteristics of R6 metabolism are transport of cobalt (LDA effect: 4, α=0.006) and nickel (LDA effect: 4.2, α=0.006). Cobalt is required for methylotrophic methanogenesis [82], whilst nickel is central to the final step of all methanogenic routes [83, 84]. A weight of literature indicates methane production increases substantially when nickel and cobalt are added [85-87].

Increased archaeal ribosome metabolism (LDA effect: 3.64, α=0.006) and reduction of quinones in energy metabolism (LDA effect: 3.52, α<0.02) are also predicted to differentiate metabolism in R6 from R1.

3.5.3 Constrained Correlation Analysis

Constrained Correlation Analysis (CCA) measured the relationships between community structure and time-points, and metabolism and time-points, in the context of specified ('constraining') process variables. Several process variables were intercorrelated, describing the same source of variation in the dataset. In particular, levels of TAN, alkalinity and total dissolve solids (TDS) were strongly intercorrelated (R=0.80 – 0.95), as were B_{ix}, biogas output and specific methane yield (SMY) (R= 0.81 – 0.97); and chloride, total salinity, chemical oxygen demand (COD), volatile solids (VS) and duration of trial (R=0.81 – 0.97). As such, three governing processes described the reactor communities: inhibitor accumulation, biogas activity, and duration of trial.

5.3.1 CCA of Communtiy Abundances

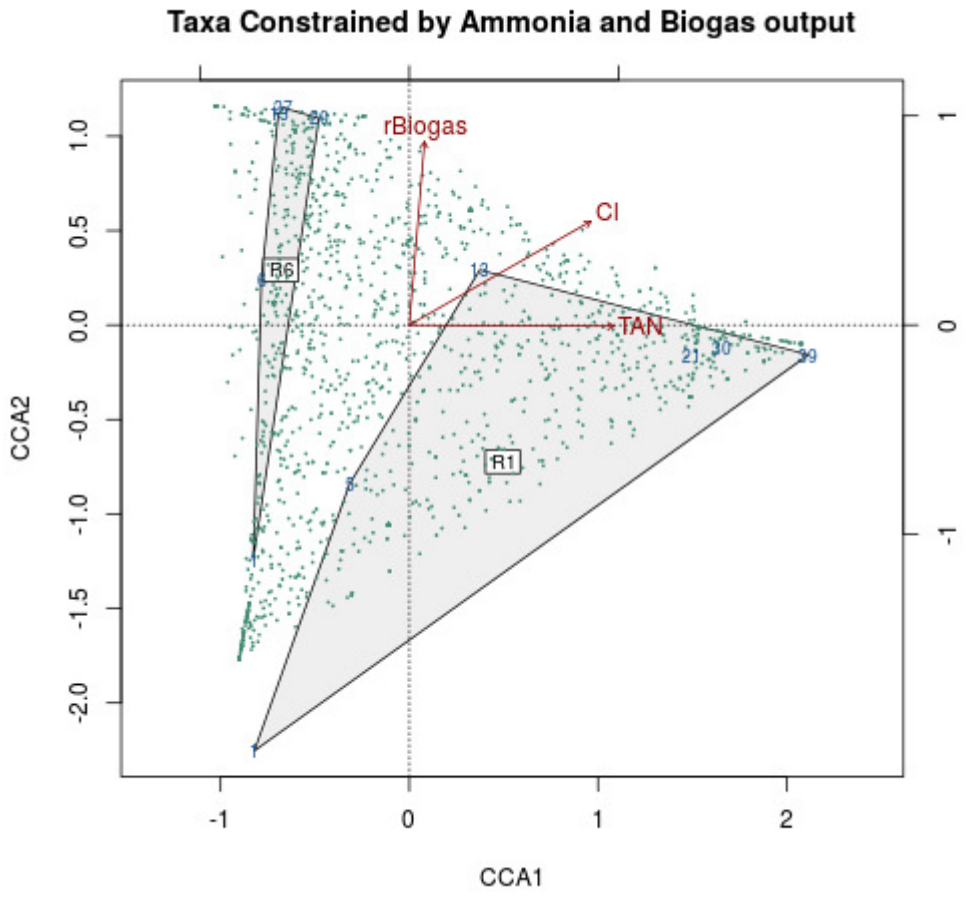


Figure 2: Constrained Canonical Analysis plot, illustrating differentiation of the reactor communities due to the strongest factors: ammonia (TAN), biogas yield (rBiogas), and chloride concentration (Cl).

CCA showed that levels of ammonia (specifically total ammoniacal nitrogen, TAN), chloride and raw biogas output had the strongest correlations with community makeup, with the most significant and non-redundant effects on taxonomic abundances (R=0.50, significance after 999 permutations: , VIF< 8). Together, these 3 parameters described 49.8% of variation in community abundance and allowed the major interactions defining these communities to be visualised via biplot (fig. ORDI). Ordination under these contraints shows clear segregation between the two samples. Although initial community and process similarities cause Week1 samples to cluster, R1 and R6 time-points diverged along X and Y axes respectively, with clustering of later time-points showing established communities. Despite low OLR in R1, accumulation of TAN exceeded 5,000mg/l in later time-points, and

was the most strongly correlated inhibitor of biogas process (X axis). R6 time-points show negligible interaction with ammonia levels or overloading along the X axis, indicating TAN levels up to 3000mg/l did not inhibit the R6 community. R6 instead correlates strongly with increasing biogas output, seen as distribution along the Y axis. Note that Week 13 of R1 correlated with biogas production (movement on Y axis) before R1 reached higher ammonia levels. Rising chloride concentrations correlate with both reactor setups, relating trial duration and a gradual accumulation of dissolved content. A stronger association with R1 is explained through a higher *U. lactuca laoding rates*, with no obvious inhibitory effects.

Correlations with OLR, Alk and TAN were up to x1.5 times stronger for Archaea, while pH, salinity, COD, VS% and Cl correlated to Bacteria more strongly (x1.5 – 2 times). Curiously, the bacterial community was more than twice as correlated to B_{ix} as the archaeal community (R: 0.21 v 0.12), reflecting the more patterned bacterial community involved in methanogenesis and/or relatively consistent methanogen components. A negative correlation between biogas output and biodiversity indices (R>-0.6) can be explained through 'niche exclusion', where taxa unsuited to anaerobic digestion are outcompeted by better-equipped taxa, causing a decrease in diversity. Excluded taxa are known to persist at low abundances and play important roles during reactor transitions [33, 88].

3.5.3.2 CCA of Predicted Metabolic Activitie

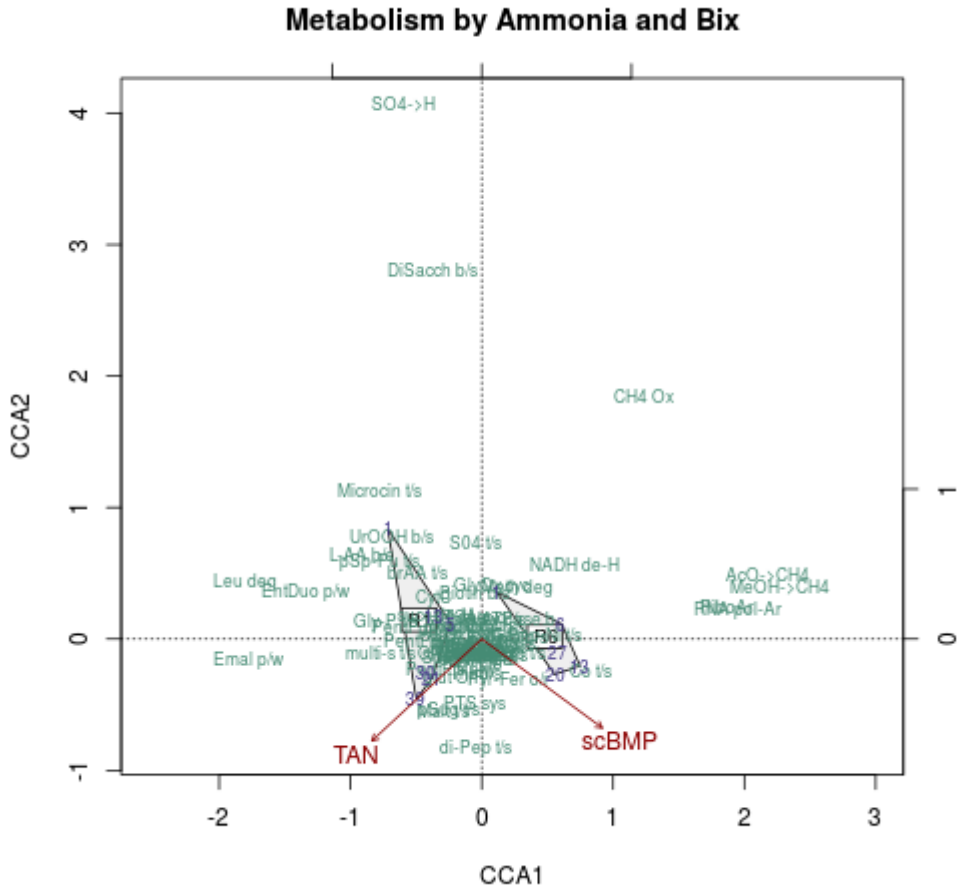


Figure 3: Constrained Canonical Analysis plot of samples and predicted metabolic abundances, diffrentiated by the strongest factors, ammonia (TAN) and specific biomethane potential (scBMP).

CCA using predicted metabolic abundances showed strongest non-redundant correlations with TAN and B_{ix} (R=0.50, VIF=1 significant after 999 permutations). Ordination under these constraints showed energy metabolism to best differentiate samples along the X axis, with methanogenesis predictions for R6 contrasting with predicted alternative anaerobic metabolic pathways (Entner-Duodoroff, ethylmalonyl, and pentose-phosphate pathways) and carbon uptake pathways (multi-saccharide transport system) in R1. Samples differentiated along the Y axis as reactors matured, with earlier metabolic diversity (e.g. sulphate reduction and transport, methane oxidation) absent in later samples as overall diversity decreased. Methanogenesis (acetate and methanol metabolism) and archaeal

translation and transcription clearly associated wit hR6, while negatively correlating with TAN levels. Predictions for Nickel and Cobalt transport also associate with R6 time-points.

4.0 Summary

U. lactuca presents a self-renewing resource with a highly accessible organic fraction, low or lacking in recalcitrant polymers (cellulose, lignin, alginates) [73, 89, 90]. However, anaerobic digestion of *U. lactuca* is prone to a high VFA yield [73, 91]. In this study, VFA accumulation was the result of several cumulative underlying inhibitors of the microbial community, correlating with trial duration. CCA showed total ammoniacal nitrogen (TAN) concentration to be the most influential variable affecting biogas community structure, causing a near-absence of methanogens and subsequently leading to high concentrations of acetic acid in R1. Biogas production was the second best descriptor of community structure, with classical biogas taxa detected in both reactors. Hydrolytic populations fluctuated in response to OLR, with rapid generation of VFAs (in particular acetic acid) by multiple acidogenic and acetogenic populations digesting the *U. lactuca* portion of feedstock.

Methanosarcina was the primary methanogen, enabling successful biogas operation in R6 through continuous degradation of acetic acid. *Methanosarcina* has been previously described as a more resilient methanogen [37, 42, 92], however this study saw early loss of all acetoclasts in R1, presumably due to high ammonia levels. *Methanosarcina* populations in R1 collapsed between Weeks 5 and 13 as TAN exceeded 3,500mg/l, while hydrogenotrophic archaea declined after TAN exceeded 5,000: these are posited as inhibitory thresholds for methanogens in *U. lactuca*-slurry digestion as operated in [Allen et al., 2014]. Sequential inhibition of acetoclastic followed by hydrogenothrpic methanogens reduced R1's ability to metabolise acetic acid directly (via acetoclasty) and indirectly (enabling oxidation by bacteria), causing accumulation of acetic acid. Methanogenesis also maintains a raised oxidation-reduction potential (ORP) in the reactor by sequestering highly-reduced carbon into the gaseous phase as methane. Reduced methanogenic activity encouraged use of alternate electron acceptors (e.g. nitrates), favouring populations of marine, halotolerant and non-obligate anaerobes known for diverse metabolism of proteins, amino acids and nitrogen (largely *Alphaproteobacteria* and *Gammaproteobacteria,* strong biomarkers for R1). Reduced methanogenic activity would

also reduce dependence on uptake of cobalt and nickel, a predictable biomarker for R6. Contrasting the overloading of R1, acetic acid availability was limiting in R6: good growth conditions and a lack of inhibition are demonstrated for *Methanosarcina* by increased abundance at raised VFA levels of 1,860 mg/l at R6 Week 13. The gradual reduction in biogas yield in R6 [Allen et al., 2014] is attributed to a decrease in acidogenic activity, possibly due to inhibition via ammonia accumulation. Strong metabolic predictions for cobalt and nickel uptake in R6 re-enforce the importance of trace elements in biogas reactors

Inhibited acetogenic metabolism of iso-valeric acid at R1 Week 5 lead to a deficit of acetic acid. Catabolism of iso-valeric acid to propionic and acetic acid during reactor inception mirrors abundance of an uncharacterised *Sphingobacterales* genus (family WCHB1-69, aquifer remediation isolate) in both R1 and R6. In R1, an unknown factor suppressed catabolism of iso-valeric acid at Week 5 leading to subsequent overload, which may be linked to the disappearance of WCHB1-69 from that reactor. *Psychrobacter* was seen to proliferate in both reactors in response to abundant propionic acid and decline when it was limiting, implicating a role in propionic acid metabolism. Although propionic acid has been demonstrated as non-inhibitory to methanogens [72] accumulation is likely to hinder equilibria of substrate degradation. Late proliferation of the *Clostridiales* taxa *Hydrogenispora* (previously isolate OPB54) suggests a lack of inhibition at high levels of ammonia, salt or acetate, and involvement in VFA metabolism. Consistently elevated *Proteiniphilum* abundances in R1 reflect the high protein content of *U. lactuca*, with a likely role in ammonia release.

5.0 Conclusion

Anaerobic digestion of *U. lactuca* indirectly inhibits acetogenic and methanogenic processes, with ammonia showing strongest causative correlation. At high *U. lactuca* volumes, decreasing OLR was not sufficient to recover the acetoclastic methanogens required to remove acetic acid and prevent overloading, nor retain hydrogenotrophic methanogens. At low *U. lactuca* volumes, inhibited acetogenesis caused *Methanosarcina* populations yields to shrink, affecting biogas yield. Chloride accumulated but did not clearly correlate with inhibition. Effects of *U. lactuca laoding rates* significantly affected

community makeup, with higher *U. lactuca* loading characterised by diverse, facultatively anaerobic, marine and halotolerant taxa, lack of methanogens, and a predicted reliance on alternative carbon metabolism.

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Appendix C: Assessing biomethane production from the organic fraction of municipal solid waste in batch and continuous operation

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Assessing the variability in biomethane production from the organic fraction of municipal solid waste in batch and continuous operation

James D. Browne [a], Eoin Allen [b,c], Jerry D. Murphy [b,c,*]

[a] Agrifood and Biosciences Institute, Hillsborough, Northern Ireland, UK
[b] School of Engineering, University College Cork, Ireland
[c] Environmental Research Institute, University College Cork, Ireland

HIGHLIGHTS

- Eight organic waste streams were examined for biochemical methane potential (BMP).
- Commercial food waste produced 560 mL CH_4 g VS^{-1} in continuous trials.
- Raising the loading rate to 4 kg VS m^{-3} day^{-1} led to a reduction in methane yield.
- The low C:N ratio led to levels of 7000 mg N L^{-1} at high loading rates.
- Free ammonia levels of 1000 mg N L^{-1} were encountered at a pH of 8.

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ABSTRACT

This paper examines the variability in biomethane potential from the organic fraction of municipal solid waste depending on source of origin. Eight organic waste streams were examined for biochemical methane potential (BMP). Specific methane yields of between 274 and 368 mL CH_4 g VS^{-1} for household waste and 491–535 mL CH_4 g VS^{-1} for commercial waste were achieved. Inclusion of garden waste reduced methane yields. A continuous trial on commercial food waste produced an average of 560 ± 29 mL CH_4 g VS^{-1} at a moderate organic loading rate (OLR) of 2 kg VS m^{-3} day^{-1} with a hydraulic retention time (HRT) of 30 days. Raising the OLR to 4 kg VS m^{-3} day^{-1} led to a reduction in specific methane yield. The low carbon to nitrogen (C:N) ratio of commercial food waste (14.4) led to process instability due to total ammonia nitrogen levels in excess of 7000 mg L^{-1} towards the end of the trial.
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1. Introduction

Many municipal organic waste streams are dominated by food waste, particularly catering premises such as restaurants, hotels and office canteens. In the Republic of Ireland, food waste accounts for approximately 25% of domestic household and 40% of commercial waste [1]. The organic fraction of municipal solid waste (OFMSW) is a term often used in Ireland and the UK to describe food and garden waste in household and commercial waste streams. In many EU countries OFMSW is simply referred to as biowaste. National and European legislation places restrictions on the amount of OFMSW which may be sent to landfill [2] while the current EU Waste Framework Directive [3] seeks to encourage waste separation at source and biological treatment of OFMSW. Anaerobic digestion (AD) is a mature biotechnology which can maximise the value of organic waste. The methane component of biogas, produced from the anaerobic process, is a valuable renewable gaseous fuel. The digestate from the biogas process may be used as a mineral rich fertilizer and reduce synthetic fertilizer consumption [4].

One of the objectives of this paper is to outline the variability in methane yields from OFMSW depending on the waste source and type of collection. A selection of organic waste samples from domestic, commercial and food processing waste streams were investigated. The biochemical methane potential (BMP) test was used to assess the methane yield for each substrate. In addition to the BMP tests, a continuous AD trial was carried out for 25 weeks using commercial canteen food waste as substrate to examine the effects of organic loading rate and hydraulic retention time on the specific methane yield.

* Corresponding author at: School of Engineering, University College Cork, Ireland. Tel.: +353 21 490 2286.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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Assessing biomethane production from the organic fraction of municipal solid waste in batch and continuous operation

James Brown, Eoin Allen[a], Jerry D. Murphy[a,b]

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] School of Engineering, University College Cork, Cork, Ireland

Abstract:

This paper examines the variability in biomethane potential from the organic fraction of municipal solid waste (OFMSW) depending on source of origin. Eight organic waste streams were examined for biochemical methane potential (BMP). Specific methane yields of between 274-368 L CH_4 kg^{-1} VS for household waste and 491-535 L CH_4 kg^{-1} VS for commercial waste were achieved. Inclusion of garden waste reduced methane yields. A semi continuous trial on commercial food waste produced an average of 560 ± 29 L CH_4 kg^{-1} VS at a moderate organic loading rate (OLR) of 2 kg VS m^{-3} d^{-1} with a hydraulic retention time (HRT) of 30 days. Raising the OLR to 4 kg VS m^{-3} d^{-1} led to a reduction in specific methane yield. The low carbon to nitrogen (C:N) ratio of commercial food waste (14.4) led to process instability due to levels in excess of 7000 mg l^{-1} towards the end of the trial.
Keywords: anaerobic digestion; food waste; BMP; CSTR

1 Introduction

Food waste accounts for approximately 25% of domestic household waste in Ireland [1]. Many commercial organic waste streams are also dominated by food waste, particularly catering premises such as restaurants, hotels and office canteens. National and European legislation place restrictions on the amount of organic waste which may be sent to landfill [2]. The current EU Waste Framework Directive [3] seeks to encourage waste separation at source and biological treatment of organic waste. Anaerobic digestion is a vector which can maximise the value of organic waste. The methane component of biogas, produced from the anaerobic process, is a valuable renewable gaseous fuel. The digestate from the biogas process may be used as a mineral rich fertilizer and reduce synthetic fertilizer consumption [4]. This paper seeks to outline the variability in methane yields from OFMSW depending on the waste source and type of collection. A selection of organic waste samples from domestic, commercial and food processing waste streams were investigated. The biochemical methane potential (BMP) test was used to assess the methane yield for each substrate. Based on the results of the BMP test the waste stream with the highest BMP was chosen as the feedstock for a semi continuous anaerobic digestion trial. This trial was used to assess the long term process stability at increasing organic loading rates.

2 Materials and methods

2.1 Collection, preparation and characterisation of waste samples Samples were collected in a large centralised facility (Acorn Recycling Ltd.) licensed to treat 45,000 tonnes per annum of organic municipal waste (referred to as brown bin waste in Ireland). This facility treats a wide range of municipal organic waste streams from across the province of Munster in Ireland (population circa 1.25 million people). As shown in Figure 8.1, a total of 8 different waste streams were sampled; 4 household, 2 commercial and 2 food processing streams. Each sample consisted of approximately 10 kg of material sampled across a large bulk quantity of each waste stream. The German VDI guidelines were followed on sampling solid material [5]. The samples were screened for non organic material and were then passed through a Buffalo food mincer to a particle size of less than 5mm. All samples were stored in a freezer at -20°C until required as previously described by [6]. A proximate and

elemental analysis was carried out in triplicate on samples from each waste stream as shown in Table 8.1.

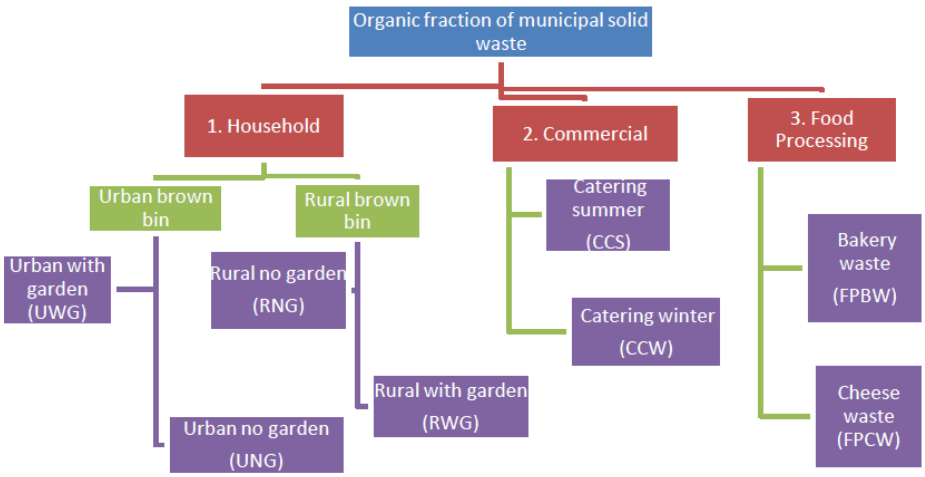


Figure 1 Illustration of samples taken from the organic fraction of municipal solid waste (with and without garden waste)

Table 1 Characterisation of OFMSW samples

Samples	Total solids (%)	Volatile solids (% TS)	Total carbon (%TS)	Total hydrogen (%TS)	Total nitrogen (%TS)	C:N
Household brown bin						
Rural with garden (RWG)	33.4 (0.4)	82.3 (0.2)	43.3 (0.2)	5.9 (0.1)	2.7 (0.1)	16
Rural no garden (RNG)	30.6 (3.3)	88.4 (0.4)	44.9 (0.2)	6.6 (0.1)	3.1 (0.2)	14.5
Urban with garden (UWG)	25.66 (0.1)	73.6 (0.4)	41.3 (0.2)	5.2 (0.1)	2.6 (0.4)	16
Urban no garden (UNG)	31.0 (2.4)	93.8 (0.3)	46.5 (0.2)	7.3 (0.1)	3.7 (0.1)	12.6
Commercial waste						
Commercial canteen summer (CCS)	32.8 (0.1)	92.6 (0.3)	49.0 (0.6)	7.0 (0.1)	3.4 (0.2)	14.4
Commercial canteen winter (CCW)	23.8 (0.5)	90.0 (0.3)	48.2 (0.2)	7.0 (0.04)	3.6 (0.2)	13.4
Food processing						
Food processing bakery waste (FPBW)	45.7 (0.4)	91.9 (0.6)	52.7 (0.2)	8.2 (0.05)	2.8 (0.2)	18.8
Food processing cheese waste (FPCW)	15.9 (0.1)	55.6 (0.3)	24.9 (0.1)	4.1 (0.03)	4.6 (0.03)	5.4

All values are presented as mean and (standard deviation)

2.2 BMP tests

The apparatus used to conduct the BMP tests was the Automatic Methane Potential Test System II (Bioprocess Control Sweden AB). This laboratory instrument is specially designed for determination of the BMP of a substrate. The AMPTS II system consists of three major parts as follows:

- A temperature controlled water bath with 15 bottle reactors of 500 ml volume, each equipped with a mixer that can be run in either continuous or intermittent mode.
- A carbon dioxide fixing unit with an alkaline solution (3N sodium hydroxide) that absorbs the carbon dioxide and hydrogen sulphide produced during the anaerobic digestion process.
- A gas measuring unit consisting of 15 parallel operating cells, where the gas is measured through water displacement. When approximately 10 ml of gas has been accumulated each cell opens and releases the gas. For each opening, the time, temperature and pressure are registered and stored locally in an embedded Central Processing Unit (CPU). Based on these measurements, normalised (0°C, 1 atm and dry gas) accumulated gas production and gas flow rate are calculated.

The BMP tests were performed with a working volume of 400 ml. The ratio of inoculum to substrate was chosen to be 2:1 on a volatile solids (VS) basis. The inoculum to substrate ratio is a critical parameter in conducting a BMP test according to the Anaerobic Digestion Specialist Group of the International Water Association [7]. A ratio of 2:1 or greater of inoculum to substrate on a VS basis is recommended for BMP trials to limit any inhibitory effects due to the chemical composition of the substrate such as inhibition associated with accumulation of ammonia and volatile fatty acids (VFA) [8]. All samples were tested for BMP in triplicate. A BMP test of the inoculum alone (referred to as a blank) was conducted in triplicate. The average methane yield from the blanks was subtracted from the samples of OFMSW with inoculum to accurately assess the BMP yields from the samples only. A triplicate BMP test was also carried out on cellulose for quality control as the maximum BMP from cellulose is known and can be compared with the BMP yield. The percentage volatile solids destroyed, during the batch process was calculated as follows:

$$\% \text{ VS destruction} = 100 \cdot (1 - (VS_f - VS_{fb})/(VS_i - VS_{ib}). \quad \text{(Eq. 1)}$$

Where; VS_i is the amount of total input VS (g),

VS_f is the amount of total VS at the end of the BMP test (g),

VS_{ib} is the amount of VS (g) in the inoculum (blank) at the beginning of the BMP test,

VS_{fb} is the amount of VS (g) in the inoculum (blank) at the end of the test.

The Buswell equation was used to calculate the theoretical maximum methane potential [9].

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \qquad \text{(Eq. 2)}$$

Where;

n is the number of atoms of carbon;

a is the number of atoms of hydrogen;

b is the number of atoms of oxygen;

The biodegradability index is the ratio of the measured BMP divided by the theoretical methane yield according to the Buswell equation and is used to assess the level of biodegradability of a substrate.

2.3 Source and characteristics of inoculum for BMP tests

The inoculum for the BMP tests was obtained from a lab scale 300L digester treating mostly cattle slurry and a small portion of food waste operating at mesophilic temperatures (35 °C). After an incubation period of one week the inoculum had a pH of 7.9, total solids (TS) of 34.2 gVS kg^{-1} and volatile solids (VS) content of 21.4 gVS kg^{-1} after passing through a 2mm sieve. Inoculum from both rounds was tested using cellulose as a standard control substrate ($C_{12}H_{20}O_{10}$). The maximum theoretical methane yield from cellulose according to the Buswell equation is 415 L CH_4 kg VS^{-1}. The specific methane yield produced from the cellulose was 371±4 LCH_4 kgVS^{-1}. This is almost 90% of the theoretical maximum indicating that a healthy inoculum.

2.4 Kinetic modelling of BMP tests

Two first order kinetic models were used to fit the cumulative methane production data from the BMP tests. Assuming first-order kinetics for the hydrolysis of particulate organic matter, the cumulative methane production can be described by means of the following equation:

$$Y(t) = Y_m \cdot \left(1 - \exp^{(-kt)}\right) \qquad \text{(Eq. 3)}$$

Where,

Y(t) is the cumulative methane yield at digestion time t days (L CH_4 kg^{-1} VS),

Y_m is methane potential of the substrate (L CH_4 kg^{-1} VS),

k is methane production rate constant (first order disintegration rate constant) (d^{-1}),

t is the time (days).

The duration of the lag phase is also an important factor in determining the efficiency of anaerobic digestion. The lag phase (k) can be calculated with the modified Gompertz model as described by [10] as follows:

$$M(t) = P \cdot \exp\{-\exp[\frac{R_{max} \cdot e}{P}(\Delta - t)] + 1\} \qquad \text{(Eq. 4)}$$

Where,

M is the cumulative methane yield at a given time (L CH_4 g VS^{-1}),

P is the max methane potential (L CH_4 kg^{-1} VS) from the BMP test,

R_{max} is the maximum methane production rate (L CH_4 kg^{-1} VS d^{-1}),

e is the mathematical constant = 2.7183,

λ is the lag phase for methane production to begin (days),

t is the time (days).

A nonlinear least-square regression analysis was performed using Excel to determine λ, R_{max}, k, and the predicted methane yield. The predicted methane yield obtained from the

regression analysis was plotted with the measured methane yield. The statistical indicators, Correlation coefficient (R_2) and root mean square error (RMSE) were calculated to assess the goodness of fit [11].

2.5 Statistical analysis

The significance of differences in the average methane yields was determined by using single factor Analysis of Variance (ANOVA) in Excel software 2007. If the calculated F value was higher than the tabulated F value, the minimum significant difference (MSD) was calculated to judge whether two or more averages were significantly different or not (Tuckey test). MSD was calculated at P = 0.05 ($MSD_{0.05}$) [12].

2.6 Semi-continuous trial

The semi continuous trial was carried out in a continuously stirred tank reactor (CSTR) with a total volume of 5L (working volume of 4L) and ran for a period of 25 weeks. The reactor was maintained at a temperature of 37 + 1^{o}C and was continuously stirred at a rate of 100 rpm. The reactor was constructed out of thick walled plastic with a vertically mounted stirring mechanism as shown in Figure 8.2. The reactor was placed inside a coiled copper pipe frame which was heated by a thermo-circulator. Biogas flow was measured using a tipping bucket mechanism whereby the number of tips was recorded and multiplied by the calibrated gas volume of the tipping bucket (78 ml per tip). Biogas was sampled downstream of the gas flow tipping meter in 1L Tedlar gas bags and analysed for methane, carbon dioxide and hydrogen sulphide.

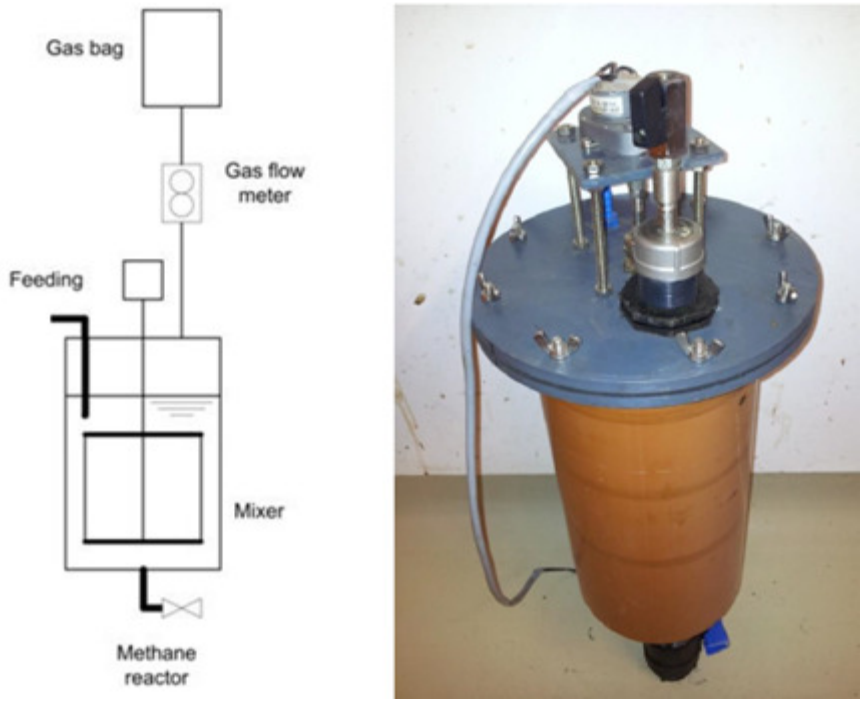


Figure 2 Continuously stirrer tank reactor (5L) used for semi continuous trial

2.7 Analytical methods Total solids and volatile solids were determined gravimetrically following the standard methods (APHA, 2005). The biogas composition in the semi continuous trial was measured by infra red gas analyser (Status Scientific Control I-R biogas analyzer). The instrument was calibrated before the commencement of the trial and showed an accuracy of ± 1% when tested weekly on a standard mixture of 65% methane 35% carbon dioxide provided by BOC specialty gases. All methane yields were adjusted to standard temperature of 273 K and 1 atmosphere (1013 hPa). Volatile organic acids and total alkalinity were measured using the Nordmann titration method (1978) using 0.1N sulphuric acid and a Titronic Universal Titrator. The pH of the digestate was measure daily using a Jenway 3510 pH meter. Total ammonia was measured using the Hach NH_3-N vials and spectrophotometer DR 3900.

3 Results and Discussion

3.1 Results from the BMP tests

The results from the BMP test are shown in Table 8.2. Household waste streams ranged in methane potential from 274 – 368 L CH_4 kg^{-1} VS, commercial waste samples ranged from 491 – 535 L CH_4 kg^{-1} VS while food processing samples exhibit the largest difference between samples (529 L CH_4 kg^{-1} VS for bakery waste and only 188 L CH_4 kg^{-1} VS for cheese waste activated sludge). The commercial waste samples exhibited a much higher degree of biodegradability and volatile solids reduction than the household waste samples. In particular the household waste streams which consisted of mostly garden waste had a much lower biodegradability index than waste streams without garden waste. The BMP result for the cheese waste was much lower than expected. In a previous study by the authors [6] a sample of cheese processing treatment sludge from a different location yielded 461 L CH_4 kg^{-1} VS. This demonstrates that the type of existing biological waste treatment processes at dairy plants can produce waste sludge with hugely different biomethane potential. A one way Anova analysis suggests a statistical difference between the mean biomethane potential results depending on the source of OFMSW ($F_{7,16} = 332.6$, $P < 0.01$.Where there are 7 degrees of freedom between samples and 16 degrees of freedom within samples. Multiple comparisons using the Tuckey test ($MSD_{0.05} = 34.4$ mL gVS^{-1}, $P < 0.05$) revealed that there is a significant difference in biomethane potential between almost 90% of the waste samples depending on source, however there were some notable exceptions. In the household waste stream there was no significant difference between urban and rural samples that came from a similar collection system ($P > 0.05$). However there was a significant difference in methane potential depending whether garden waste was included or not. For example samples without garden waste gave higher methane yields than samples which consisted mostly of garden waste. Canteen waste samples gave significantly higher BMP yields than from household waste streams. However there was a significant difference between canteen waste samples depending on the season. Samples taken from the same waste collection run in summer (June 2012) gave 9% higher BMP yields than winter (December 2012). In the food processing stream bakery waste samples gave vastly greater methane yields (529 L CH_4 kg^{-1} VS) than from cheese waste activated sludge (188.5 L CH_4 kg^{-1} VS). Interestingly the bakery waste sample did not

differ significantly from the canteen waste (CCS). The results from the commercial waste samples are similar to previously reported BMP yields from canteen food waste (480-530 L CH_4 $kgVS^{-1}$) [13].

Table 2 Spectrum of food waste – samples collected

Source	BMP (30 days) (ml CH_4 g VS^{-1})	Theoretical BMP Buswell equation	Biodegradability Index	Volatile solids destruction as measured (% VS)
Rural with garden (RWG)	274.1 (4.6)	577	0.48	47
Rural no garden (RNG)	367.8 (6.2)	566	0.65	69
Urban with garden (UWG)	296.7 (6.1)	625	0.47	51
Urban no garden (UNG)	343.7 (2.7)	564	0.61	60
Commercial canteen summer (CCS)	534.5 (5.0)	620	0.86	81
Commercial canteen winter (CCW)	490.9 (4.8)	620	0.79	80
Food processing bakery waste (FPBW)	529.2 (25.4)	696	0.76	81
Food processing cheese waste (FPCW)	188.5 (1.2)	530	0.36	42

3.2 Kinetic study results

The results of the kinetics analysis using the first order kinetic model and the modified Gompertz model are summarised in Table 8.3 (a) and (b) respectively. The first order kinetic model gave k values ranging from 0.12 – 0.17 d^{-1} for household samples, 0.07 - 0.09 d^{-1} for commercial samples and 0.08 - 0.13 d^{-1} for food processing samples. The commercial food waste samples had higher percentages of proteins and lipids which take longer to

digest than carbohydrates therefore resulting in lower k values [14]. The modified Gompertz model showed a lag time of 1.2 and 3 days for all samples tested. The time taken to reach 90% of the maximum BMP value was shown to range from 9 – 15 days indicating that all OFMSW substrates were readily degradable. Both models exhibited a good fit when plotted against the measured data with the coefficient of determination (R_2) ranging from 0.93 -0.95 for the first order model and 0.99 for the Gompertz model. The RMSE ranged from 10.7 – 48.8 L CH_4 kg^{-1} VS for the first order model while the Gompertz model gave lower values of over 0.7 – 9.9 L CH_4 kg^{-1} VS. Both models can be used to predict the maximum methane potential. The modified Gompertz model gave slightly lower predicted maximum BMP yields than the measured data ranging from -0.8 to -9.3% while the first order model generally gave higher predicted methane yields than measured ranging from -1.8 to +19.2%. In 87.5% of cases the model Gompertz gave a more accurate predicted max biomethane yield than the first order equation. Based on the statistical indicators (RMSE and R_2) the modified Gompertz model was found to demonstrate the best fit for the samples tested. The cumulative methane yields of the BMP tests are shown in Figure 8.3. The first order kinetic model fits are shown in dashed curves while the modified Gompertz model fits are shown in unbroken curves.

Table 3 (a) Results of BMP kinetic analysis using the first order kinetic equation

Sample	BMP measured (mL CH_4 gVS^{-1})	BMP predicted (mL CH_4 gVS^{-1})	Difference (%)	RMSE (mL CH_4 gVS^{-1})	R^2	k (day^{-1})
Household waste						
RWG	274.1 (4.6)	292	+ 6.5	21.6	0.93	0.12
RNG	367.8 (6.2)	388	+ 5.5	28.7	0.93	0.14
UWG	296.7 (6.1)	302	+ 1.8	18.0	0.95	0.17
UNG	343.7 (2.7)	369	+ 7.4	27.2	0.93	0.12
Commercial waste						
CCS	534.5 (5.0)	603	+12.8	40.2	0.94	0.09
CCW	490.9 (4.8)	585	+ 19.2	36.7	0.95	0.07
Food processing waste						
FPBW	529.2 (25.4)	623	+ 17.7	48.8	0.92	0.08
FPCW	188.5 (1.2)	185	- 1.8	10.7	0.95	0.13

Table 8.3(b) Results of BMP kinetic analysis using the modified Gompertz equation

Sample	BMP measured (mL CH_4 gVS^{-1})	BMP predicted (mL CH_4 gVS^{-1})	Difference (%)	R^2	RMSE (mL CH_4 gVS^{-1})	Lag phase (days)	T_{90} (days)
Household waste							
RWG	274.1 (4.6)	268	- 2.2	0.99	2.9	2.2	11
RNG	367.8 (6.2)	363	- 1.3	0.99	4.1	2.0	10.2
UWG	296.7 (6.1)	288	- 2.9	0.99	0.8	1.3	8.7
UNG	343.7 (2.7)	338	- 1.7	0.99	3.3	2.2	11.3
Commercial waste							
CCS	534.5 (5.0)	530	- 0.8	0.99	3.4	2.3	13.4
CCW	490.9 (4.8)	484	- 1.4	0.99	0.7	2.5	15.3
Food processing waste							
FPBW	529.2 (25.4)	528	- 0.2	0.99	9.9	3.0	14.3
FPCW	188.5 (1.2)	171	- 9.3	0.97	0.7	1.2	10.8

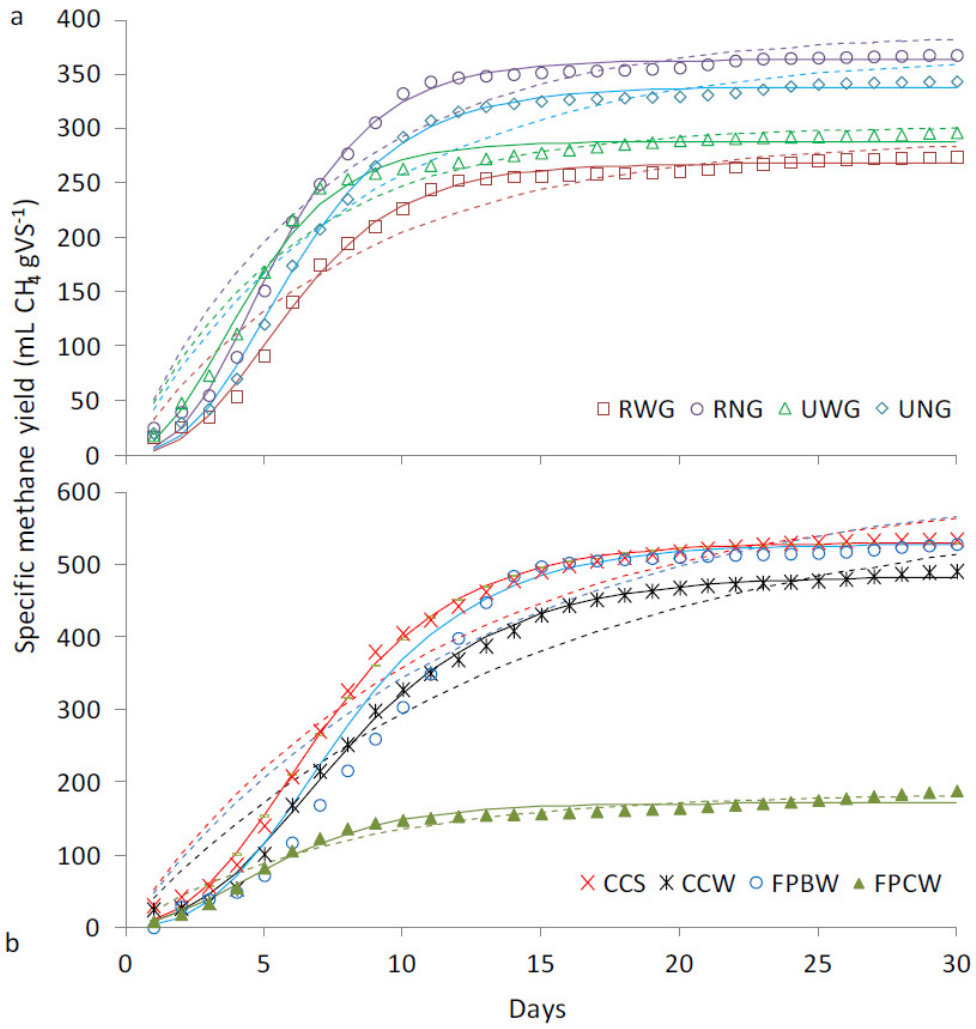


Figure 3 BMP cumulative methane yields for (a) household samples and (b) commercial & processing samples

4 Results from semi continuous trial

4.1 Specific methane yields in period 1

The semi continuous trial was operated for 176 days using commercial canteen food waste from the same collection as sample CCS in the BMP trials. This waste stream was chosen as a substrate for the semi-continuous trial because the same material had been used in separate AD trials conducted by the authors [15]. The semi-continuous system was started at a moderate organic loading rate (OLR) of 2 kg VS

m^{-3} d^{-1}. The hydraulic retention time (HRT) was initially set at 30 days. This was achieved by adding a portion of digestate back in with the input feed keeping the total solids content of the input feed to 10% which facilitated easy stirring of digester contents. The reactor was maintained at this OLR for 3 HRTs (period 1). The first HRT incorporated the start-up and acclimatisation period. By the end of the first HRT the system had reached a steady state of methane production. Methane yields from the second and third HRT were used to calculate average specific methane yield for period 1 (OLR of 2 kg VS m^{-3} d^{-1}) which was 560.1 ± 29.3 L CH_4 kg^{-1} VS. The standard deviation in the second and third HRT was only 5% of the total yield and clearly showed that the reactor was in steady state. The weekly average specific methane yield is shown in Figure 8.4 (a). The daily percentage methane in the biogas is shown in Figure 8.4(b). In the start-up period the percentage methane increased from 40.4% to 60% over the first 30 days with the weighted average methane percentage in the biogas remaining at 60 ± 1.3 % for period 1.

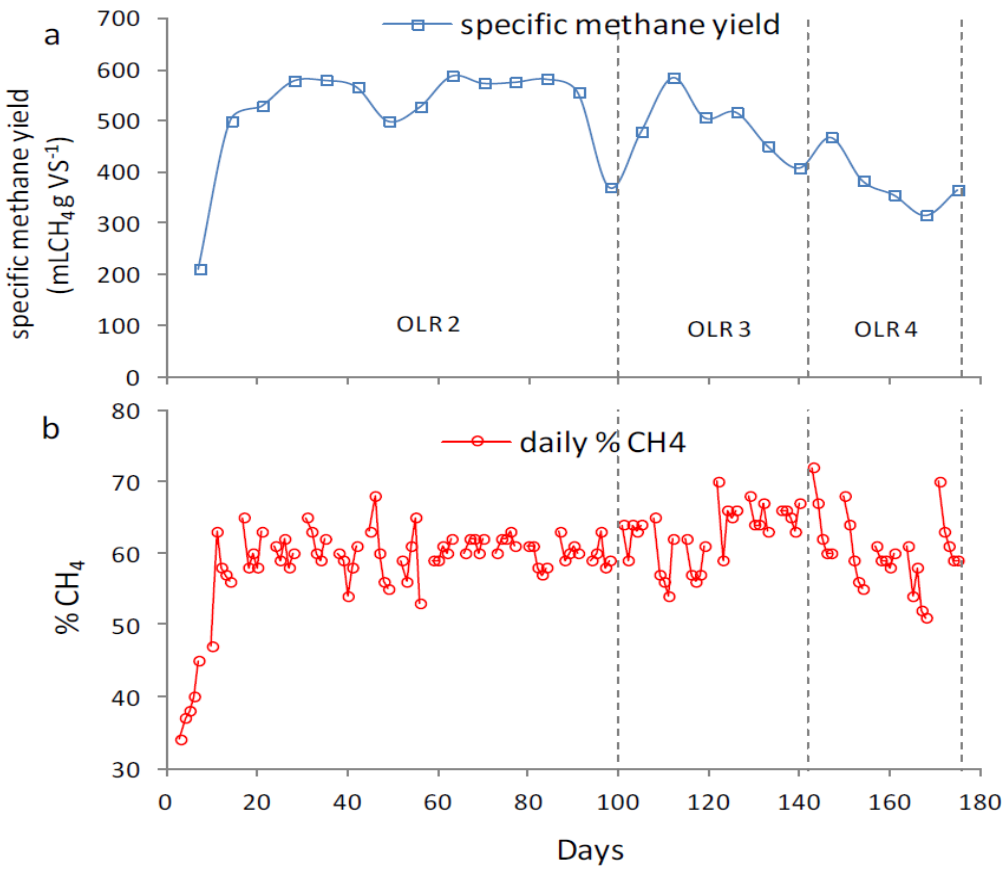


Figure 4 (a) Weekly average specific methane yield and (b) daily methane percentage

4.2 Specific methane yields in period 2

After completing 3 HRTs at the initial feeding rate, the OLR was increased to 3 kg VS m^{-3} d^{-1} at day 99. By increasing the OLR to 3 the HRT was reduced to 21 days as the solids content of the input material was kept at 10% TS by recirculation of an increased amount of digestate. The OLR was maintained at 3 kg VS m^{-3} d^{-1} for 2 HRTs (42 days). The average SMY for period 2 was 484 ± 72.0 L CH_4 g VS^{-1}. This is a reduction of about 13% from the previous SMY in period 1. The standard deviation in period 2 is approximately 15% of the average SMY and shows that there was greater fluctuation in daily gas production at the higher OLR of 3 kg VS m^{-3} d^{-1}. The weighted average methane content in the biogas increased to 61.5 ± 2.8 % in period 2.

4.3 Specific methane yields in period 3

On day 142 the OLR was further increased to 4 kg VS m^{-3} d^{-1} which resulted in a reduced HRT of 17 days. The trial was completed on day 176. The average SMY in the final period was 381.5 ± 52.0 L CH_4 g VS^{-1} which was a 21% decrease in SMY from period 2 and a 32% decrease from period 1. The average methane content was 60.7±3.6%.

4.4 Conversion of volatile solids to gas

To assess the conversion of VS to gas the following equation 8.5 taken from [16] is used:

$M_R = L_N \cdot ((16 \cdot CH_4\%) + (44 \cdot CO_2\%))/22.413$ (Eqn. 5)

Where;

MR is the daily average mass of volatile solids removed (g VS),

L_N is the average daily normalised biogas volume (L) at standard temperature and pressure (STP),

CH_4 %, is the methane content in the biogas,

CO_2 is the carbon dioxide content in the biogas.

There are 22.413 L per mole of gas at STP. According to this equation the average removal of VS in period 1 was 84% with a HRT of 30 days. This decreased to 72% in period 2 with a HRT of 21 days and further reduced to 54% in period 3 with a HRT of 17 days. The average concentration of total solids in the digestate increased from 5.1 ± 0.5 % TS in period 1 to 5.5 ± 0.3 % TS in period 2 and 6.7 ± 0.9 % TS in the final period. This indicates that reducing the HRT also reduces the degradation of volatile solids. However the large drop in specific methane yield towards the end of the trial may not be entirely as a result of the reduced HRT as signs of process instability emerged towards the end of the trial at an OLR of 4 kg VS $m^{-3}\,d^{-1}$.

4.5 Monitoring process stability in semi-continuous trial

During the semi-continuous trial the total volatile fatty acids (VFA), total alkalinity (TA), pH and total ammonia nitrogen (TAN) were monitored to assess the stability of the digestion process. The average results from the three time periods are shown in Table 8.4. In Period 1 (OLR 2 kgVS $m^{-3}\,d^{-1}$) the concentration of total VFAs was 1128 ± 281 mg $Ac_{eq}\,l^{-1}$. A small increase was observed during Period 2 (OLR of 3 kgVS $m^{-3}\,d^{-1}$) with an average of 1511 ± 77 mg $Ac_{eq}\,l^{-1}$. The concentration of VFAs rose sharply towards the end of the trial during Period 3 (OLR of 4 kgVS $m^{-3}\,d^{-1}$) as shown in Figure 8.5 (a), with an average of 2595 ± 750 mg $Ac_{eq}\,l^{-1}$. The sharp increase in VFA concentration indicated that the biological system was stressed.

Table 4 Summary of results from semi-continuous AD trial of canteen food waste

Period	1	2	3
OLR (kg VS m^{-3} day^{-1})	2	3	4
HRT (days)	30 x 3	21 x 2	17 x 2
SYM (mL CH4 g VS^{-1})	560.1 (29.3)	483.9 (72.0)	381.5 (52.0)
% CH4 (Weighted Average)	60.1 (1.3)	61.5 (2.8)	60 (3.6)
% VS conversion to gas	84	72	54
% TS (digestate)	5.1 (0.5)	5.5 (0.3)	6.7 (0.9)
% VS (digestate)	3.5 (0.3)	3.9 (0.2)	4.7 (0.7)
pH	7.7 (0.13)	7.9 (0.14)	8.1 (0.11)
TAN (mg L^{-1})	3543 (525)	5342 (485)	7205 (280)
NH_3 (mg L^{-1})	237 (50)	433 (185)	952 (75)
VFAs (mg HAc_{eq} L^{-1})	1128 (281)	1511 (77)	2595 (750)
Alkalinity (mg $CaCO_3$ L^{-1})	8093 (970)	9830 (159)	10230 (185)
VFA/TA	0.14	0.15	0.25

Results are indicated as a mean with standard deviation is in brackets

The average total alkalinity for the period 1 was 8093 ± 970 mg $CaCO_3$ l^{-1}. This increased to 9839 ± 159 mg $CaCO_3$ l^{-1} in period 2 and 10230 ± 185 mg $CaCO_3$ l^{-1} in period 3. The ratio of TVFA/TA is often used to assess the stability of the AD process. A ratio of 0.4 or less indicates that the process is stable while ratios over 0.8 indicate organic overloading and process instability. During the trial the VFA/TA ratio remained below 0.4, however it is clear that even though the ratio was within stable limits, high concentrations of total ammonia nitrogen (TAN) coupled with a large decrease in SMY towards the end of the trial indicate that a state of semiinhibited methanogenesis had been reached.

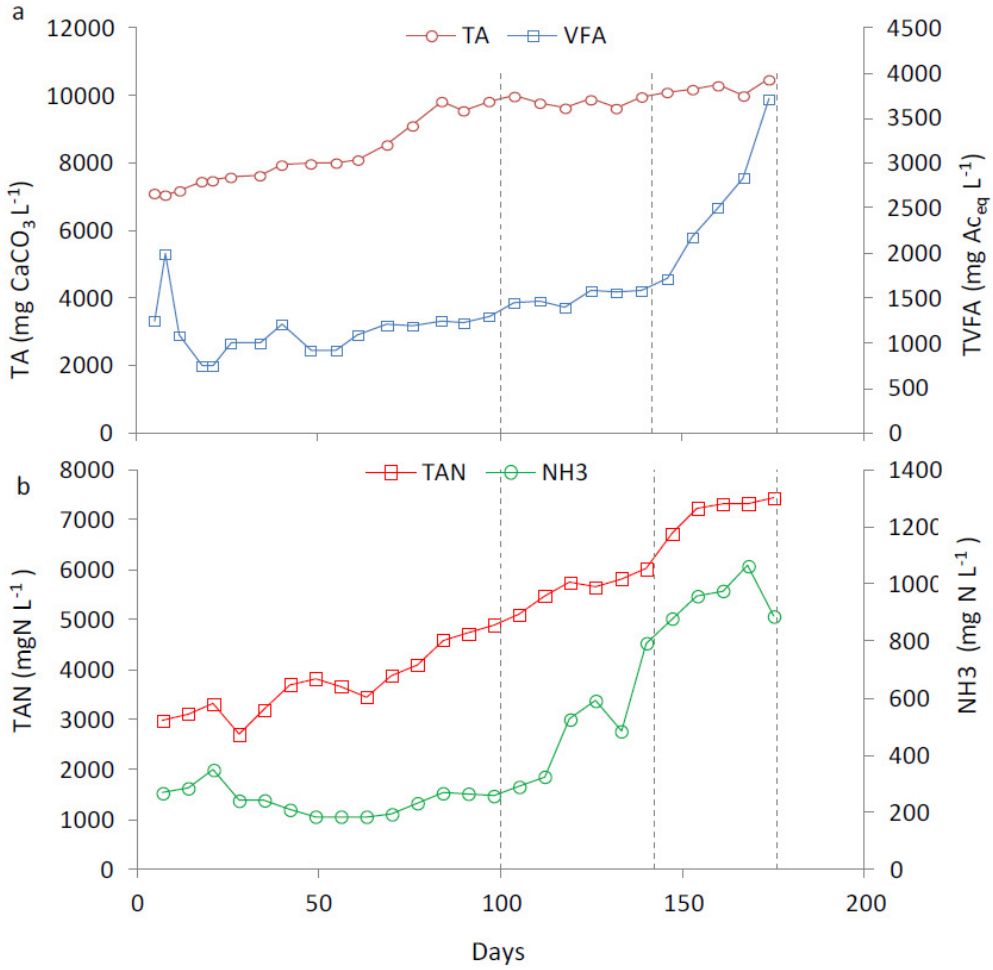


Figure 5 (a) Monitoring of total alkalinity (TA) and total volatile fatty acids. (TVFA) (b) total ammonia nitrogen (TAN) and free ammonia (NH_3) in the semi-continuous trial.

4.6 The inhibitory effects of high ammonia concentrations

There is a linear relationship between decreased specific methane yield and increasing concentrations of free ammonia in the liquid phase. Total ammonia nitrogen (TAN) contributes to the buffering capacity of the system but can be toxic to methanogens at higher pH values. A rise in pH from 7 to 8 can result in a 10 fold increase in the concentration of free ammonia. During the trial the pH increased from an average of 7.7 ± 0.1 in period 1 to 8.1 ± 0.1 in the final period. The high pH is of concern when combined with high levels of TAN as the relationship between ionised ammonium (NH_4^+) and unionised (free) ammonia (NH_3) is pH and temperature dependent. The concentration of

TAN increased linearly for the duration of the trial with final concentrations in excess of 7000 mg N l^{-1}, as shown in Figure 8.5(b). This is a very high concentration of TAN and would be considered to be well in the toxicity range for methane production [17]. It is well documented that high concentrations of free ammonia (NH_3) can cause inhibition to methane production, [18]. [19] reported that free ammonia concentrations above 1000 mg N l^{-1} are inhibitory for methanogenesis. Banks and colleagues (2012) reported high concentrations of total ammonia at high organic loading rates using source separated food waste. They showed that at elevated levels of total ammonia the acetoclatic methanogens were virtually nonexistent with the methane production coming from the hydrogenotrophic route. To overcome the inhibitory effects of high levels of ammonia the addition of trace elements such as iron, cobalt, selenium and molybdenum were successfully shown to improve methane yields at high organic loading rates (e.g. 5 kg VS m^{-3} d^{-1}) [20]. 8.4.7 Comparison of methane yield from food waste The specific methane yield (SMY) produced during period 1 of the semi continuous trial was relatively high in comparison to other reported methane yields from food waste. The highest average SMY of 560 ± 29.3 L CH_4 kg^{-1} VS was achieved at an OLR of 2 kg VS m^{-3} d^{-1} and HRT of 30 days. This is 90.3% of the Buswell Equation value. It is however 7% higher than the average BMP result from the same sample. This indicates that at moderate organic loading rates a continuous AD process may equal or even exceed methane yields from the BMP test. This may be due to acclimatisation of the inoculum with time. Other workers have recorded higher SMYs in continuous digestion than in BMP mode [21]. Zhang and colleagues (2012) achieved 425 L CH_4 kg^{-1} VS from continuous digestion of source segregated food waste at an OLR of 2 kg VS m^{-3} d^{-1}. The same material gave BMP results of between 445-456 L CH_4 kg^{-1} VS [22]. Davidsson and colleagues (2007) reported methane yields of between 300-400 L CH_4 kg^{-1} VS for a large number of source sorted OFMSW samples which had all been through different pre-treatment processes [23]. Separate trials by the authors [15] on a two phase AD system involving sequentially fed leach beds connected to an upflow anaerobic sludge blanket, treating the same commercial food waste, produced 384 L CH_4 kg^{-1} VS which corresponded to 72% of the value obtained in the BMP test. This is approximately 70% of the highest average methane yield achieved in the semi continuous trial and suggests that a conventional CSTR may be the best reactor configuration for maximising methane yield from food waste.

5 Conclusions

The characteristics of OFMSW can vary largely depending on the source and type of collection with BMP values of between 274 - 535 L CH_4 kg^{-1} VS. A semi continuous trial on commercial food waste produced an average of 560 ± 29 L CH_4 kg^{-1} VS at a moderate OLR of 2 kg VS m^{-3} d^{-1} with a HRT of 30 days. At higher OLRs (4 kg VS m^{-3} d^{-1}) increasing concentrations of VFAs (2595 mg l^{-1}) coupled with high concentrations of free ammonia (952 mg l^{-1}) led to a greatly reduced average specific methane yield (344 L CH_4 kg^{-1} VS).
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Appendix D:Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems

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Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems

J. McEniry [a], E. Allen [b], J.D. Murphy [b], P. O'Kiely [a,*]

[a] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland
[b] Bioenergy and Biofuels Research Group, Environmental Research Institute, University College Cork, Ireland

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ABSTRACT

Grassland biomass is likely to be harvested and stored as silage to ensure a predictable quality and a constant supply of feedstock to an anaerobic digestion facility. Grass (*Phleum pratense* L. var. Erecta) was ensiled following the application of one of six contrasting additive treatments or a 6 h wilt treatment to investigate the effects of contrasting silage fermentation characteristics on CH_4 yield. In general, silage fermentation characteristics had relatively little effect on specific CH_4 yield (from 344 to 383 Nl CH_4 kg^{-1} volatile solids). However, the high concentrations of fermentation products such as ethanol and butyric acid following clostridial and heterofermentative lactic acid bacterial fermentations resulted in a numerically higher specific CH_4 yield. While the latter fermentation products of undesirable microbial activity have the potential to enhance specific CH_4 yield, the numerically higher specific CH_4 yield may not compensate for the associated total solids and energy losses during ensiling.

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1. Introduction

Grassland biomass can be an excellent feedstock for biogas production and will likely be the dominant feedstock for on-farm anaerobic digestion in temperate Northwest Europe [1,2]. In order to ensure a predictable quality and a constant supply of grass to an anaerobic digestion facility, it is most likely to be harvested and stored as silage [1]. The main objective of ensilage is the efficient preservation of the energy content of a crop and this is achieved by the combination of an anaerobic environment and the bacterial fermentation of sugar. The lactic acid produced in the latter process lowers the pH and prevents the proliferation of spoilage microorganisms [3].

However, fermentation under farm conditions is not a controlled process and silage fermentation characteristics will depend on the nutrients fermented and the microorganisms responsible [4]. Silage which has undergone a desirable fermentation is generally characterised by a low pH, high lactic acid content and low concentrations of butyric acid and ammonia-N [5,6]. Furthermore, the ensiled energy is almost completely recoverable in a closed lactic acid dominant fermentation [7]. In contrast, and despite the negligible loss of energy, the production of ethanol by yeast during fermentation is undesirable because no acidification occurs [8]. Similarly, under sub-optimal ensiling conditions a secondary clostridial fermentation may lead to considerable total solids (TS) and energy losses due to extensive production of CO_2 and H_2 from the fermentation of lactate and hexose sugars [3].

A range of fermentation products formed during ensiling can influence specific CH_4 yield. For example, the specific CH_4 yield of some silages has been reported to be higher than for the original parent material due to the proportionately greater loss of TS than energy during the formation of fermentation products such as ethanol and 1,2-propanediol [9–11]. Similarly, a more heterofermentative lactic acid bacteria (LAB) fermentation with higher concentrations of acetic acid has been reported to enhance CH_4 production [12,13]. However, in all these cases, the potential losses occurring during fermentation must also be taken into account in order to make a more complete assessment of the overall effects of silage fermentation.

However, in general, only a limited number of studies [10,12,14] have provided information on the impact of grass silage fermentation characteristics on CH_4 production. Thus, the objective of this study was to investigate the effects of contrasting grass silage

* Corresponding author. Tel.: +353 46 9061100; fax: +353 46 9026154.
E-mail addresses: josephmceniry@gmail.com (J. McEniry), padraig.okiely@teagasc.ie (P. O'Kiely).

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Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems

Joe McEniry[a], Eoin Allen[b], Jerry D. Murphy[b,c], P.O'Kiely[a].

[a] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

[b] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[c] School of Engineering, University College Cork, Cork, Ireland

Abstract

Grassland biomass is likely to be harvested and stored as silage to ensure a predictable quality and a constant supply of feedstock to an anaerobic digestion facility. Grass (Phleum pratense L. var. Erecta) was ensiled following the application of one of six contrasting additive treatments or a 6 h wilt treatment to investigate the effects of contrasting silage fermentation characteristics on CH_4 yield. In general, silage fermentation characteristics had relatively little effect on specific CH_4 yield (from 344 to 383 L CH_4 kg^{-1} VS). However, the high concentrations of fermentation products such as ethanol and butyric acid following clostridial and heterofermentative lactic acid bacterial fermentations resulted in a numerically higher specific CH_4 yield. While the latter fermentation products of undesirable microbial activity have the potential to enhance specific CH_4 yield, the numerically higher specific CH_4 yield may not compensate for the associated total solids and energy losses during ensiling.

1. Introduction

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However, in general, only a limited number of studies [10,12,14] have provided information on the impact of grass silage fermentation characteristics on CH_4 production. Thus, the objective of this study was to investigate the effects of contrasting grass silage fermentation characteristics on CH_4 yield. Methane productionwas determined in two contrasting biomethane potential (BMP) test systems.

2. Materials and methods

2.1. Approach

Grass was ensiled following the application of one of six different additive treatments or a 6 h wilt treatment to generate seven silages with markedly contrasting fermentation characteristics. The effect of grass silage fermentation characteristics on specific CH_4 yield (i.e. normalised litre per kilogram volatile solids; L CH_4 kg^{-1} VS) was subsequently determined using two contrasting BMP test systems. Silage aerobic stability and the impact of aerobic deterioration on specific CH_4 yield were also determined, since these potentially impact on the efficiency of CH_4 production and are affected by the silage fermentation process.

2.2. Harvest and ensiling

Timothy (Phleum pratense L. var. Erecta) was grown in four field plots (each 20 m2) at Grange (53_ 520 N, 06_ 660 W) under an inorganic fertiliser N input of 125 kg N ha_1 and harvested on 24 May 2010. The herbage from each plot was harvested using a Haldrup forage plot harvester (J. Haldrup, Løgstor, Denmark) to an average 6 cm stubble height and passed through a precision-chop harvester (MEX V1, Pottinger; nominal chop-length of 19 mm) immediately prior to ensiling.

Prior to filling laboratory silos, seven randomly selected samples of chopped herbage (each 8 kg) from each plot were assigned to each of the following treatments: (1) Control (i.e. no additive applied), (2) Formic acid based additive (Add SafeR_, 70 g ammonia and 640 g formic acid per 1 kg additive; Trouw Nutrition, UK), 5 L t_1, (3) Sucrose, 10 kg t_1, (4) Calcium carbonate (CaCO3; Sigma, Dublin, Ireland), 10 kg t_1, (5) Homofermentative LAB inoculant (Ecosyl 100_, Lactobacillus plantarum MTD1, 1 _106 colony forming units g_1 herbage; Ecosyl Products Ltd., North Yorkshire, U.K.) plus sucrose (20 kg t_1) and CaCO3 (4 kg t_1), (6) Heterofermentative LAB inoculant (Pioneer 11A44_, Lactobacillus buchneri, 1 _ 105 colony forming units g_1 herbage; Southern Farm and Fuel Supplies, Cork, Ireland) plus sucrose (20 kg t_1) and CaCO3 (4 kg t_1) and (7) 6 h wilting period. Herbage for the 6 h wilt treatment was wilted outdoors on sheets of polythene with frequent manual tedding. There was no rainfall during harvesting or wilting. A constant weight (5 kg) of each herbage was then ensiled in laboratory silos [15] for 110 days. No effluent was produced during

storage. Representative samples of the herbage pre- and postensiling were stored at _18 _C prior to chemical analyses and determination of specific CH_4 yield (silage samples only).

2.3. Aerobic stability

After each of the silages had been weighed and sampled on day 110, the remainder of the silage was used to assess aerobic stability and deterioration [16]. Briefly, each silage was placed in a polythene-lined polystyrene box within an insulated room (4.35 m _ 3.66 m _ 2.80 m) where the ambient temperature was held at 20 _ 1 _C. Thermocouples were placed in the middle of the silage in each box and the temperature was recorded every hour (for 192 h) by a data logger (SQ ELTEK 80 T, Eurolec Instrumentation Ltd., Dundalk, Ireland). Uninsulated plastic containers of water (4 _ 3.8 L) stored near the silage acted as reference temperatures to which all silage temperatures were compared. The main indices of aerobic stability and deterioration were expressed as (a) the interval in hours until the temperature increased more than 2 _C above the reference temperature (b) maximum temperature rise and (c) the accumulated temperature rise (_C) up to 192 h of aerobiosis. The similar water content of the six unwilted silage treatments would confer similar specific heat characteristics on them, so that recording changes in their temperatures during exposure to air should reflect their relative heat production. The lower water content of the Wilt 6 h treatment necessitates some caution when comparing its aerobic stability or deterioration index values to the other treatments.

A representative sample of each silage was taken after 8 days (i.e. 192 h) exposure to air and samples were stored at _18 _C prior to chemical analyses and determination of specific CH_4 yield (using the micro-BMP system only).

2.4. Chemical analysis

Representative herbage samples pre- and post-ensiling were dried at 98 and 85 _C, respectively, for 16 h in an oven with forced air circulation to estimate TS content, and the values for silage samples were corrected for the loss of volatiles [17]. Replicate samples were also dried at 40 _C for 48 h before being milled (Wiley mill; 1 mm screen). Dried, milled samples were used for the determination of in vitro total solids digestibility and neutral detergent fiber, acid detergent fiber, crude protein, ash and water soluble carbohydrate concentrations and buffering capacity (herbage pre-ensiling only) as

previously described by King et al. [18]. Herbage VS concentration was subsequently determined (VS ¼ TS _ ash). Using silage samples taken prior to drying, the pH was determined from an aqueous extract using a handheld pH meter (R 315 pH, Reagecon Diagnostics Ltd., Dublin, Ireland). Further silage juice was extracted for the analysis of lactic acid, volatile fatty acids (i.e. acetic acid, propionic acid and butyric acid), ethanol and ammonia-N as previously described by McEniry et al. [19].

The pH and TS concentration of the silages after 8 days exposure to air and the TS (85 _C for 16 h) and VS content of the sludge inoculum used in subsequent BMP tests were also determined using methods described above.

2.5. BMP test systems

Two contrasting BMP test systems were used to investigate the effects of silage fermentation characteristics on specific CH_4 yield as outlined below. The impact of exposure of silage to air on specific CH_4 yield was determined in the micro-BMP system only.

2.5.1. Micro-BMP

Dried, milled samples were used to determine the specific CH_4 yield of each silage in 160 ml micro-BMP tests, in accordance with VDI 4630 [20] and as described previously by McEniry et al. [2]. Drying the silage samples facilitated their preservation and the processing of a relatively large representative sample of undried herbage to provide a smaller representative sub-sample for analyses [21]. Briefly, inoculum and substrate were added to 160 ml incubation bottles at a VS inoculum to substrate ratio of 2:1 and at a final VS concentration of 10 g kg_1. The inoculum (pH ¼ 7.98; 4 g TS kg_1, 2 g VS kg_1) was obtained from a farm digester treating cattle manure (Agri-Food and Biosciences Institute, Hillsborough, Northern Ireland). Micro- and macro- mineral solutions were added to ensure that nutrient conditions were not limiting [2] and distilled water was added to each bottle to adjust the final volume to 70 ml.

In order to determine the CH_4 yield of the inoculum, six replicate bottles with no substrate (i.e. blanks) were incubated under the same conditions. The final pH in each bottle was adjusted to 7.2 with 1 M hydrochloric acid, before the contents were flushed with N2 gas

for 1 min and sealed with butyl rubber stoppers and aluminium crimp seals. Bottles were incubated at 38 _C for 35 days and hand-mixed daily.

Using a detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK), the gas headspace pressure inside each bottle was recorded after 2, 5, 8, 13, 18, 27 and 35 days. The total amount of biogas produced was estimated using the following equation: Gas production (ml) ¼ (vh/Pa) _ Pt; where vh is the headspace volume (ml), Pa is the atmospheric pressure (hPa) and Pt is the gas headspace pressure (hPa). Following the determination of biogas volume, a 0.8 ml sample of gas was used to determine CH_4 concentration by gas chromatography [2]. After sampling the gas pressure inside each bottle was released.

Evaluation of these data included the following steps [20]: (a) headspace correction of the biogas values on day 2, as inert gas in the headspace at the beginning (day 0) of the batch digestion test causes a dilution of the biogas components, (b) subtraction of the volume of CH_4 produced by the inoculum (i.e. blank) from the volume of CH_4 produced in the batch digestion test with substrate and inoculum and (c) normalising the CH_4 volume to standard temperature and pressure conditions (i.e. dry gas, 273 K, 1013 hPa). The specific CH_4 yield was calculated as the cumulative sum of the CH_4 volume produced over the 35 day incubation period relative to the substrate VS concentration added to the test. Methane yield (L CH_4 kg TS^{-1}) was also expressed relative to the substrate TS concentration added to the test and this was subsequently adjusted to reflect silage TS recovered (i.e. expressed based on grass TS ensiled and taking account of in-silo losses).

2.5.2. Large-BMP

The specific CH_4 yield of each silage was also determined using an Automated Methane Potential Test System (AMPTS II; Bioprocess Control; Lund, Sweden), in accordance with VDI 4630 [20]. When required for analysis, individual silage samples were thawed at 4 _C for 24 h. Inoculum and substrate were added to 500 ml incubation bottles at a VS inoculum to substrate ratio of 2:1 and at a final VS concentration of 56 g kg_1. The inoculum (pH ¼ 7.56; 6 g TS kg_1, 4 g VS kg_1) was obtained from a farm digester treating cattle and poultry manure (Shanagolden, Co. Limerick, Ireland). Distilled waterwas added to each bottle to adjust the final volume to 400 ml.

In order to determine the CH_4 production of the inoculum, three replicate bottles with no substrate (i.e. blanks) were incubated under the same conditions. The incubation bottles were sealed with a screw-on cap and silicon spray before the contents were flushed with N2 gas for 1 min. Bottles were incubated at 38 _C for 33 days and the contents of each bottle were mixed with a slow rotating mixing rod (60 r.p.m.; every second minute). Methane production was recorded remotely over the 33 day incubation period. The biogas produced in each bottle passes through a second unit of bottles (one for each incubation bottle) containing 3M NaOH solution, which allows CH_4 to pass through while retaining CO_2 and H_2S. The CH_4 is then passed through a third unit comprising a gas measuring tipping mechanism (one for each bottle). A specific volume of CH_4 causes the tipping device to tip. This movement is recorded via a digital pulse and output is recorded in a software package as volume of CH_4 produced. These data were evaluated as described previously for the micro-BMP system.

2.6. Statistical analysis

Means and standard deviations (s.d.) were calculated for grass chemical composition pre-ensiling. Appropriate silage data and CH_4 yield data from BMP tests were analysed by one-way analysis of variance using the Proc GLM procedure of SAS, Version 9.1.2. Methane yield data from the two BMP test systems were analysed separately. The Tukey adjustment for multiple comparisons was used in testing for differences between means. The changes in silage chemical composition and specific CH_4 yield as a result of exposure to air were calculated by subtracting values for silage after 8 days exposure to air from silages at silo opening, respectively. These data were analysed according to the same procedure as outlined above.

3. Results

3.1. Grass composition pre-ensiling

Mean (s.d.) grass composition pre-ensiling is presented in Table 1. The 6 h wilt treatment resulted in a numerical increase in herbage TS concentration to 265 g kg_{-1} and had little effect on any of the other variables measured. 3.2. Silage composition Although relatively small differences in silage total solids digestibility were observed between treatments,

these differences were not significantly different ($P > 0.05$) across treatments (Table 2). Neutral detergent fibre and acid detergent fibre concentrations were lower ($P < 0.01$) for the silages from the homofermentative LAB treatment compared with the control, $CaCO_3$ (acid detergent fibre only), heterofermentative LAB and 6 h wilt treatments. Herbage crude protein concentration was lower ($P < 0.001$) and ash concentration higher ($P < 0.001$) for the silages from the $CaCO_3$ treatment compared with all other treatments. There was a major decline in herbage water soluble carbohydrate concentration during ensiling with the concentration of water soluble carbohydrate remaining in the silages being higher ($P < 0.001$) for the formic acid treatment compared with all other treatments.

Silage pH was highest (P < 0.001) for the CaCO3 treatment followed by the control and heterofermentative LAB treatments, with the pH for other treatments being similarly low (3.9 to 4.09; Table 2). In contrast, lactic acid concentrationwas lower (P < 0.001) for the silages from the CaCO3, control and heterofermentative LAB treatments compared with the homofermentative LAB treatment. Acetic acid (P < 0.001), propionic acid (P < 0.01; with the exception of control and CaCO3 treatments) and ethanol (P < 0.01) concentrations were all higher for the silages from the heterofermentative LAB treatment. Although butyric acid concentration was numerically higher for the control and the CaCO3 treatments (16.2 and 20.6 g kg_1 TS), this difference was not significant (P > 0.05). This reflects the heterogeneous nature of silage feedstocks and suggests that preservation was particularly poor for specific replicates of some treatments (e.g. CaCO3 treatment), but that this trendwas not evident across all replicates. Total fermentation products concentration was higher (P < 0.001) for the homofermentative LAB, sucrose and heterofermentative LAB treatments compared with the CaCO3 treatment. Similarly, the proportion of lactic acid in total fermentation products was higher (P < 0.001) for the formic acid, sucrose, homofermentative LAB and 6 h wilt treatments (0.76 to 0.81) compared with the CaCO3 treatment (0.31), with the other two treatments being intermediate (0.45 to 0.55). Finally, ammonia-N concentration was highest (P < 0.01) for the CaCO3 additive treatment compared with the other six treatments. Although TS recovery was numerically higher for the homofermentative LAB and sucrose treatments and lowest for the CaCO3 treatment, these differences were not significant (P > 0.05).

Table 1
Mean (s.d.) grass total solids concentration (TS; g kg^{-1}) and chemical composition (g kg^{-1} TS, unless indicated otherwise in footnotes) pre-ensiling.

Wilt (h)	TS	Chemical composition[a]						
		TSD	NDF	ADF	CP	Ash	WSC	BC
0	201 (10.1)	754 (11.0)	575 (11.0)	318 (6.0)	162 (12.3)	82 (1.7)	96 (8.5)	461 (8.6)
6	265 (15.3)	738 (13.2)	578 (9.0)	319 (10.2)	162 (2.6)	81 (2.0)	96 (6.6)	456 (5.1)

[a] TSD = total solids digestibility (g kg^{-1}); NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; WSC = water soluble carbohydrates; BC = buffering capacity (m. Eq. kg^{-1} TS).

3.3. Aerobic stability

The additive treatments and the 6 h wilt treatment had little effect ($P > 0.05$) on the indices of aerobic stability measured (Table 3). However, although not statistically significant, the interval in hours until the temperature increased more than 2 _C above the reference temperature was numerically lower for the sucrose (159 h) and homofermentative LAB (162 h) treatments, with all other silages appearing to be stable after 184e192 h of aerobic exposure. Similarly, the maximum temperature rise (4.8 and 6.7 _C for sucrose and homofermentative LAB treatments, respectively) and the accumulated temperature rise up to 192 h of aerobiosis (10.0 and 13.0 _C) were numerically higher for these silages.

3.4. BMP test systems-specific CH_4 yield

On average in the micro-BMP system, 0.78 of total CH_4 production over the 35 day incubation period was produced by day 13 of the batch digestion test. The specific CH_4 yield for the dried, milled silage samples varied from 283 to 314 L CH_4 kg^{-1} VSVS and did not differ ($P > 0.05$) between treatments (Table 4). In the large-BMP system the average specific CH_4 yield for the silages ranged from 344 to 383 L CH_4 kg^{-1} VSVS (Table 4). Despite the higher numerical specific CH_4 yield of the $CaCO_3$ and heterofermentative LAB treatments (370 and 383 L CH_4 kg^{-1} VSVS, respectively), there was no difference ($P > 0.05$) observed between the silages from different treatments. In contrast, when CH_4 yield is expressed per kg_1 silage $TS_{recovered}$ (Tables 2 and 4), the CH_4 yield of the $CaCO_3$ treatment was lower ($P < 0.01$) than all treatments (with the exception of the control and 6 h wilt treatments).

3.5. Exposure to air e changes in chemical composition and specific CH_4 yield

In general, exposure of the silages to air resulted in a numerically small increase in silage pH (þ0.39) and a small decrease in TS concentration (_2.9 g kg_1) and specific CH_4 yield (_11 L CH_4 kg^{-1} VSVS; but with the exception of the heterofermentative LAB and 6 h wilt treatments). However, these changes were relatively small and they were not significantly different ($P > 0.05$) across treatments (Table 5).

4. Discussion

4.1. Silage fermentation characteristics

The seven treatments produced a range of silages with contrasting fermentation characteristics. With the exception of the control, $CaCO_3$ and heterofermentative LAB treatments, all silages exhibited a lactic acid dominant fermentation (i.e. >0.75 lactic acid /fermentation products) with little or no clostridial activity as evidenced by butyric acid and ammonia-N concentrations of <10 g kg_{-1} TS and 100 g kg_{-1} N, respectively. This was indicative of a satisfactory preservation [6]. The increase in ammonia-N concentration with the formic acid based additive (127 g kg_{-1} N) is a result of the direct contribution of ammonia-N from the additive thus overestimating the extent of proteolysis during ensiling [22]. In contrast, silages from the control and $CaCO_3$ treatments showed evidence of both saccharolytic and proteolytic clostridial activity as indicated by the high pH (4.81 and 5.62 for control and $CaCO_3$ treatments, respectively) and high concentrations of butyric acid (16.2 and 20.6 g kg_{-1} TS) and ammonia-N (158 and 368 g kg_{-1} N) [5].

Table 2
Treatment effects on silage total solids concentration (TS; g kg^{-1}), chemical composition (g kg^{-1} TS, except pH and unless indicated otherwise in footnotes) and TS recovery (TSR; g kg^{-1}).

Treatment[a]	TS	Chemical composition[b]														TSR[c]
		pH	TSD	NDF	ADF	CP	Ash	WSC	LA	AA	PA	BA	EtOH	FP	LA/FP	NH_3–N	
Control	192^{A}	4.81^{B}	705	571^{A}	367^{A}	168^{A}	$96^{A, B}$	7.5^{A}	$70^{A, B}$	17.1^{A}	$5.2^{A, B}$	16.2	12.0^{A}	$121^{A, B}$	$0.55^{A, B}$	158^{A}	965
Formic acid	200^{A}	3.90^{A}	734	$531^{A, B}$	$337^{A, B}$	175^{A}	86^{A}	32.9^{B}	$101^{A, C}$	13.4^{A}	2.0^{A}	2.5	6.6^{A}	$126^{A, B}$	0.81^{A}	127^{A}	968
Sucrose	206^{A}	3.91^{A}	702	$523^{A, B}$	$336^{A, B}$	167^{A}	88^{A}	10.3^{A}	$131^{A, C}$	21.4^{A}	2.9^{A}	3.4	12.6^{A}	$172^{A, C}$	0.76^{A}	92^{A}	999
$CaCO_3$	207^{A}	5.62^{C}	678	$540^{a, B}$	369^{A}	139^{B}	152^{C}	6.9^{A}	30^{B}	22.5^{A}	$5.8^{A, B}$	20.6	14.4^{A}	93^{B}	0.31^{B}	368^{B}	805
Homofermentative LAB	$217^{A, B}$	3.92^{A}	750	494^{B}	313^{B}	167^{A}	$99^{A, B}$	13.6^{A}	154^{C}	20.1^{A}	3.8^{A}	9.0	11.1^{A}	198^{C}	0.78^{A}	88^{A}	1000
Heterofermentative LAB	206^{A}	4.57^{B}	702	555^{A}	355^{A}	166^{A}	112^{B}	9.5^{A}	$70^{A, B}$	42.7^{B}	11.4^{B}	3.3	28.8^{B}	$157^{A, C}$	$0.45^{A, B}$	126^{A}	985
Wilt 6 h	260^{B}	4.09^{A}	731	549^{A}	353^{A}	171^{A}	86^{A}	8.1^{A}	$96^{A, C}$	13.8^{A}	1.7^{A}	2.7	11.4^{A}	$126^{A, B}$	0.76^{A}	96^{A}	965
s.e.m.[d]	9.0	0.085	17.4	9.4	6.8	3.8	3.3	2.40	11.2	2.37	1.12	4.14	2.21	8.6	0.064	32.0	67.3
Significance	***	***	NS	***	***	***	***	***	***	***	***	NS	***	***	***	***	NS

[a] Formic acid = Add SafeR® additive; homofermentative LAB = Ecosyl 100® lactic acid bacterial inoculant plus sucrose and $CaCO_3$ heterofermentative LAB = Pioneer 11A44® lactic acid bacterial inoculant plus sucrose and $CaCO_3$.

[b] TSD = total solids digestibility (g kg^{-1}); NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; WSC = water soluble carbohydrates; LA = lactic acid; AA = acetic acid; PA = propionic acid; BA = butyric acid; EtOH = ethanol; FP = total fermentation products (LA + AA + PA + BA + ethanol); LA/FP = LA as a proportion of total FP; NH_3–N = ammonia-N (g kg^{-1} N).

[c] Proportion of silage TS weight removed from the laboratory silos relative to herbage TS weight ensiled.

[d] s.e.m. = standard error of the mean; *** = $P < 0.001$, NS = not significant; means within a column without a capital letter superscript(s) in common differ ($P < 0.01$).

Table 3
Treatment effects on indices of silage aerobic stability and deterioration.

Treatment[a]	Interval (h) to temperature rise > 2 °C	Maximum temperature rise (°C)	Accumulated temperature rise to 192 h (°C)
Control	192	1.3	1.9
Formic acid	192	0.6	3.1
Sucrose	159	4.8	10.0
$CaCO_3$	184	1.4	2.1
Homofermentative LAB	162	6.7	13.0
Heterofermentative LAB	192	0.2	2.2
Wilt 6 h	184	3.2	1.9
s.e.m.[b]	13.9	1.69	4.60
Significance	NS	NS	NS

[a] Formic acid = Add SafeR® additive; homofermentative LAB = Ecosyl 100® lactic acid bacterial inoculant plus sucrose and $CaCO_3$ heterofermentative LAB = Pioneer 11A44® lactic acid bacterial inoculant plus sucrose and $CaCO_3$.
[b] s.e.m. = standard error of the mean; NS = not significant.

Table 4
Treatment effects on silage specific CH_4 yield (Nl CH_4 kg^{-1} VS) and CH_4 yield per kilogram of silage total solids (TS) recovered (Nl CH_4 kg^{-1} $TS_{recovered}$) in two contrasting biomethane potential (BMP) test systems.

Treatment[a]	Specific CH_4 yield		CH_4 yield kg^{-1} TS recovered[b]	
	Micro-BMP	Large-BMP	Micro-BMP	Large-BMP
Control	294	344	261	$305^{A, B}$
Formic acid	309	356	278	319^{A}
Sucrose	309	355	286	328^{A}
$CaCO_3$	310	370	209	242^{B}
Homofermentative LAB	314	350	288	320^{A}
Heterofermentative LAB	306	383	272	341^{A}
Wilt 6 h	283	345	254	$311^{A, B}$
s.e.m.[c]	10.3	19.3	22.1	18.9
Significance	NS	NS	NS	*

[a] Formic acid = Add SafeR® additive; homofermentative LAB = Ecosyl 100® lactic acid bacterial inoculant plus sucrose and $CaCO_3$ heterofermentative LAB = Pioneer 11A44® lactic acid bacterial inoculant plus sucrose and $CaCO_3$.
[b] Expressed based on grass TS ensiled and taking account of in-silo losses.
[c] s.e.m. = standard error of the mean; NS = not significant; * = $P < 0.05$; means within a column without a capital letter superscript(s) in common differ ($P < 0.01$).

In the case of the control treatment, the relatively low TS (201 g kg_1) and water soluble carbohydrate (96 g kg_1 TS) concentrations of the herbage pre-ensiling and the relatively high buffering capacity (461 m. Eq. kg_1 TS) would have presented an elevated challenge to preservation. This was further evidenced by a fermentation coefficient (FC ¼ TS (g 100 g_1) þ 8 water soluble carbohydrate/ buffering capacity) value of 31, which is considerably lower than the critical value of 45 considered to be essential for the production of anaerobically stable silage free of butyric acid [23]. This combination of factors appeared to be conducive to permitting undesirable clostridial activity.

However, the direct acidification and antimicrobial effect of the formic acid treatment allowed LAB to dominate the fermentation (i.e. 0.81 lactic acid/fermentation products) and produced silages with more desirable fermentation characteristics. This treatment resulted in a low pH and likely resulted in a greater inhibition of Enterobacteria and Clostridia as evidenced by reduced concentrations of ethanol, butyric acid and ammonia-N compared with the control treatment [5,8]. Adding sucrose also improved silage preservation, further suggesting that the low water soluble carbohydrate concentration in the herbage pre-ensiling presented an elevated challenge to preservation. The sucrose provided an additional supply of fermentable substrate to the microbial population, counteracting the negative ensilability effects of low TS concentration and high buffering capacity, and resulted in an almost two-fold increase in lactic acid concentration and a decrease in pH.

Calcium carbonate was included to act as a buffer against acidification. The resulting silages were very poorly preserved showing evidence of high levels of clostridial activity which resulted in high (0.195) TS losses. As no signs of excessive respiration (e.g. mould growth) were evident and no effluent was produced for any of the silages, TS loss was largely reflective of the efficiency and extent of fermentation. In contrast, the addition of a homofermentative LAB inoculant (including sucrose and CaCO3) resulted in a lactic acid dominant fermentation, with a high concentration of lactic acid (154 g kg_1 TS), reduced proteolysis and a greater TS recovery. The inclusion of small amounts of sucrose and CaCO3 with the latter inoculant would have increased the total fermentation products concentration by supplying additional substrate for fermentation and increasing the amount of lactic acid required to lower the pH, respectively.

The heterofermentative LAB treatment (including sucrose and CaCO3) resulted in the production of high concentrations of a number of fermentation products. Acetic acid, propionic acid and ethanol concentrations were all high (42.7, 11.4 and 28.8 g kg_1 TS, respectively) in these silages, with lactic acid contributing only 0.45 of total fermentation products. Similar to the formic acid treatment, the 6 h wilt treatment produced silages with a more restricted fermentation as evidenced by the lower concentration of total fermentation products. The reduced concentrations of butyric acid and ammonia-N, compared with the control treatment, demonstrates the inhibitory effect of higher TS concentration on Clostridia [24].

Table 5
Treatment effects on the changes (silage after 8 days exposure to air−silage at silo opening) in silage pH, total solids (TS; g kg^{-1}) concentration and specific CH_4 yield (Nl CH_4 kg^{-1} VS) in the micro biomethane potential test system after 8 days exposure to air. A negative value indicates a decrease due to aerobic exposure of silage.

Treatment[a]	pH	TS	Specific CH_4 yield
Control	0.11	−1.1	−8.6
Formic acid	0.06	−5.0	−8.8
Sucrose	1.07	−3.3	−5.9
$CaCO_3$	0.14	−0.4	−17.1
Homofermentative LAB	1.04	−5.9	−15.2
Heterofermentative LAB	0.12	−4.4	8.7
Wilt 6 h	0.20	0.0	23.1
s.e.m.[b]	0.451	4.37	13.53
Significance	NS	NS	NS

[a] Formic acid = Add SafeR® additive; homofermentative LAB = Ecosyl 100® lactic acid bacterial inoculant plus sucrose and $CaCO_3$ heterofermentative LAB = Pioneer 11A44® lactic acid bacterial inoculant plus sucrose and $CaCO_3$.
[b] s.e.m. = standard error of the mean; NS = not significant.

4.2. Silage fermentation characteristics and specific CH_4 yield

Despite the large differences in both the extent and direction of fermentation among the seven grass silages, no significant differences were observed in specific CH_4 yield. However, the numerically higher specific CH_4 yield values of the CaCO3 and the heterofermentative LAB treatments likely reflect the higher potential CH_4 yield from fermentation products such as ethanol and butyric acid [11]. For example, there is no difference in the theoretical CH_4 yield of lactic acid and acetic acid (355 L CH_4 kg^{-1}), whereas the theoretical CH4 yield for

ethanol (693 L CH_4 kg^{-1}), butyric acid (604 L CH_4 kg^{-1}) and propionic acid (503 L CH_4 kg^{-1}) are significantly higher [9,25].

Furthermore, the CH_4 content of the biogas produced during anaerobic digestion can be altered by the chemical composition of the fermentation products [11]. For example, the theoretical CH_4 content (expressed as a proportion of biogas volume) for ethanol, butyric acid and propionic acid are 0.75, 0.63 and 0.58, respectively [9]. Thus, the specific CH_4 yield of the CaCO3 and the heterofermentative LAB treatments was numerically higher due to the accumulation of fermentation products showing a higher CH_4 yield than those of the other dominant fermentation products measured (i.e. lactic acid and acetic acid). This suggests that the numerically higher specific CH_4 yield of the heterofermentative LAB treatment may be a result of the increased concentrations of fermentation products such as ethanol and propionic acid, as opposed to the higher proportion of acetic acid [12,13]. However, an increase in the specific CH_4 yield expressed on a VS

basis may not reflect the CH_4 yield per mass unit of silage material and the losses occurring during ensilage must also be taken into account [11,12]. When considering TS recovered and in-silo losses, the lowest CH_4 yield was observed for the CaCO3 treatment. This reflects the high levels of clostridial activity which resulted in high TS losses due to extensive production of CO_2 and H2 from the fermentation of lactate and hexose sugars [5]. Neurieter et al. [26] also reported that a clostridial fermentation could improve specific CH_4 yield, but that the high losses during storage negatively compensated for the higher specific CH_4 yield. In the current study, however, the positive effect of enhanced specific CH_4 yield was outweighed by the large TS losses during the ensiling process.

Heterofermentative lactic acid bacterial fermentations are generally associated with higher TS losses than homofermentative lactic acid fermentations [3]. This was not particularly evident in the current study with differences in TS losses during ensiling being minimal. Furthermore, the CH_4 yield for the heterofermentative LAB treatments, expressed on a TS recovered basis, was also numerically higher (on average 24 L CH_4 kg^{-1} VSTSrecovered, excluding the CaCO3 treatment) than all other treatments. This may suggest that under good ensiling conditions, with minimal TS losses, that there may be some potential to increase CH_4 yield through the promotion of a heterofermentative lactic acid bacterial fermentation.

4.3. Aerobic stability and specific CH_4 yield Aerobic microorganisms can be reactivated on exposure to air causing spoilage [3]. These microorganisms multiply and contribute to heating and chemical changes within the silage, indicated at its simplest by a reduction in lactic acid concentration and a corresponding rise in pH. Aerobic deterioration is undesirable and losses can amount to 30e50 g TS kg_1 within 1 day of exposure to air [27], as well as to losses in available energy content.

In the current study, all silages appeared to be relatively stable on exposure to air, with only relatively small increases in silage pH (from 0.06 to 1.07) and small losses in TS concentration (from _0.4 to 5.9 g kg_1) observed. The presence of acetic acid (from 13.4 to 42.7 g kg_1 TS) and propionic acid (from 1.7 to 11.4 g kg_1 TS) in all silages may have resulted in the inhibition of yeast activity and this may have enhanced silage stability on exposure to air [28]. Furthermore, the numerically higher temperature rise and the shorter interval until temperature increased by more than 2 _C above the reference temperature for the sucrose and homofermentative LAB treatments may be explained by the high concentrations of lactic acid in these silages possibly acting as a substrate for lactate-assimilating yeast [29].

Reflecting this high aerobic stability, only a small decrease in specific CH_4 yield was observed for the silages following 8 days exposure to air and this likely reflects small losses in residual water soluble carbohydrate and fermentation products concentrations as a result of aerobic microbial activity. However, this trend was not consistent across all treatments and a small increase in specific CH_4 yield was observed for the heterofermentative LAB and 6 h wilt treatments following exposure to air. An explanation for this trend is not apparent.

4.4. Comparison of BMP test systems

A major difference between the two BMP test systems was the use of dried, milled silage samples in the micro-BMP test. Thermal drying can change the chemical composition of a feedstock and these changes may impact on specific CH_4 yield. For example, continued activity by plant enzymes during thermal drying at low temperatures can result in organic matter losses, while drying at high temperatures can result in the production of indigestible ‘Maillard’ products [21].

More importantly, thermal drying can result in the loss of volatile compounds such as fermentation acids and alcohols, with Porter and Murray [17] reporting volatility coefficients of 0.09, 0.55 and 0.99 for lactic acid, volatile fatty acids and alcohols fermentation products when thermal drying at 60 _C, respectively. Although the volatility of the silage at 40 _C would have been considerably lower than when drying at 60e100 _C [17,30], some partial losses would still have occurred and this likely contributed to the numerically lower specific CH_4 yield for each silage in the micro- BMP test system.

However, despite the numerically lower specific CH_4 yield data observed for the micro-BMP test system, no significant differences in specific CH_4 yield were observed between the silages in either of the BMP test systems. This may suggest that the micro-BMP test system is useful for the comparison and ranking of samples relative to one another, but is less suitable for the determination of the maximum specific CH_4 yield.

5. Conclusions

Grass silage fermentation characteristics appear to have relatively little effect on specific CH_4 yield. However, the relatively high concentrations of fermentation products such as ethanol and butyric acid in the CaCO3 and the heterofermentative LAB treatments resulted in a numerically higher specific CH_4 yield. For the CaCO3 treatment, the positive effect of enhanced specific CH_4 yield with an undesirable clostridial fermentation was outweighed by the large TS losses occurring during ensiling. While some of the fermentation products (i.e. ethanol and butyric acid) of undesirable microbial activity have the potential to enhance specific CH_4 yield, this numerically higher specific CH_4 yield may not compensate for the associated TS and energy losses occurring during ensiling.

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Appendix E: Additional peer reviewed journal papers with minor contributions

Waste Management 33 (2013) 2470–2477

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Waste Management

journal homepage: www.elsevier.com/locate/wasman

Improving hydrolysis of food waste in a leach bed reactor

James D. Browne, Eoin Allen, Jerry D. Murphy *

Department of Civil and Environmental Engineering, University College Cork, Ireland
Environmental Research Institute, University College Cork, Ireland

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ABSTRACT

This paper examines the rate of degradation of food waste in a leach bed reactor (LBR) under four different operating conditions. The effects of leachate recirculation at a low and high flow rate are examined with and without connection to an upflow anaerobic sludge blanket (UASB). Two dilution rates of the effective volume of the leach bed reactors were investigated: 1 and 6 dilutions per LBR per day. The increase in dilution rate from 1 to 6 improved the destruction of volatile solids without connection to the UASB. However connection to the UASB greatly improved the destruction of volatile solids (by almost 60%) at the low recirculation rate of 1 dilution per day. The increase in volatile solids destruction with connection to the UASB was attributed to an increase in leachate pH and buffering capacity provided by recirculated effluent from the UASB to the leach beds. The destruction of volatile solids for both the low and high dilution rates was similar with connection to the UASB, giving 82% and 88% volatile solids destruction respectively. This suggests that the most efficient leaching condition is 1 dilution per day with connection to the UASB.

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1. Introduction

1.1. Introduction to municipal biowaste treatment

Municipal biowaste often referred to as the organic fraction of municipal solid waste (OFMSW) consists of food and garden waste from domestic, commercial and street cleanings. It is the main cause of smell and nuisance in municipal solid waste (MSW) and is responsible for most of the environmental hazards associated with municipal waste management, such as the formation of polluting leachate and methane gas under anaerobic conditions. As EU states are obliged to divert biodegradable waste from landfill under the terms set out in the Landfill Directive 1999 (EC, 1999), new treatment methods are sought in many countries to treat OFMSW in the most environmentally and economically sound way.

The use of anaerobic digestion (AD) in treating OFMSW is becoming increasingly popular across Europe (Mata-Alvarez, 2003). However OFMSW is a complex and heterogeneous material and many questions still remain about the most effective AD process for OFMSW digestion and even if it is suitable for long term continuous mono-digestion (Banks et al., 2011). A significant portion of OFMSW consists of food waste with a total solids (TS) content of 20–30% (Davidsson et al., 2007). As of June 2010, commercial premises in Ireland which produce greater than 50 kg of food waste per week are legally required to provide designated bins for source separated food waste (SSFW) (Dept of Environment, 2009). It is estimated that over a million tonnes per annum of OFMSW will have to be diverted from landfill in Ireland by 2016 to meet the EU Landfill Directive (EC, 1999). Currently alternative waste treatment infrastructure is insufficient to meet this demand (EPA, 2009). Due to the recent EC proposal (EC, 2012) to limit biofuels from food crops to 2011 levels (ca. 5%) the potential to upgrade biogas from food waste to biomethane (Budzianowski, 2012) and use as a transport fuel (Murphy et al., 2013) can help EU states to meet the 10% renewable energy in transport target.

1.2. Anaerobic digestion technology

The most commonly known and used digester type is the continuously stirred tank reactor (CSTR) which is operated at a low total solids content, typically 5–10% TS (Banks et al., 2011; Climenhaga and Banks, 2008; Nizami et al., 2009). Food waste has a total solids content of between 20% and 30% therefore wet AD systems may require dilution with water or agricultural slurry to facilitate homogenization and mixing. As water has a relatively large specific heat capacity (4.2 kJ/kg/°C) the required heat energy would be larger due to the increased volumes to be treated as would the energy required for pumping and mixing (Jagadabhi et al., 2011). It is commonly cited in the waste management industry that food waste even when it is collected in a designated bin, can be heavily contaminated with other household waste materials

* Corresponding author at: Environmental Research Institute, University College Cork, Ireland. Tel.: +353 21 490 2286.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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The resource of biomethane, produced via biological, thermal and electrical routes, as a transport biofuel

Jerry D. Murphy [a,b,*], James Browne [a,b], Eoin Allen [a,b], Cathal Gallagher [c]

[a] Environmental Research Institute, University College Cork, Cork, Ireland
[b] Department of Civil and Environmental Engineering, University College Cork, Cork, Ireland
[c] Bord Gais Energy, Gas Works Road, Cork, Ireland

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ABSTRACT

Biomethane is an energy vector suitable for renewable transport fuel which may derive energy through three different methodologies: thermal gasification; biological anaerobic digestion; and conversion of electricity to hydrogen (via electrolysis) and on to methane as described by the Sabatier Equation. Thermal gasification to produce methane (based on "hard" feed stock) tends to require significant scale, of the order of 400 MW. Biological anaerobic digestion (based on "soft" feed stock) is typically of scale less than 1 MW. Systems based on the Sabatier Equation convert hydrogen to methane exothermically and sequester carbon. The resource is assessed at 19% of energy in transport in Ireland. Adopting the approach of the EU Renewable Energy Directive (for example double credit for biofuels from residues and lignocellulosic feed stock) biomethane can supply 40% renewable energy supply in transport (RES-T). The resource is sufficient to supply 30% of the private transport fleet with indigenous sustainable gaseous biofuel.

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1. Introduction

There tends to be a preoccupation with renewable electricity when discussing renewable energy. This tends to follow the desire for a silver bullet technology [1]; one solution to solve the problems of peak oil (and peak fossil fuel?), security of supply, global warming and climate change. Considering that electricity is approximately 20% of final energy demand [1] on average, a simplistic solution would suggest that electricity capacity is increased considerably (up to a factor of five if no energy reduction takes place) and that green electricity is used to satisfy the three main energy demands (electricity, heat and transport). An intelligent examination of the problem may suggest that electricity is predominately produced by coal and gas (which tend not to be on the critical path for peak fuel) while oil (which probably has peaked) is the dominant fuel for transport (petrol and diesel) and in many countries for heat (as kerosene). Thus the most pressing problem for the world may be renewable transport and thermal energy supply. Pragmatically a number of solutions are required to replace depleting oil reserves for a growing world population with improving standards of living. Initially natural gas may be seen as the first abundant source of substitution for oil [2]. Natural gas has a far higher hydrogen composition than oil (25% versus 13%) and as such produces less carbon dioxide per unit of energy. Local air quality is far better served using natural gas vehicles (NGVs) than diesel in buses or petrol in cars. There are over 12 million NGVs on the world's roads, many in developing countries such as Pakistan. Many of the vehicles are buses which use far more fuel than a private vehicle [2]. Biomethane may be blended with or used as a substitute for natural gas in NGVs. Obviously biomethane may also displace natural gas as a source of thermal energy. All thorough investigations of renewable energy resources must examine the potential market size and the technology [3,4]. Three technologies are considered here: biological and thermal production of methane is relatively well understood; the third source is as a storage mechanism for green electricity.

Anaerobic digestion is a ubiquitous technology; however the feed stocks utilised are ever expanding. Initially residues such as sewage, slurry, organic waste dominated; now however crops and crop residues are greatly enhancing the potential bioresource. There are over 6000 biodigesters in Germany alone; Maize is the dominant feed stock [5]. As of late gas grid injection is the distribution system of choice allowing optimal energy and financial returns [6,7]. Countries such as Ireland with an extensive natural gas grid and large bioresource (91% of agricultural land under grass) see huge potential for biomethane; 2.5% of grass land is potentially surplus and available for biomethane production [7]. The EU Renewable Energy Directive [8] allows double credit for biofuels produced from lignocellulosic feed stock and residues.

* Corresponding author. Environmental Research Institute, University College Cork, Cork, Ireland. Tel.: +353 21 490 2286.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry

David M. Wall [a,b,d], Eoin Allen [a,b], Barbara Straccialini [c], Padraig O'Kiely [d], Jerry D. Murphy [a,b,*]

[a] Bioenergy and Biofuels Research Group, Environmental Research Institute, University College Cork, Cork, Ireland
[b] School of Engineering, University College Cork, Cork, Ireland
[c] Department of Agricultural, Food and Environmental Science, Perugia University, Perugia, Italy
[d] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

HIGHLIGHTS

- Higher grass silage input maximises potential biomethane output.
- At 4 kg VS m^{-3} d^{-1} the SMY for mono-digestion of grass silage reduces by 12%.
- Biomethane efficiencies remain optimal at high OLRs with addition of 20% slurry.
- HRT should exceed 20 days for effective anaerobic digestion of grass silage.

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ABSTRACT

This study investigated the feasibility of mono-digesting grass silage, dairy slurry and the co-digestion of the two substrates at a range of concentrations with a specific focus on digester performance while increasing organic loading rate (OLR). The results show that the higher the proportion of grass silage in the substrate mix the higher the specific methane yield (SMY) achieved. Optimum conditions were assessed for 100% grass silage at an OLR of 3.5 kg VS m^{-3} d^{-1} generating a SMY of 398 L CH_4 kg^{-1} VS equating to a biomethane efficiency of 1.0. For co-digestion of grass silage with 20% dairy slurry the optimum condition was noted at an OLR of 4.0 kg VS m^{-3} d^{-1} generating a SMY of 349 L CH_4 kg^{-1} VS and a biomethane efficiency of 1.01. Hydraulic retention times of less than 20 days proved to be a limiting factor in the operation of farm digesters.

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1. Introduction

1.1. Use of grass to meet renewable energy targets through anaerobic digestion

Whereas the predominant crop feedstock for anaerobic digestion in Germany and Austria is maize silage (IEA, 2014), Ireland, with a temperate climate, is more suited to the production of grass and can potentially achieve high yields per hectare (O'Donovan et al., 2011). Thus, grass silage is the primary source of conserved feed for ruminants in the country (O'Mara, 2008). Excess grass silage, surplus to livestock requirements, has been identified as a potential source for biomethane production which would significantly contribute to upcoming renewable energy targets (McEniry et al., 2013; Wall et al., 2013). The successful operation of grass-fed digesters is of utmost importance to the establishment of an anaerobic digestion industry in the country.

Mono-digestion of grass silage has been reported to give difficulties due to a deficiency in essential trace elements over long term operation of a reactor (Jarvis et al., 1997; Thamsiriroj et al., 2012). Ireland has an abundance of slurry, derived from faeces and urine, collected from ruminant and monogastric farm livestock accommodated indoors. This is a potential co-substrate attributable to its relatively high content of trace elements. However, the addition of slurry to a digester theoretically reduces potential biomethane yields (Wall et al., 2013) and therefore it is important to find the right balance with respect to the stable operation of a reactor and its economic feasibility. Digesters fed solely with slurry have proved economically challenging with low methane yields (Gerin et al., 2008).

* Corresponding author at: Bioenergy and Biofuels Research Group, Environmental Research Institute, University College Cork, Cork, Ireland.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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The effect of trace element addition to mono-digestion of grass silage at high organic loading rates

David M. Wall [a,b,d], Eoin Allen [a,b], Barbara Straccialini [c], Padraig O'Kiely [d], Jerry D. Murphy [a,b,*]

[a] Environmental Research Institute, University College Cork, Cork, Ireland
[b] School of Engineering, University College Cork, Cork, Ireland
[c] Department of Agricultural, Food and Environmental Science, Perugia University, Perugia, Italy
[d] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

HIGHLIGHTS

- Dairy slurry when co-digested with grass silage provided sufficient trace elements.
- Low addition of slurry (20% VS) exhibited stable VFA profiles and high SMYs.
- Mono-digestion of grass silage at high loading rates required trace element addition.
- Supplementation of cobalt, nickel and iron to mono-digestion increased SMY by 12%.

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ABSTRACT

This study investigated the effect of trace element addition to mono-digestion of grass silage at high organic loading rates. Two continuous reactors were compared. The first mono-digested grass silage whilst the second operated in co-digestion, 80% grass silage with 20% dairy slurry (VS basis). The reactors were run for 65 weeks with a further 5 weeks taken for trace element supplementation for the mono-digestion of grass silage. The co-digestion reactor reported a higher biomethane efficiency (1.01) than mono-digestion (0.90) at an OLR of 4.0 kg VS m^{-3} d^{-1} prior to addition of trace elements. Addition of cobalt, iron and nickel, led to an increase in the SMY in mono-digestion of grass silage by 12% to 404 L CH_4 kg^{-1} VS and attained a biomethane efficiency of 1.01.

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1. Introduction

1.1. Role of nutrients in anaerobic digestion

The role of nutrients in the anaerobic digestion process is a key aspect of digester performance and stability. Macronutrients (N, P, K, Na, Ca and Mg) are primarily associated with the digestate, and their potential role is as a fertiliser substitute or other valued added end products. They also act as important biological components in digestion systems. Micronutrients, or trace elements (TEs), are aligned to the operational performance of the reactor and any deficiency in such TEs can have a detrimental effect on potential biomethane yields. The bio-availability of TEs is primarily dependent on the chemical form in which they are present, and on the balance between individual macro-/micro-nutrients.

1.2. Benefit of trace elements in grass silage digestion

Grass silage, produced in excess of livestock requirements, is an essential substrate in the establishment of an anaerobic digestion industry in Ireland. In a previous paper by the authors (Wall et al., 2014), continuous mono-digestion of grass silage (termed R6 in the paper) was shown to give high specific methane yields (SMY) of 398 L CH_4 kg^{-1} volatile solids (VS) at an organic loading rate (OLR) of 3.5 kg VS m^{-3} d^{-1}. However, as the OLR was increased to 4.0 kg VS m^{-3} d^{-1}, the SMY decreased to 360 L CH_4 kg^{-1} VS; a drop of 12%. The system employed recirculation of effluent liquor (<25 g dry solids (DS) kg^{-1}) to ensure the reactor remained at a desirable solids content (<100 g DS kg^{-1}). This led to a shortened hydraulic retention time (HRT) of 19 days, which is postulated as a reason for the drop off in SMY.

To maintain high SMYs for mono-digestion of grass silage it is suggested that specific TEs be added to the reactor. Alternatively co-digestion with dairy cow slurry, an abundant agricultural resource in Ireland may be utilised. The addition of 20% dairy slurry

* Corresponding author at: Environmental Research Institute, University College Cork, Cork, Ireland.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage

D.M. Wall [a,d], B. Straccialini [a,c], E. Allen [a], P. Nolan [d], C. Herrmann [a,b], P. O'Kiely [d], J.D. Murphy [a,b,*]

[a] Environmental Research Institute, School of Engineering, University College Cork, Cork, Ireland
[b] Science Foundation Ireland (SFI), Marine Renewable Energy (MaREI) Centre, Ireland
[c] Department of Agricultural, Food and Environmental Science, Perugia University, Perugia, Italy
[d] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

HIGHLIGHTS

- Treatments to stimulate hydrolysis were assessed on a high fibre grass silage.
- Particle size reduction and rumen fluid addition were assessed by methane yields.
- Batch tests did not reveal the impact of these variables reporting similar yields.
- Operation of continuous digestion of grass >3 cm was mechanically problematic.
- The best case was <1 cm silage with rumen fluid addition yielding 371 L CH_4 kg^{-1} VS.

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ABSTRACT

This work examines the digestion of advanced growth stage grass silage. Two variables were investigated: particle size (greater than 3 cm and less than 1 cm) and rumen fluid addition. Batch studies indicated particle size and rumen fluid addition had little effect on specific methane yields (SMYs). In continuous digestion of 3 cm silage the SMY was 342 and 343 L CH_4 kg^{-1} VS, respectively, with and without rumen fluid addition. However, digester operation was significantly affected through silage floating on the liquor surface and its entanglement in the mixing system. Digestion of 1 cm silage with no rumen fluid addition struggled; volatile fatty acid concentrations rose and SMYs dropped. The best case was 1 cm silage with rumen fluid addition, offering higher SMYs of 371 L CH_4 kg^{-1} VS and stable operation throughout. Thus, physical and biological treatments benefited continuous digestion of high fibre grass silage.

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1. Introduction

To meet the mandatory EU transport targets set under the Renewable Energy Directive (EC, 2009), a number of digestible feedstocks have been identified for gaseous biofuel production in Ireland including food waste (Browne and Murphy, 2013), green seaweed and slurry (Allen et al., 2014). Grass silage, a substantial crop resource, has also been recognised for its potential contribution (McEniry et al., 2013). It has been reported that digesting grass silage and dairy slurry on a 1:1 volatile solids (VS) basis can achieve over 10% renewable energy supply in transport (RES-T) using just 1.1% of grassland in the country (Wall et al., 2013). However, grass is not a homogenous feedstock and its chemical characteristics can vary significantly (McEniry and O'Kiely, 2013). Grass silage harvested at an advanced growth stage will typically have higher lignocellulosic content and lower dry solids digestibility (DSD). Optimising the digestion of this type of crop can potentially improve the knowledge employed by farmers and developers in tailoring the design of their technologies and maximising biogas production. Two treatments are investigated in this work to improve the digestibility of low DSD grass silage: particle size reduction and rumen fluid addition.

Limited literature is available on the optimum particle size of grass silage for anaerobic digestion. Previous batch digestion tests suggested that a particle size of approximately 1 cm may be optimum (Kaparaju et al., 2002). Other crop substrates such as maize, sorghum, forage rye, winter rye and triticale have been examined for the effect of particle size in batch trials, using both fresh and ensiled substrates (Herrmann et al., 2012a). Shorter chopping lengths were shown to increase the availability of fermentable

* Corresponding author at: Environmental Research Institute, School of Engineering, University College Cork, Cork, Ireland. Tel.: +353 86 0554493.
E-mail address: jerry.murphy@ucc.ie (J.D. Murphy).

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Investigating two-phase digestion of grass silage for demand-driven biogas applications: effect of particle size and rumen fluid addition

David Wall[a], Eoin. Allen[a], Ricahrd O'Shea, P.O'Kiely[b], Jerry D. Murphy[a,c].

[a] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

[b] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

[c] School of Engineering, University College Cork, Cork, Ireland

Abstract:

High lignocellulose content grass silage was investigated for two-phase digestion (leaching followed by UASB) for application to demand-driven biogas production. Leaching trials were undertaken investigating the effects of particle size reduction and rumen fluid addition on the hydrolysis and acidogenesis phases. Reducing grass silage particle size to <1 cm was not suited to leaching as particles could not be fully entrained in the system; this was not an issue at >3 cm particle size. Rumen fluid addition increased production of VFAs but reduced pH levels, which subsequently hindered hydrolysis of volatile solids (VS). When electricity demand is low, it is recommended to operate in leach only mode with grass silage particle size >3 cm and with rumen fluid addition; this limits VS destruction to 30 % while maintaining a high VFA yield. When electricity demand is high, connection of the UASB generates 61 % destruction of VS maximising biogas production.

Keywords: grass silage, particle size, rumen fluid, demand driven biogas

A perspective on algal biogas

Jerry D Murphy
Bernhard Drosg
Eoin Allen
Jacqueline Jerney
Ao Xia
Christiane Herrmann



In future energy systems, with a growing world population, it will be essential to optimise bioenergy production whilst minimising the use of finite agricultural land. Algae are suggested as a biomass source with significant growth rates, which may be cultivated in the ocean (seaweed) or on marginal land (microalgae). Biogas is suggested as a beneficial route to sustainable energy; however the scientific literatue on algae biogas is relatively sparse.

There are numerous strains and species of both macroalgae (also known as seaweed) and of microalgae. The composition of algae is highly variable depending on species, cultivation, harvest method, time of harvest and nutrients in the growing waters.

The yields of biogas vary significantly. Some seaweeds are very amenable to biogas and an argument may be made that the technology could be commercialised for co-digestion with other substrates. Some algae are not readily amenable to mono-digestion due, for example, to high levels of sulphur and sodium, or low carbon to nitrogen ratios. Many microalgae possess thick cell walls, which significantly reduces the biogas yield.

This report has an ambition of synthesising the literature and establishing a state of the art in algal biogas.

The report is aimed at an audience of academics and energy policy makers and was produced by IEA Bioenergy Task 37. Task 37 is a part of IEA Bioenergy, which is one of the 42 Implementing Agreements within IEA. IEA Bioenergy Task 37 addresses the challenges related to the economic and environmental sustainability of biogas production and utilisation.

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