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Biomethane production from grass silage: laboratory assessment to maximise yields

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

Supervisors: Professor Jerry Murphy & Dr. Pádraig O'Kiely

Head of School: Professor Nabeel Riza

June 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: David Wall

Date: 22nd June 2015

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Abstract

On-farm biogas production is typically associated with forage maize as the biomass source. Digesters are designed and operated with the focus of optimising the conditions for this feedstock. Thus, such systems may not be ideally suited to the digestion of grass. Ireland has ca. 3.85 million ha of grassland. Annual excess grass, surplus to livestock requirements, could potentially fuel an anaerobic digestion industry. Biomethane associated with biomass from 1.1 % of grassland in Ireland, could potentially generate over 10 % renewable energy supply in transport. This study aims to identify and optimise technologies for the production of biomethane from grass silage.

Mono-digestion of grass silage and co-digestion with slurry, as would occur on Irish farms, is investigated in laboratory trials. Grass silage was shown to have 7 times greater methane potential than dairy slurry on a fresh weight basis (107 m^3 t^{-1} v 16 m^3 t^{-1}). However, comprehensive trace element profiles indicated that cobalt, iron and nickel are deficient in mono-digestion of grass silage at a high organic loading rate (OLR) of 4.0 kg VS m^{-3} d^{-1}. The addition of a slurry co-substrate was beneficial due to its wealth of essential trace elements.

To stimulate hydrolysis of high lignocellulose grass silage, particle size reduction (physical) and rumen fluid addition (biological) were investigated. In a continuous trial, digestion of grass silage of <1 cm particle size achieved a specific methane yield of 371 L CH_4 kg^{-1} VS when coupled with rumen fluid addition.

The concept of demand driven biogas was also examined in a two-phase digestion system (leaching with UASB). When demand for electricity is low it is recommended to disconnect the UASB from the system and recirculate rumen fluid to increase volatile fatty acid (VFA) and soluble chemical oxygen demand (SCOD) production whilst minimising volatile solids (VS) destruction. At times of high demand for electricity, connection of the UASB increases the destruction of volatiles and associated biogas production.

The above experiments are intended to assess a range of biogas production options from grass silage with a specific focus on maximising methane yields and provide a guideline for feasible design and operation of on-farm digesters in Ireland.

Thesis output

Chapters which have been published as papers or are currently under review in peer-reviewed journals:

Chapter 3:

Wall, D.M., O'Kiely, P., Murphy, J.D., 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. Bioresource Technology 149, 425-431.

Chapter 4:

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D., 2014. Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry. Bioresource Technology 173, 422-428.

Chapter 5:

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D., 2014. The effect of trace element addition to mono-digestion of grass silage at high organic loading rates. Bioresource Technology 172, 349-355.

Chapter 6:

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

Chapter 7:

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

Chapters from peer-reviewed conference papers:

Chapter 2:

Wall D.M., Murphy J.D., O'Kiely, P., 2013. Second generation biofuel: biomethane from co-digestion of grass and slurry. 17th EGF Symposium, 23-26 June, Hof Conference Centre, Akureyri, Iceland.

Co-authored peer-reviewed publications:

Appendix A:

Allen, E., Wall, D.M., Herrmann, C., Murphy, J.D., 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.

Appendix B:

Allen, E., Wall, D.M., Herrmann, C., Xia, A., Murphy, J.D., 2015. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? Energy 81, 352-360.

Contribution to the papers

Chapter 2: I was the first author of the paper and responsible for researching and collating the relevant data.

Chapter 3: I was the first author of the paper and was responsible for the experimental design, undertaking of laboratory work and data analysis.

Chapter 4: I was the first author of the paper and was responsible for experimental design, operation of the laboratory trials for approximately 16 months, sample analysis, collating the data and providing an evaluation. Gathering and processing of raw materials was undertaken in conjunction with my colleagues.

Chapter 5: I was the first author of the paper and principally responsible for the decision making in the approach of the study. I was also responsible for undertaking the laboratory trials and performing analysis within the experimental period.

Chapter 6: I was the first author of this paper and responsible for the experimental design of the study, laboratory trials taking place over a 9 month period, sample analysis and evaluation, and data collation. The administering of raw materials was achieved in conjunction with my colleagues.

Chapter 7: I was first author of this paper and was responsible for the planning and execution of the laboratory trials over a 7 month period. I performed the experimental measurements, data compilation, analysis and evaluation.

Nomenclature

B. Ef	biomethane efficiency
BI	biodegradability index
BMP	biomethane potential assay
CH_4	methane gas
CHP	combined heat and power
CO_2	carbon dioxide gas
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
DS	dry solids (or dry matter)
EU	European Union
FOS/TAC	Flüchtige organische säuren /totales anorganisches carbonat
G:S	grass silage to slurry ratio
H_2	hydrogen gas
ha	hectare
HRT	hydraulic retention time
LBR	leach bed reactor
M t	million tonnes
NH_3	free ammonia
OLR	organic loading rate
RES-T:	renewable energy supply in transport
SCOD	soluble chemical oxygen demand
SLBR-UASB	sequential leach bed reactor/upflow anaerobic sludge blanket
SMY:	specific methane yield
TAN	total ammonical nitrogen
TEs	trace elements
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VS	volatile solids
WTE	with trace elements
wwt	wet weight

1. Introduction

1.1 Introduction and background to thesis

Anaerobic digestion is a mature technology that can provide a source of renewable gaseous fuel. The digestion process involves the conversion of organic matter to biogas (50-60 % methane) by a consortium of microorganisms in an oxygen-free environment. The generated biogas can be upgraded to biomethane (>97 % methane), which has an energy content equivalent to that of natural gas. Thus, biomethane derived from anaerobic digestion can be utilised in the same manner for applications such as heat production, electricity generation and as a compressed natural gas (CNG) transport fuel. The distribution system for biomethane can be provided through direct injection into the existing natural gas grid. Efforts to provide a more sustainable energy infrastructure in the EU has seen six countries (Denmark, Sweden, Belgium, Netherlands, France and Switzerland) agree to supply 100 % carbon neutral gas in the natural gas grids by 2050 under the green gas commitment. The EU Renewable Energy Directive requires that 10 % of all energy in transport be renewable by 2020 (known as the RES-T). Biomethane production through anaerobic digestion can significantly contribute to such targets as once injected into the grid it can be sold off-site as a transport fuel. A number of digestible feedstocks have been identified including commercial wastes, industrial wastes, agricultural residues, energy crops and macro-algae. The use of first-generation biofuels such as biodiesel from rapeseed and ethanol from wheat or maize has now been capped at 7 % in contributing to the RES-T (EC, 2015). Thus second-generation biofuels are now being sought as they do not directly compete with food production. EU regulations also permit a double weighting for second-generation biofuels contributing towards proposed RES-T targets (EC, 2009).

Germany, with over 8,000 digesters, remains the world leader in anaerobic digestion with a predominant crop feedstock of maize. In Ireland the most abundant indigenous crop is grass with approximately 3.85 m ha of grassland in the country. The majority of this grassland is used by the Irish beef and dairy industries, however, substantial quantities of grass silage in Ireland are available annually surplus to livestock requirements. Even allowing for government initiatives (such as Food Harvest 2020) that seek increases in beef and dairy production, sufficient quantities of grass are available to facilitate anaerobic digestion, provided grass management and production practices are more efficient. The resource of grass, if desired, could thus be considerable and initiate a green gas industry.

Grass is a second-generation biofuel and potentially an excellent source of biomethane. As a feedstock it has a high solids content and a high specific methane capacity. The concept of digesting grass varies greatly with multiple reactor configurations and changing substrate characteristics such as species type, date of harvest and effective pre-treatments. Digester operation can also vary in terms of loading rates, retention times and co-digestion with other available substrates. It has been suggested that mono-digestion of grass can be problematic and unstable over time. Furthermore, co-digestion with slurry has been recommended to give a more stable digestion process. This may be serendipitous as in general, slurry and silage co-exist on most Irish farms. Slurry residues are an abundant resource in Ireland. As of 2007 it was reported that over 35 M t yr^{-1} of slurry was generated in Ireland, consisting of 30.5 M t from cattle, 2.35 M t from pigs, 1.84 M t from poultry and 0.19 M t from sheep. This thesis investigates the anaerobic digestion of grass silage to achieve the highest attainable methane yields. Both batch and continuous laboratory assessments were undertaken. Aspects such as mono-digestion of grass

silage, co-digestion with dairy slurry, addition of trace elements, reactor design, hydrolysis treatments and potential demand driven biogas applications are investigated.

1.2 Thesis aims and objectives

The aims and objectives of the thesis were as follows:

- To calculate the specific methane yields (L CH_4 kg^{-1} VS) for mono-digestion of grass silage, mono-digestion of dairy slurry and co-digestion of grass silage with dairy slurry in biomethane potential (BMP) assays and in continuously stirred tank reactors (CSTRs).
- To estimate the potential for biomethane to satisfy RES-T targets in Ireland using a bioresource matrix based on quantities of excess grass available.
- To highlight the potential process limitations in producing biomethane as might occur on Irish farms. This is effected by investigating the limits of organic loading rate and hydraulic retention time in mono-digestion of grass silage and co-digestion of grass silage with dairy slurry in CSTRs.
- To develop comprehensive micronutrient profiles which compare mono-digestion of grass silage to co-digestion of grass silage with dairy slurry.
- To identify potentially deficient trace elements in mono-digestion of grass silage at high organic loading rates and furthermore, supplement these trace elements to examine the effect on biogas production and system efficiency.
- To examine the effect of particle size reduction and rumen fluid addition (in stimulating hydrolysis) on an advanced growth stage grass silage and calculate the specific methane yields (L CH_4 kg^{-1} VS) in BMPs and CSTRs.

- To examine the effect of particle size reduction and rumen fluid addition in a two-phase digestion process such as leach bed reactors with upflow anaerobic sludge blanket (LBR-UASB system) and assess its potential to operate as a demand driven biogas concept.

1.3 Thesis outline and link between chapters

The thesis is comprised of 8 chapters and 2 additional appendices. The overall theme of the chapters is the production of biomethane from grass silage through anaerobic digestion processes. Chapter 2 examines the scientific literature and succinctly reviews previous work undertaken for the digestion of grass while highlighting the upcoming EU renewable energy targets relevant to Ireland. Chapters 3 to 7 exhibit the majority of the laboratory work undertaken over a 3 year period. Chapters 3, 4, 5 and 6 are peer-reviewed journal papers and appear in the thesis as per published manuscripts. Chapter 7 is currently under review for publication. The thesis follows the academic paper model, that is, a succession of published journal papers that can be read independently or as a whole. A summary of chapters 2 to 7 is given below:

Chapter 2: *A review of grass digestion*

This chapter was adapted from a conference paper presented at the 17th European Grassland Federation conference held in Akureyi, Iceland in 2013. It focuses on the significant potential of grass as a source of biomethane, however indicates that the concept of digesting grass for gaseous biofuel varies greatly in the literature. This is due to multiple reactor configurations, a diverse range of loading rates and whether co-digestion with other substrates was examined. Feedstock related variations such as the specific grass species, date of harvest, ensilage conditions and application of

pre-treatments are also highlighted. The abundance of literature has left a wide range of results in terms of reported specific methane yields (SMYs). It is highlighted that mono-digestion of grass can be somewhat problematic and unstable over time. This is thought to be due to a deficiency of essential trace elements in long term operation; co-digestion with slurry is proposed as providing a more stable digestion process. The relevant EU renewable transport targets are also emphasised. The Renewable Energy Directive of the European Union states that 10 % of all energy in transport must be renewable by 2020 (RES-T). As a second-generation biofuel, grass qualifies for a double weighting (EC, 2009) under the RES-T in considering the 2020 target of 10 % RES-T. Thus, to satisfy Ireland's EU target of 10 %, only 5 % RES-T would be required if the industry was founded on the digestion of grass. The distribution system for the gaseous biofuel is recommended to be the existing natural gas grid.

Chapter 3: *The potential for biomethane from grass and slurry to satisfy renewable energy targets*

Chapter 3 documents the potential for excess grass silage produced in Ireland to contribute towards renewable transport targets set by the EU. Biomethane potential (BMP) assays are undertaken to illustrate the SMYs obtainable from grass silage (107 m^3 CH_4 t^{-1}), dairy slurry (16 m^3 CH_4 t^{-1}) and co-digestion of the two substrates at various volatile solids mixture ratios (27−79 m^3 CH_4 t^{-1}). BMP assessments at a range of co-digestion ratios indicate methane yields were between 4−11 % lower than the pro-rata values calculated from mono-digestion. The calculated methane yields were then matched to quantified resources of grass silage in Ireland to create a bioresource matrix of potential scenarios for producing biomethane. The paper

suggests that co-digestion of the majority of slurry produced from dairy cows in Ireland with grass silage quantities equivalent to 1.1 % of grassland on a 50:50 volatile solids basis would generate over 10 % renewable energy supply in transport. The industry proposed would equate to 170 digesters each treating 10,000 t a^{-1} of grass silage and 40,000 t a^{-1} of slurry from dairy cows.

Chapter 4: *Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry*

Chapter 4 further develops the work undertaken in Chapter 3, focusing on continuous digestion of the substrates using 5 L continuously stirred tank reactors (CSTRs) to give a more realistic interpretation of full-scale digestion processes. Specific focus is put on digester performance whilst increasing the reactors organic loading rate (OLR). Operational parameters that could not be evaluated at BMP scale could now be assessed in detail. Six CSTRs were operated for a period of 62 weeks and reported SMYs for the mono-digestion of grass silage, mono-digestion of dairy slurry and co-digestion of the two substrates (at volatile solids ratios 20:80, 40:60, 60:40, and 80:20). The results indicate that higher proportions of grass silage in the substrate mix, generated higher SMYs. Mono-digestion of grass silage could be undertaken successfully to an OLR of 3.5 kg VS m^{-3} d^{-1} generating a SMY of 398 L CH_4 kg^{-1} VS. Increasing the OLR to 4.0 kg VS m^{-3} d^{-1} was not as effective and the SMYs dropped. If the grass silage was co-digested with 20 % dairy slurry the OLR could be increased to 4.0 kg VS m^{-3} d^{-1} generating a SMY of 349 L CH_4 kg^{-1} VS. Higher OLRs force the hydraulic retention time (HRT) to decrease; and a retention of less than 20 days proved to be a limiting factor in the operation of grass digesters.

Chapter 5: *The effect of trace element addition to mono-digestion of grass silage at high organic loading rates*

Chapter 5 expands upon the work carried out in Chapter 4 by considering the effect of trace element addition in digestion. Two reactors that were operated in Chapter 4 are further investigated, specifically, mono-digestion of grass silage and co-digestion of grass silage with 20 % dairy slurry (VS basis). These two reactors were most promising in terms of generating the highest SMYs with increased organic loading rates. Trace element profiles are developed for both reactors from a low loading rate of 2.0 kg VS m^{-3} d^{-1} to a high loading rate of 4.0 kg VS m^{-3} d^{-1}. The addition of dairy slurry in co-digestion is found to provide sufficient trace elements for the digestion process as indicated through stable volatile fatty acid (VFA) profiles and high SMYs. Three trace elements are found to be undersupplied in the mono-grass digester in comparison, namely, cobalt, iron and nickel. Supplementation of the identified trace elements to the mono-grass digester, at rates equivalent to that found in the co-digestion reactor, led to an increase in SMY by 12 % to 404 L CH_4 kg^{-1} VS and provided a much more settled VFA profile.

Chapter 6: *Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage*

Whereas Chapters 3, 4 and 5 dealt with good quality grass silage with high dry solids digestibility (DSD), Chapter 6 focuses on the anaerobic digestion of advanced growth stage grass silage with lower DSD. Overcoming potential digestion difficulties is trialled using two treatments to stimulate hydrolysis of the high fibre crop. A physical treatment is effected by chopping the silage to two different particle

sizes – less than 1 cm (<1 cm) and greater than 3 cm (>3 cm). A biological treatment of rumen fluid addition, at a specific rate, is also examined. Laboratory trials are assessed through BMPs and furthermore, the operation of two 5 L CSTRs to attain SMYs and evaluate the potential benefits of the hydrolytic treatments. The BMP trials indicated little difference in terms of treatments added as methane yields were at a similar range. Continuous digestion in CSTRs was found to offer a more valuable insight. The longer particle size (>3 cm) instigated serious mechanical issues such as inadequate mixing in the reactor and floating of grass silage on the liquor surface. Shorter particle sizes (<1 cm) were deemed more appropriate for digestion in the CSTRs, however, the microorganisms struggled to breakdown the high fibre crop without the addition of rumen fluid. An SMY of 371 L CH_4 kg^{-1} VS and a stable digestion process was obtained for digestion at <1 cm particle size with rumen fluid added at a rate of 50 mL per kg silage.

Chapter 7: *Investigating two-phase digestion of grass silage for demand-driven biogas applications: effect of particle size and rumen fluid addition*

The application of two-phase digestion as a demand driven biogas concept is examined in Chapter 7. The grass silage used is again of advanced growth stage with low DSD with treatments of particle size (<1 cm and >3 cm) and rumen fluid addition once again employed. Four leaching trials are undertaken demonstrating the first phase (hydrolysis and acidogenesis) of two-phase digestion using leach bed reactors (LBRs). Reduction of particle size to <1 cm was not suitable in the LBRs due to a combination of mechanical issues. Considerations of pH range, VFA production and soluble COD production were undertaken to identify the best conditions for producing a high quality leachate. Rumen fluid addition increased

VFA production but hampered hydrolysis of volatile solids (VS) due to low pH. VS destruction for >3 cm particle size grass silage without rumen fluid addition (42 %) was better than the same grass with rumen fluid addition (30 %). The destruction rate increased to 61 % when the LBRs were run in tandem with an upflow anaerobic sludge blanket (UASB). A demand driven biogas process is recommended as follows: when electricity demand is low the system can go “off-line”, with leachate recirculation and rumen fluid addition, to maximise leachate potential while minimising VS destruction; when electricity demand is high, connection to the UASB increases destruction of VS and increases biogas production.

References

EC. 2009. Directive 2009/28/EC of The European Parliament and of The Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official Journal of the European Union.

EC, 2015. European Parliament: Environment committee backs compromise on cleaner biofuels. Press release. Available from: *http://www.europarl.europa.eu/news/en/news-room/content/20150413IPR41649/html/Environment-committee-backs-compromise-on-cleaner-biofuels*

2. A review of grass silage digestion

2.1 First and second generation biofuels

First-generation biofuels are based on food crops and typically include ethanol produced from wheat or maize, or biodiesel produced from rapeseed oil. Second-generation biofuels are based on non-edible crops, including wood, straw, residues and perennial grass. The Renewable Energy Directive of the European Union (EC, 2009) requires 10 % of all energy in transport to be renewable by 2020. As of April 2015, the use of food-based first-generation biofuels has been capped at 7 % in contributing to EU renewable transport targets (EC, 2015), thus stimulating the development of second-generation biofuels from non-edible feedstocks (EC, 2012). The Renewable Energy Directive also requires biofuels to emit a minimum of 60 % less greenhouse gases than the fossil fuel they replace. First-generation liquid biofuels will struggle to satisfy this target. Typical values of targets listed in the Renewable Directive (EC, 2009) are 32 % for wheat ethanol and 45 % for rapeseed biodiesel. Utilising grass as a source of biomethane negates the need for tillage, and allows for carbon sequestration. Korres *et al.* (2010) have shown that grass biomethane, when used as a transport fuel, can effect a 75 % reduction in emissions when allowing for the fact that permanent grasslands can sequester 0.6 t C ha^{-1} yr^{-1}. Smyth *et al.* (2009) have shown that the energy production per hectare (both gross and net) for grass biomethane is significantly higher than for first-generation liquid biofuels. This suggests that the quantity of land required to satisfy energy in transport is significantly less than that required if first-generation biofuels are used. Biogas has been used across Europe principally as a source of combined heat and power (CHP). More recently, the concept of upgrading biogas to biomethane and supplying it to the gas grid for use as a direct replacement for natural gas (in producing electricity, heat and transport fuel) has become popular. As of 2012,

Europe had 137 biogas plants with biomethane upgrading systems for injection into natural gas grids or for use as a transportation fuel (Oechsner *et al.*, 2015).

2.2 Anaerobic digestion process

Anaerobic digestion is a process whereby microorganisms break down organic matter in the absence of oxygen to produce end products of biogas and digestate. The process is split into four phases – hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis occurs first as the carbohydrates, fats and proteins of the feedstock are broken down by hydrolytic bacteria into smaller constituents (monomers) of sugars, fatty acids and amino acids. Further breakdown occurs in the second phase (acidogenesis) where acidogenic bacteria convert the products of hydrolysis to carbon dioxide (CO_2), hydrogen (H_2), ammonia, volatile fatty acids (VFA) and alcohols. The third step (acetogenesis) occurs as acetogens (or acetogenic bacteria) convert the products of acidogenesis to predominantly acetic acid as well as CO_2 and H_2. Finally in the methanogenesis step, the products from acetogenesis are converted to methane (CH_4) and CO_2 via strictly anaerobic methanogens (methanogenic archaea). The resultant biogas from an anaerobic digestion process is typically in the range of 50−60 % CH_4 and 40−50 % CO_2. Digestate is the effluent produced from the end of the digestion process, which can be separated into solid and liquid components and used as a fertiliser or bedding material.

2.3 Grass silage as a feedstock for anaerobic digestion

It has been reported that approximately 1,500 M t of agricultural biomass is available for digestion in the EU each year, half of which is crop material (Lehtomäki *et al.*, 2008a). While maize is the predominant crop used, grass silage is accounted for in

over 50 % of digesters operating in Germany and Austria (Prochnow *et al*., 2009). Perennial ryegrass is the principal species of reseeded temperate grassland in Europe, but other species, such as Italian ryegrass, timothy, cocksfoot and tall fescue, are also common (McEniry & O'Kiely, 2013; Nizami *et al.*, 2009), while permanent pastures of mixed botanical composition are widespread in many regions. Grass is ensiled to ensure a year-round supply of feedstock (Koch *et al.*, 2009). Good ensiling, where lactic acid production dominates the fermentation, will efficiently conserve grass as a feedstock for anaerobic digestion. In contrast, poor storage conditions can lead to over 50 % losses in potential methane yield (Pakarinen *et al.*, 2008). Ensiling very wet herbage can result in a loss of leachate, but this can be collected and beneficially added to the digester (McEniry *et al.*, 2011).

The specific methane yield (SMY), measured in litres of methane per kg volatile solids (L CH_4 kg^{-1} VS), attainable from a specific grass substrate is subject to its characteristic and seasonal variations (Prochnow *et al*., 2005). In general, the SMY of grass silage will increase the earlier the harvest date while the yield of biomass obtainable per unit grassland area will depend on the attainable growth of the crop (Prochnow *et al*., 2009). This has been demonstrated in different studies where the SMYs of first-harvest grasses (leafy/vegetative stage) are higher than their respective second harvests (stemmy/flowering stage) (Lehtomäki *et al.*, 2008b; Seppälä *et al.*, 2009). The increase in lignified fibre structure, due to later a harvest date, leads to a slower rate of degradation and also increases the required hydraulic retention time (HRT) (McEniry & O'Kiely, 2013; Prochnow *et al.*, 2005). However, on the contrary, species of timothy clover and reed canary grass have been shown to give higher SMYs with advancing crop maturity due to a decreased water content (Lehtomäki *et al*., 2008b).

2.4 Reactor configurations

The dry solids (DS) content of a particular grass substrate can vary from less than 150 g kg^{-1} to greater than 500 g kg^{-1} meaning both wet and dry digestion technologies can be employed (Lehtomäki *et al.*, 2008a; Lehtomäki *et al.*, 2007). The traditional system for wet anaerobic digestion is a continuously stirred tank reactor (CSTR). This is a one-phase system where all four steps of the digestion process occur in one vessel that is constantly mixed. Operative temperatures can be mesophilic (37°C) or thermophilic (55°C). CSTRs receive a continuous supply of feed while having a similar amount of substrate (digestate) removed simultaneously. This is the most conventional type of digester due to its simple design and relatively low cost. However CSTRs are limited in operation by the DS content, with typical values within the reactor kept below 120 g kg^{-1} to ensure effective mixing. Wet digestion of grass silage may require a high input of water for dilution, which increases energy requirements for pumping and heating the material (Jagadabhi *et al.*, 2011; Lehtomäki *et al.*, 2008a). In wet processes with inefficient mixing, grass substrates have a tendency to float on the surface of the liquor in a reactor and form layers of scum (Lehtomäki *et al.*, 2007; Thamsiriroj & Murphy, 2010).

Alternative systems to the traditional CSTR are available. Leach bed reactors (LBR) in conjunction with an upflow anaerobic sludge blanket (UASB) reactor may be used as a two-phase system. This process separates the hydrolysis/acidogenesis phase of digestion from acetogenesis/methanogenesis phase. This is achieved by loading the feedstock into LBRs, which are subsequently sprinkled with continuously recirculated leachate. The leachate is ultimately sent to the UASB for biogas production (Jagadabhi *et al.*, 2011; Lehtomäki *et al.*, 2008a; Nizami & Murphy, 2011). The overriding advantages of two-phase systems are a reduced HRT,

improved hydrolysis rates and higher methane concentrations in the biogas (Jagadabhi *et al.*, 2011; Nizami *et al.*, 2012; Yu *et al.*, 2002). Nizami *et al.* (2012) produced a biogas with a methane composition of 71 % from a leach bed-UASB system while a 52 % methane composition was reported for the same grass silage in a CSTR. Aslanzadeh *et al.* (2013) also demonstrated that the ratio of methane to carbon dioxide was increased for a UASB in comparison to a CSTR.

2.5 Physical, chemical and biological treatments

The rate-limiting step in the digestion of grass silage is the hydrolysis of its lignocellulosic components (Cirne *et al.*, 2007; Wang *et al.*, 2009; Xie *et al.*, 2011b). The structure of grass silage is comprised primarily of cellulose, hemicellulose and lignin (up to 75 % of its dry matter content) and hence, microbes are restricted in the breakdown of such fibrous material (Wang *et al.*, 2009; Xie *et al.*, 2011a). Various chemical, biological and physical treatments have been documented in order to increase digestibility (Jagadabhi *et al.*, 2008; Nizami *et al.*, 2009). Physical treatment typically involves the maceration of grass silage to more suitable particle sizes. A particle size of 1 cm has been reported to be optimal for anaerobic digestion (Kaparaju *et al.*, 2002). A study on the effects of combined thermal and alkali pre-treatment showed that application of 100°C combined with addition of 5 % NaOH (5.0 g 100 g^{-1}) in the feedstock could enhance the biodegradability of such lignocellulosic materials and increase SMYs by as much as 38 % (Xie *et al.*, 2011a). However, such chemical pre-treatments that rely on higher input temperatures increase the process energy requirements and hence result in additional costs (Xie *et al.*, 2011a). The effect of a biological treatment additive on methane production at ensiling stage has also been investigated. It was found that the additive, containing

both lactic acid bacteria and enzymes (cellulose, pectinase and xylanase), did not enhance methane yields with various grass crops (Pakarinen *et al.*, 2008). Another study focused on the recirculation of alkali-treated solids from the digestate to assess the impact on methane production (Jagadabhi *et al.*, 2008). However, they were not found to be effective in destroying lignocellulosic structures.

Aside from the aforementioned pre-treatments, supplementation of trace elements is another potential route in maximising digester efficiency and performance. The addition of cobalt as a trace element to a system digesting grass-clover silage has been shown to increase methane yields by increasing the conversion rate of acetate. A lower limiting critical concentration for cobalt of 0.02 mg L^{-1} was advised for mono-digestion of grass (Jarvis *et al.*, 1997).

2.6 Co-digestion of grass silage with slurry

Co-digestion of grass silage with animal residues, or slurries, provides an alternative option in biogas production (Jagadabhi *et al.*, 2010; Lehtomäki *et al.*, 2008a; Lehtomäki *et al.*, 2007). Mono-digestion of farm slurries is typically low yielding as the vast majority of the substrate's energy content has already been eliminated through the digestive tract of the animal (Lehtomäki *et al.*, 2007; Weiland, 2003). This is exacerbated by the low DS content. If the DS content of the slurry is at 80 g kg^{-1} with 750 g VS kg^{-1} DS, then the methane production may be as low as 6 m^3 t^{-1}. The primary incentive for mono-digestion of grass silage is high volumetric yields of methane. It is, however, prone to process imbalance and hence co-digestion with slurry could alleviate some of these issues (Jagadabhi *et al.*, 2010). Co-digestion of substrates can potentially provide a synergistic effect by balancing of nutrients and enhancing the digestion process (Pagés-Díaz *et al.*, 2014). Addition of grass silages

to slurry in co-digestion has been shown to increase methane production yields (Koch *et al.*, 2009; Wang *et al.*, 2009). The addition of slurry to grass silage in a digester can potentially stabilise pH, counter-act ammonia inhibition and provide a more optimum C:N ratio for the process; all of which promote methanogenesis (Xie *et al.*, 2011b). As stated, methane yields from grass silage digestion are significantly in excess of that achievable from the digestion of farm slurries. A developer of a digester may hesitate in diluting the yield of methane generated in grass silage digestion through addition of farm slurry. However, the rationale for co-digestion is potentially increasing the SMY relative to the calculated pro-rata yield; that is, provide a synergistic relationship by combining the two substrates. Co-digestion may also provide a more stable process whilst utilising two co-existing feedstocks on Irish farms.

A number of studies have investigated the grass-slurry mix ratio for co-digestion but with varying results. In the literature, there is a tendency to use the term manure when describing what the authors would consider slurry, with a DS content of less than 100 g kg^{-1}. A co-digestion study of cow manure (65 g DS kg^{-1} and 51 g VS kg^{-1}) and grass silage (75 % timothy, Phleum pratense and 25 % meadow fescue, Festuca pratensis) indicated that a 30 % crop share, on a VS basis, was optimum in obtaining the highest SMY (Jagadabhi *et al.*, 2008). An increase to 40 % crop share in the co-digestion mix was suggested to negatively impact the SMYs by as much as 12 % (Lehtomäki *et al.*, 2007). A biomethane potential (BMP) assay that focused on co-digestion of pig manure and grass silage indicated a mix ratio of 1:1 (VS pig manure: VS grass silage) was best to achieve a high SMY with relatively short lag phase (Xie *et al.*, 2011b). If the source of slurry is dilute, one option is to concentrate the solid material before introducing it to the system as a co-substrate to ensure a higher solids

loading and, in return, more biogas per unit volume than raw manure (Asam *et al.*, 2011; Xie *et al.*, 2011b; Xie *et al.*, 2012). Also, as biomass transport costs can potentially be high, separating the solid content in the slurry can provide a cost-saving alternative. A study on the co-digestion of solid pig manure and dried grass silage in continuously stirred tank reactors (CSTRs) suggested that crop shares of 20 %, 30 % and 40 % (VS basis) could all be operated successfully but showed little difference in terms of methane output with a low loading rate of 1 kg VS m^{-3} d^{-1} giving the highest VS destruction (Xie *et al.*, 2012). Additional literature has, on the contrary, recommended higher grass silage proportions of up to 75 % with cow manure (Comino *et al.*, 2010). However, 80 % share of crop was suggested to have an inhibitive effect.

2.7 Operational considerations in biogas production from grass silage

The organic loading rate (OLR) is a critical parameter in an anaerobic digestion system. If the OLR is too high, it can lead to system failure through volatile fatty acid accumulation and/or ammonia inhibition (Xie *et al.*, 2012). Specific studies related to the effect of OLR on grass digestion or co-digestion of grass silage with slurry are limited. However, it has been reported that doubling the OLR from 2.0 to 4.0 kg VS m^{-3} d^{-1} decreased SMYs in the digestion of cow manure and grass silage (40 % VS crop share on a VS basis) from 268 to 186 L CH_4 kg^{-1} VS (Lehtomäki *et al.*, 2007). This was attributed to an inadequate retention time, which did not allow for effective degradation. Hence the hydraulic retention time (HRT) also becomes a critical parameter in the digestion of grass silage. In a study of solid pig manure and dried grass silage, increasing the OLR from 1.0 to 3.0 kg VS m^{-3} d^{-1} again decreased the SMYs by an average of 38 % (Xie *et al.*, 2012). The OLRs used in mono-

digestion of grass silage have primarily been kept at a low range of approximately 1.0 to 2.5 kg VS m^{-3} d^{-1}.

The tendency for grass silage to float at the top liquor level of a CSTR can be problematic in achieving stable digestion (Thamsiriroj & Murphy, 2010). Without an efficient mixing system, grass particles form a floating mass that accumulates, dries and becomes a barrier between the liquor and gas in the digester. Maceration of grass silage to a suitably small particle size reduces its tendency to float; it is also advisable that the mixing system breaks the surface and pulls the floating grass sods into the liquor (Thamsiriroj & Murphy, 2010). In such wet digestion systems, it is important to keep the DS content at levels below 120 g kg^{-1} through water dilutions or recirculation of liquor separated from the digestate. Such recirculation in a CSTR must be carefully monitored. A study digesting alfalfa silage was shown to increase pH and alkalinity initially through recirculation but this subsequently resulted in an accumulation of organic and inorganic material, which inhibited hydrolysis and methanogenesis (Nordberg *et al*., 2007). The same study reported that inhibition could be overcome by replacing half of the recirculated liquor with water, which diluted the effect. Recirculation of effluent liquor was also examined in the continuous digestion of grass silage liquor. Results illustrated that, combining an increased OLR with effluent liquor recycling, allowed for higher methane yields. This was attributed to the recycled material acting as a source of inoculum and thus helping the bacteria adapt to the process (Abu-Dahrieh *et al*., 2011). Thamsiriroj *et al*., (2012) found that recirculation of leachate from the second step of a two-stage wet stirred system, mono-digesting grass silage, removed any need for the dilution of grass, and allowed a sharing of the work by the two digesters. Overall an OLR of 2.5 kg VS $m^{-3}d^{-1}$ was achieved with a methane production of 455 L kg^{-1} VS; which

equated to over 90 % destruction of VS. Raising the OLR to 3.0 kg VS m^{-3} d^{-1} led to system failure.

2.8 Specific methane yields (SMYs) from grass silage

The SMYs for grass silage reported in past literature are documented in Table 2.1 and exhibit a broad range of values from 140 to 510 L CH_4 kg^{-1} VS. The considerable variation in methane production from grass silage can be attributable to the different species of grass, the fertility of the field, the application rates of fertilizer, the time of harvest, and the ensiling process. Issues of variability are also prevalent in slurries and manures such as the slurry source (swine, cattle, dairy), the animal housing (concrete slats or straw bedding), the diet of the animals (fed concentrate or not) and water dilution (rain water or wash water mixed with the slurry). As a result of these issues, it is difficult to directly compare data from the scientific literature. Table 2.2 outlines a selection of data from the literature on co-digestion of grass with slurries and manure. Co-digestion of these two substrates in literature is limited. The SMYs range from 143 to 304 L CH_4 kg^{-1} VS.

Table 2.1 Selected results from scientific literature reporting the SMYs from mono-digestion of grass

Substrate	Harvest/Treatment	Reactor	Yield ($L\ CH_4\ kg^{-1}\ VS$)	OLR	Temperature
Grass silage (Lehtomäki *et al.*, 2008a) 75 % timothy (*Phleum pratense*), 25 % meadow fescue (*Festuca pratensis*)	Cut at early flowering stage, pre-wilted for 24 hours, bunker ensiled with additive, chopped to 3cm	Leach Bed UASB	197	5.0 kg COD $m^{-3}\ d^{-1}$	35°C
Grass silage (Koch *et al.*, 2009)	Harvested late summer (haylage 50 % DS), pre-wilted, bunker ensiled with no additive, dried and chopped to maximum of 6mm	Loop Reactor	260	3.5 kg VS $m^{-3}\ d^{-1}$	38°C
Grass silage (Wichern *et al.*, 2009)	Untreated and pre-treated heterofermentative ensiled grass	Mono-fermenter	300-360	0.3-2.5 kg VS $m^{-3}\ d^{-1}$	38°C
Grass silage (Jagadabhi *et al.*, 2011) 75 % timothy (*Phleum pratense*), 25 % meadow fescue (*Festuca pratensis*)	Cut at early flowering stage, pre-wilted for 24hrs, bunker ensiled with additive, chopped to 5-6cm	BMP	360	NA	35°C

Table 2.1 continued

Substrate	Harvest/Treatment	Reactor	Yield ($L\ CH_4\ kg^{-1}\ VS$)	OLR	Temperature
Grass silage (Jagadabhi *et al.*, 2011) 75 % timothy (*Phleum pratense*), 25 % meadow fescue (*Festuca pratensis*)	Cut at early flowering stage, pre-wilted for 24hrs, bunker ensiled with additive, chopped to 5-6cm	Leach Bed-UASB	140	Up to 7.0 kg COD $m^{-3}\ d^{-1}$	37°C
Grass Silage (Xie *et al.*, 2011) Perennial ryegrass (*Lolium perenne*)	Harvested in June, field-wilted for 24 hours, baled in polythene film, oven dried at 60°C, chopped to 10mm, pretreated with an assortment of temperatures and NaOH concentrations	BMP	326-452	NA	35°C
Grass Silage (Amon *et al.*, 2007) Six grassland variants in mountainous and valley regions in Austria	One to four cuts per year at varying stages of vegetation, all grass was ensiled	BMP	128-392	NA	38°C
Grass mixture (Jagadabhi *et al.*, 2010) 75 % timothy (*Phleum pratense*), 25 % meadow fescue (*Festuca pratensis*)	Cut at early flowering stage, pre-wilted for 24hrs, bunker ensiled with additive, chopped to 5-6cm	BMP	400	NA	35°C

Table 2.1 continued

Substrate	Harvest/Treatment	Reactor	Yield ($L\ CH_4\ kg^{-1}\ VS$)	OLR	Temperature
Grass (Seppälä *et al.*, 2009) Four grass species – cocksfoot (*Dactylis glomerata L.*), tall fescue (*Festuca arundinaceae Schreb.*), reed canary grass (*Phalaris arundinaceae L.*), Timothy (*Phleum pratense L.*)	Harvested at different periods over 3 year period, chopped to a particle size of approximately 1cm, oven dried at 60°C	BMP	342 Cocksfoot Avg. 336 Tall fescue Avg. 310 Timothy Avg. 296 Reed canary Avg.	NA	35°C
Grass silage (McEniry & O'Kiely, 2013) Perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum*), timothy (*Phleum pratense*), cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*)	Each grass type was harvested at 3 separate dates (May, June and July), cut to an average size 6cm, dried and milled for trials	BMP	223 Cocksfoot Avg. 237 Tall fescue Avg. 244 Timothy Avg. 242 Italian ryegrass Avg. 246 Perennial ryegrass Avg.	NA	38°C
Grass silage (Pakarinen *et al.*, 2008) Two substrates: A mixture of timothy (*Phleum pratense*), red clover (*Trifolium pratense*) and meadow fescue (*Festuca arundinacea*) and ryegrass (*Lolium multiflorum)*	Harvested in June, August and September for field and laboratory trials, chopped to 5cm particle size, with and without drying at 24 and 48 hours at 20°C, ensiled with and without additive	BMP	140-510 Mixed grass 320-510 Ryegrass	NA	NA

Table 2.1 continued

Substrate	Harvest/Treatment	Reactor	Yield ($L\ CH_4\ kg^{-1}\ VS$)	OLR	Temperature
Fresh grass and grass silage (Mahnert *et al.*, 2005) Perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and meadow foxtail (*Alopecurus pratensis*)	Harvested in May (first cut), wilted for 24 hours at 25°C, both fresh and ensiled grass used	BMP	310-360	NA	35°C
Fresh grass (Mahnert *et al.*, 2005) Perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and meadow foxtail (*Alopecurus pratensis*)	Second cut, fresh grass, same quantity of each species	CSTRs	302-329	0.71-1.42 kg VS $m^{-3}\ d^{-1}$	35°C
Fresh grass (Prochnow *et al.*, 2005) Meadow Foxtail grassland vegetation (*Alopecuretum pratensis* association) mainly consisting of Couch (*Elymus repens*), Meadow Foxtail (*Alopecurus pratensis*), Smooth Meadow Grass (*Poa pratensis*), Stinging Nettle (*Urtica dioica*), Creeping Thistle (*Cirsium arvense*) and Cow Parsley (*Anthriscus sylvestris*).	Harvested monthly over 3 year period, chopped to less than 30mm	BMP	155-298	NA	35°C

Table 2.1 continued

Substrate	Harvest/Treatment	Reactor	Yield ($L\ CH_4\ kg^{-1}$ VS)	OLR	Temperature
Fresh grass (Lehtomäki *et al.*, 2008) Timothy-clover grass (67.5 % timothy *P. pratense*, 22.5 % meadow fescue *Festuca pratensis*, 10.0 % red clover *T. pratense*), Reed canary grass (*P. arundinacea*) and grass cuttings	Harvested crops at two maturity stages, grass cuttings collected early summer, chopped to 1cm particle size	BMP	370-380 Timothy-clover grass 340-430 Reed canary grass 300 Grass cuttings	NA	35°C
Grass silage (Weiland, 2003) Ryegrass	NA	NA	410	NA	NA
Grass Silage Liquor (Abu-Dahrieh *et al.*, 2011)	Sieved through 1mm mesh to remove solids	BMP	0.385 $L\ CH_4\ kg^{-1}$ COD	0.851–1.77 kg COD d^{-1}	38°C
Grass Silage (Thamsiriroj *et al.*, 2012) Perennial ryegrass (*Lolium perenne*)	First cut, field wilted 24 hours, baled in polythene stretch film, no additive	CSTR (two-stage)	455	2.5 kg VS m^{-3} d^{-1}	37°C
Grass Silage (Nizami & Murphy, 2011) Perennial ryegrass (*Lolium perenne*)	First cut, field wilted 24 hours, baled in polythene stretch film, no additive	Leach bed-UASB	341	1.9 kg VS m^{-3} d^{-1}	37°C

Table 2.2 Selected results from scientific literature reviewing the SMYs from co-digestion of grass and slurry

Substrate	Co-digestion ratio	Reactor	Yield ($L\ CH_4\ kg^{-1}$)	OLR	Temperature
Grass silage (GS) and pig manure (PM) (Xie *et al.*, 2011b)	PM:GS (VS basis) 1:0 3:1 1:1 1:3 0:1	BMP	267-304	NA	35°C
Grass silage (GS) and cow manure (CM) (Lehtomäki *et al.*, 2007) 75 % timothy (Phleum pratense), 25 % meadow fescue (Festuca pratensis); cow manure from dairy farm	CM:GS (VS basis) 10:1 5:1 10:3 5:2	CSTRs	143-268	2–4 $kg\ VS\ m^{-3}\ d^{-1}$	35°C
Grass silage (GS) and cow manure (CM) (Jagadabhi *et al.*, 2008) 75 % timothy (Phleum pratense), 25 % meadow fescue (Festuca pratensis); manure obtained from dairy farm; re-circulation of alkali treated and untreated solids	CM:GS (VS basis) 7:3	CSTRs	143-188	2–2.5 $kg\ VS\ m^{-3}\ d^{-1}$	35°C
Grass silage (GS) and cattle slurry (CM) (Mahnert *et al.*, 2005) Perennial ryegrass (Lolium perenne), cocksfoot (Dactylis glomerata) and meadow foxtail (Alopecurus pratensis); slurry from dairy farm	CM:GS (VS basis) 3:1 1:3	CSTRs	290 Avg. value from data provided	0.7-1.4 $kg\ VS\ m^{-3}\ d^{-1}$	35°C

References

Abu-Dahrieh, J., Orozco, A., Groom, E., Rooney, D. 2011. Batch and continuous biogas production from grass silage liquor. *Bioresource Technology*, 102(23), 10922-10928.

Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H., Schreiner, M., Zollitsch, W. 2007. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technology*, 98(17), 3204-3212.

Asam, Z.-u.-Z., Poulsen, T.G., Nizami, A.-S., Rafique, R., Kiely, G., Murphy, J.D. 2011. How can we improve biomethane production per unit of feedstock in biogas plants? *Applied Energy*, 88(6), 2013-2018.

Aslanzadeh, S., Rajendran, K., Jeihanipour, A., Taherzadeh, M. 2013. The Effect of Effluent Recirculation in a Semi-Continuous Two-Stage Anaerobic Digestion System. *Energies*, 6(6), 2966-2981.

Cirne, D., Lehtomäki, A., Björnsson, L., Blackall, L. 2007. Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *J Appl Microbiol.*, Sep(103(3)), 516-27.

Comino, E., Rosso, M., Riggio, V. 2010. Investigation of increasing organic loading rate in the co-digestion of energy crops and cow manure mix. *Bioresource Technology*, 101(9), 3013-3019.

EC. 2009. Directive 2009/28/EC of The European Parliament and of The Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. *Official Journal of the European Union.*

EC. 2012. EU Commission tackles the climate impacts of biofuel production. Press release. Available In: *http://ec.europa.eu/clima/policies/transport/fuel/documentation_en.htm accessed 30 October 2012*.

EC, 2015. European Parliament: Environment committee backs compromise on cleaner biofuels. Press release. Available from: *http://www.europarl.europa.eu/news/en/news-room/content/20150413IPR41649/html/Environment-committee-backs-compromise-on-cleaner-biofuels*.

Jagadabhi, P., Lehtomäki, A., Rintala, J. 2008. Co-digestion of grass silage and cow manure in a CSTR by re-circulation of alkali treated solids of the digestate. *Environ Technol.*, Oct(29(10):1085-93.).

Jagadabhi, P.S., Kaparaju, P., Rintala, J. 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology*, 101(8), 2818-2824.

Jagadabhi, P.S., Kaparaju, P., Rintala, J. 2011. Two-stage anaerobic digestion of tomato, cucumber, common reed and grass silage in leach-bed reactors and upflow anaerobic sludge blanket reactors. *Bioresource Technology*, 102(7), 4726-4733.

Jarvis, Å., Nordberg, Å., Jarlsvik, T., Mathisen, B., Svensson, B.H. 1997. Improvement of a grass-clover silage-fed biogas process by the addition of cobalt. *Biomass. Bioenerg.*, 12(6), 453-460.

Kaparaju, P., Luostarinen, S., Kalmari, E., Kalmari, J., Rintala, J. 2002. Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Sci Technol*, 45(10), 275-80.

Koch, K., Wichern, M., Lübken, M., Horn, H. 2009. Mono fermentation of grass silage by means of loop reactors. *Bioresource Technology*, 100(23), 5934-5940.

Korres, N.E., Singh, A., Nizami, A.-S., Murphy, J.D. 2010. Is grass biomethane a sustainable transport biofuel? *Biofuels, Bioproducts and Biorefining*, 4(3), 310-325.

Lehtomäki, A., Huttunen, S., Lehtinen, T.M., Rintala, J.A. 2008a. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresource Technology*, 99(8), 3267-3278.

Lehtomäki, A., Huttunen, S., Rintala, J.A. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resour. Conserv. Recy.*, 51(3), 591-609.

Lehtomäki, A., Viinikainen, T.A., Rintala, J.A. 2008b. Screening boreal energy crops and crop residues for methane biofuel production. *Biomass and Bioenergy*, 32(6), 541-550.

Mahnert, P., Heiermann, M., Linke, B. 2005. Batch- and Semi-continuous Biogas Production from Different Grass Species. *CIGR E-Journal* Volume 7(1682-1130).

McEniry, J., O'Kiely, P., Crosson, P., Groom, E., Murphy, J.D. 2011. The effect of feedstock cost on biofuel cost as exemplified by biomethane production from grass silage. *Biofuels, Bioproducts and Biorefining*, 5(6), 670-682.

McEniry, J., O'Kiely, P. 2013. Anaerobic methane production from five common grassland species at sequential stages of maturity. *Bioresource Technology*, 127(0), 143-150.

Nizami, A.-S., Korres, N.E., Murphy, J.D. 2009. Review of the Integrated Process for the Production of Grass Biomethane. *Environmental Science & Technology*, 43(22), 8496-8508.

Nizami, A.-S., Murphy, J.D. 2011. Optimizing the Operation of a Two-Phase Anaerobic Digestion System Digesting Grass Silage. *Environmental Science & Technology*, 45(17), 7561-7569.

Nizami, A.S., Orozco, A., Groom, E., Dieterich, B., Murphy, J.D. 2012. How much gas can we get from grass? *Applied Energy*, 92(0), 783-790.

Nordberg, Å., Jarvis, Å., Stenberg, B., Mathisen, B., Svensson, B.H. 2007. Anaerobic digestion of alfalfa silage with recirculation of process liquid. *Bioresource Technology*, 98(1), 104-111.

Oechsner, H., Khanal, S.K., Taherzadeh, M. 2015. Advances in Biogas Research and Application. *Bioresource Technology*, 178(0), 177.

Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M.J., Sárvári-Horváth, I., Lundin, M. 2014. Anaerobic co-digestion of solid slaughterhouse wastes with agro-

residues: Synergistic and antagonistic interactions determined in batch digestion assays. *Chemical Engineering Journal*, 245(0), 89-98.

Pakarinen, O., Lehtomäki, A., Rissanen, S., Rintala, J. 2008. Storing energy crops for methane production: Effects of solids content and biological additive. *Bioresource Technology*, 99(15), 7074-7082.

Prochnow, A., Heiermann, M., Drenckhan, A., Schelle, H. 2005. Seasonal Pattern of Biomethanisation of Grass from Landscape Management. *CIGR E-Journal*, Volume 7(1682-1130).

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J. 2009. Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource Technology*, 100(21), 4931-4944.

Seppälä, M., Paavola, T., Lehtomäki, A., Rintala, J. 2009. Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresour. Technol.*, 100(12), 2952-2958.

Smyth, B.M., Murphy, J.D., O'Brien, C.M. 2009. What is the energy balance of grass biomethane in Ireland and other temperate northern European climates? *Renewable and Sustainable Energy Reviews*, 13(9), 2349-2360.

Thamsiriroj, T., Murphy, J.D. 2010. Difficulties Associated with Monodigestion of Grass as Exemplified by Commissioning a Pilot-Scale Digester. *Energy & Fuels*, 24(8), 4459-4469.

Thamsiriroj, T., Nizami, A.S., Murphy, J.D. 2012. Why does mono-digestion of grass silage fail in long term operation? *Appl. Energ.*, 95(0), 64-76.

Wang, H., Lehtomäki, A., Tolvanen, K., Puhakka, J., Rintala, J. 2009. Impact of crop species on bacterial community structure during anaerobic co-digestion of crops and cow manure. *Bioresource Technology*, 100(7), 2311-2315.

Weiland, P. 2003. Production and Energetic Use of Biogas from Energy Crops and Wastes in Germany. *Appl Biochem Biotechnol.*, Apr-Jun(109(1-3):263-74).

Wichern, M., Gehring, T., Fischer, K., Andrade, D., Lübken, M., Koch, K., Gronauer, A., Horn, H. 2009. Monofermentation of grass silage under mesophilic conditions: Measurements and mathematical modeling with ADM 1. *Bioresource Technology*, 100(4), 1675-1681.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., Zhan, X. 2011a. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. *Bioresource Technology*, 102(19), 8748-8755.

Xie, S., Lawlor, P.G., Frost, J.P., Hu, Z., Zhan, X. 2011b. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresour. Technol.*, 102(10), 5728-5733.

Xie, S., Wu, G., Lawlor, P.G., Frost, J.P., Zhan, X. 2012. Methane production from anaerobic co-digestion of the separated solid fraction of pig manure with dried grass silage. *Bioresource Technology*, 104(0), 289-297.

Yu, H.W., Samani, Z., Hanson, A., Smith, G. 2002. Energy recovery from grass using two-phase anaerobic digestion. *Waste Management*, 22(1), 1-5.

3. The potential for biomethane from grass and slurry to satisfy renewable energy targets

The potential for biomethane from grass and slurry to satisfy renewable energy targets

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Abstract

A biomethane potential (BMP) assessment of grass silage yielded 107 m^3 CH_4 t^{-1}. Long term mono-digestion of grass silage can suffer due to a deficiency in essential nutrients; this may be overcome by co-digesting with slurry. Mono-digestion of slurry achieved a low yield of 16 m^3 CH_4 t^{-1}. BMP assessments at a range of co-digestion ratios indicated methane yields were between 4 % and 11 % lower than the values calculated from mono-digestion. This paper suggests that co-digestion of the majority of slurry produced from dairy cows in Ireland with grass silage quantities equivalent to 1.1 % of grassland on a 50:50 volatile solids basis would generate over 10 % renewable energy supply in transport (RES-T). The industry proposed would equate to 170 digesters each treating 10,000 t a^{-1} of grass silage and 40,000 t a^{-1} of slurry from dairy cows.

Keywords: Gaseous biofuel, grass silage, slurry, biogas, BMP

3.1 Introduction

3.1.1 First- and second- generation biofuels

First-generation biofuels are derived from food crops and include ethanol from wheat, and biodiesel from rapeseed oil. Second-generation biofuels are based on non-edible crops such as perennial grass and agricultural residues. The Renewable Energy Directive requires that 10 % of all energy in transport must be renewable by 2020 (EC, 2009). The directive states that biofuels must emit a minimum of 60 % less greenhouse gases (GHGs) than the fossil fuels they replace. Both wheat ethanol and rapeseed biodiesel struggle to satisfy this reduction with reference values of 32 % and 45 % (EC, 2009), respectively. Furthermore, as of October 2012, new EU proposals may limit the use of food-based biofuels to 5 % of energy in transport in order to stimulate the application of second-generation biofuels (EC, 2012). The renewable energy supply in transport (RES-T) target for Ireland is 10 % by 2020. Ireland's forecasted energy in transport in 2020 is 188 PJ (Murphy and Thamsiriroj, 2011). Second-generation biofuels derived from lignocellulosic materials (such as grass silage) and residues (including agricultural slurries) shall be considered at 2 and 4 times their energy content (EC, 2012), respectively, when considering compliance with renewable energy targets.

3.1.2 Resource of grass and slurry in Ireland

Ireland has approximately 4.19 million hectares of agricultural land of which 92 % is under grass (McEniry *et al.*, 2013). Annual yields of grass in Ireland are potentially high in a European context – values of ca. 12 to 16 t dry solids (DS) per hectare may be achieved (O'Donovan *et al.*, 2011). The requirement from grassland in Ireland is set to increase. Food Harvest 2020 is a government initiative to increase exports,

particularly in the beef and dairy industries. A targeted increase of 50 % in milk production and 20 % in beef has been assigned (DAFF, 2010).

McEniry *et al.* (2013) estimated that using current production practices and excluding Food Harvest 2020, an annual average of approximately 1.7 million t DS of grass is available in excess of current livestock requirements. This would equate to silage produced from 155,000 ha or 3.9 % of grassland in Ireland assuming an average yield of 11 t DS ha^{-1} a^{-1} (Smyth *et al.*, 2009), that would potentially be available for anaerobic digestion.

If nitrogen fertiliser was applied to the limit permitted by the EU Nitrates Directive and if the grazed grass utilisation rate of cattle was increased from 0.60 to 0.80 kg DS ingested per kg DS grown, the excess grass available could be increased to 12.2 million t DS a^{-1} (McEniry *et al.*, 2013), even when factoring for Food Harvest 2020. This quantity of silage is equivalent to that which could be produced on 1.1 million ha or 28 % of Irish grassland – a significantly higher quantity of material available for anaerobic digestion.

According to the Central Statistics Office (2010) there were 1,070,755 dairy cows in Ireland. A single dairy cow produces 0.33 m^3 of slurry per week (DAFF, 1994). Farmers are obliged to store slurry for ca. 20 weeks over the winter period. This would generate 7.07 M t a^{-1}. Slurry is typically applied to land in its raw form. The majority of slurry systems do not include for straw and are primarily comprised of faeces and urine with a dry solids content of 6–10 % of which 75–85 % would be volatile solid. There is currently no significant biogas industry in Ireland; at most there are 4 small farm scale digesters.

3.1.3 Grass biomethane

An advantage of grass silage for biomethane is the familiarity of the crop with farmers and the avoidance of arable land use (Smyth *et al.*, 2009). Grass is a perennial crop that negates the need for tillage. When including for carbon sequestration in pasture land, grass biomethane has been shown to effect a 75 % reduction in GHG emissions compared to the full life cycle analysis of diesel when used as a transport fuel (Korres *et al.*, 2010). Grass is now utilised in over 50 % of digesters operating in Germany and Austria (Prochnow *et al.*, 2009), although very rarely in a mono-digestion process. Although timothy, cocksfoot and tall fescue are sometimes used, perennial ryegrass is the principal species used in many countries (Smyth *et al.*, 2009; McEniry and O'Kiely, 2013). The digestion of grass silage has been widely reported in literature (Prochnow *et al.*, 2005; Lehtomäki *et al.*, 2008b; Seppälä *et al.*, 2009). Various digestion systems have been examined for maximising biomethane output from grass silage; these include batch leach-bed reactors (LBR) (Jagadabhi *et al.*, 2010), two-phase continuously stirred tank reactors (CSTR) (Thamsiriroj and Murphy, 2010) and sequencing LBRs coupled with an upflow anaerobic sludge blanket (SLBR– UASB) (Lehtomäki *et al.*, 2008a; Nizami *et al.*, 2011). The yields reported for mono-digestion of grass are quite varied, ranging from 200 to 450 L CH_4 kg^{-1} VS (Pakarinen *et al.*, 2008; Koch *et al.*, 2009; Nizami and Murphy, 2011). Grass silage has, however, been reported to be deficient in some essential trace elements for longterm mono-digestion (Thamsiriroj *et al.*, 2012).

3.1.4 Co-digestion of grass silage and dairy slurry

Co-digestion of grass silage and slurry has been somewhat less extensively covered in literature (Kaparaju *et al.*, 2002; Xie *et al.*, 2011, 2012). Slurry produces much

lower yields of methane than grass in mono-digestion. Values range from 136 to 239 L CH_4 kg^{-1} VS (Allen *et al.*, 2013). This is due to the majority of energy rich substrates having already been eliminated through the digestive tract of the animal (Weiland, 2003; Lehtomäki *et al.*, 2007). Therefore co-digestion with an energy crop is seen as the preferred alternative as it increases the biomethane yield. The specific methane yields from co-digestion of grass silage and slurry are once again quite varied with values reported from 140 to 300 L CH_4 kg^{-1} VS (Mahnert *et al.*, 2005; Lehtomäki *et al.*, 2007; Jagadabhi *et al.*, 2008).

There is limited literature that thoroughly assesses the optimum ratio of co-digestion of grass silage and dairy slurry, and little uniformity can be determined from the results. In an Irish context, Xie *et al.* (2011) suggested an optimal co-digestion ratio of 1:1 for concentrated pig manure and grass silage. However the substrates used were very different to those examined in this study (concentrated pig manure versus raw dairy slurry, clamp or pit silage versus baled silage).

3.1.5 Objectives

1. Calculate the specific methane potential (L CH_4 kg^{-1} VS) for grass silage, dairy slurry and various ratios of co-digestion of grass silage and slurry using biomethane potential (BMP) assays.

2. Obtain the first- and second-order kinetics of the different substrates investigated in the BMP assays.

3. Estimate the potential bioresource in Ireland of second-generation gaseous biofuel associated with a matrix of scenarios based on quantities of potential excess grassland as established by McEniry *et al.* (2013).

3.2 Methods

3.2.1 Inoculum

The inoculum used for the experiment was sourced from two existing digesters in Ireland. The first digester operated on food waste while the second operated on a mix of poultry and cattle manure. An equal share of digestate from both digesters was used as the inoculum. The DS and VS of the inoculum are outlined in Table 3.1. The inoculum for the BMP was acclimatised by heating at 40°C for 3 days prior to experimental start-up. Cellulose powder (Sigma Aldrich, CAS Number: 9004-34-6) was used as a standard to assess the efficiency of the inoculum.

3.2.2 Substrates

The grass silage was made from the first cut of a perennial ryegrass (*Lolium perenne*) sward that was harvested at an early maturity stage (low lignocellulosic content) and field wilted for 24 h prior to being baled and wrapped in plastic film. The bales were ensiled for 5 weeks and subsequently assembled into smaller 25 kg bales that were also wrapped in plastic film. These bales were stored anaerobically at room temperature until use. The silage was cut to a particle size of approximately 1 cm using a mincer (Buffalo Heavy Duty Mincer, Code: ECD400, 250 kg/h).
Fresh slurry was collected from a dairy farm in the month prior to start-up. The farm consisted of a dairy herd of 180 cows. The slurry consisted of faeces and urine (no wash water) from bovines; it was scraped from a cubicle housing by a mechanical scraper into a reception channel. The slurry assessed was taken from this reception channel and put into 25 L drums. The drums were stored at -20°C until required for the BMP assay. Slurry was thoroughly mixed to ensure a homogenous and representative sample.

The DS and VS content of the grass silage and slurry are outlined in Table 3.1 and were measured according to Standard Methods 2540 G (APHA, 2005).

Table 3.1 Characteristics of substrates and inoculums

Substrate	DS (g kg^{-1})	VS (g kg^{-1})	VS/DS (%)	C:N
Grass silage	292.7 ± 3.4	268.4 ± 2.8	91.7	26:1
Slurry	87.5 ± 2.1	66.9 ± 1.8	76.5	19:1
Inoculum	30.0	18.9	63.3	-

3.2.3 Ultimate analysis and Buswell

An ultimate analysis calculates the percentage carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) in a substrate. This allows each substrate to be described by a stoichiometric equation. The C:N ratio can also be calculated (Table 3.1). The analysis of C, H, N and O in the substrates was performed in triplicate with an elemental analyser using a thermal conductivity detector (Exeter Analytical, CE 440 Model). The grass silage sampled may be described as $C_{30}H_{50}O_{23}$ and the slurry sampled as $C_{22}H_{34}O_{19}$. The Buswell equation (Buswell and Hatfield, 1936), allows the maximum theoretical methane yield be assessed. To investigate the effects of co-digestion, the following grass:slurry (G:S) ratios were assessed on a VS mass basis: 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100.

3.2.4 Biomethane potential (BMP) assays

The Bioprocess™ automatic methane potential test system (AMPTS) was used to carry out BMP assays in triplicate on the different G:S samples, a cellulose standard and inoculum control. The batch digestion system employed 27 bottles, each of 500 mL total volume (400 mL working volume), with each bottle individually mixed.

Temperature for all units was held constant at 37°C by means of a large heated water bath. A calculated quantity of substrate (3.18 g) and inoculum (6.36 g) was initially added to each bottle; this calculation was based on a 2:1 inoculum-to-substrate ratio which is recommended to overcome any problems with inhibition (Angelidaki *et al.*, 2009). Deionised water was added to bring the level in the bottle to 400 mL. Carbon dioxide and other trace gases were removed from the produced biogas using 3 M sodium hydroxide solution. The upgraded gas was sent to a flow measurement device which measures gas through water displacement. When approximately 10 mL of gas has accumulated the cell opens and releases gas. For each opening the time, pressure and temperature is recorded. These data allow gas flow measurement at standard temperature and pressure (STP). All data are recorded on a bespoke software package. The BMP assays ran for 30 days.

The biodegradability index (BI) is defined as the biomethane yield from the BMP test expressed as a fraction of the maximum theoretical value based on the Buswell equation. This index reflects the methane conversion efficiency of the substrate.

3.2.5 Kinetics

An important parameter when evaluating the digestion of different substrates is the decay constant or k-value. This can be determined using first-order kinetics:

$$y(t) = y_m \times \left(1 - e^{(-kt)}\right)$$

where, y(t) is the cumulative specific methane yield at time t (mL CH_4 g^{-1} VS), y_m is the specific methane yield at the end of the 30 day test (mL CH_4 g^{-1} VS), t is the time (days) and k is the first order decay constant (1/day). Using second-order kinetics, parameters such as the lag phase and the maximum biomethane production rate can be evaluated. The modified Gompertz model is calculated as follows:

$$y = y_{max}.exp\left\{-exp\left[\left(\frac{u.e}{y_{max}}\right) \times (\lambda - t) + 1\right]\right\}$$

where, y is the cumulative specific methane yield (mL CH_4 g^{-1} VS), y_{max} is the predicted specific methane yield at the end of the 30 day test (mL CH_4 g^{-1} VS), u is the maximum specific biomethane production rate (mL CH_4 g^{-1} VS day^{-1}), λ is the lag phase (days) and t is the time (days).

First and second order kinetics were run using Matlab R2009a software. The half-life (T_{50}) was also calculated using Matlab and is defined as the time taken (days) to produce 50 % of the biomethane production.

3.2.6 Bioresource scenarios

In the following scenarios, present potential is defined as excess grass resource equivalent to approximately 1.7 million t DS a^{-1} and future potential is defined as approximately 12.2 million t DS a^{-1} (McEniry *et al.*, 2013). This is outlined in Section 3.1.2. Four different scenarios were investigated:

- Scenario 1: Grassland resource of 166,965 t DS a^{-1}. This is 10 % of present potential excess availability and is equivalent to silage that could be produced on 15,200 ha or 0.4 % of grassland.
- Scenario 2: Grassland resource of 500,894 t DS a^{-1}. This is 30 % of present potential excess availability and is equivalent to silage that could be produced on 45,500 ha or 1.1 % of grassland.
- Scenario 3: Grassland resource of 1,220,380 t DS a^{-1}. This is 10 % of future potential excess availability and is equivalent to silage that could be produced on 111,000 ha or 2.8 % of grassland.

- Scenario 4: Grassland resource of 3,661,140 t DS a^{-1}. This is 30 % of future potential excess availability and is equivalent to silage that could be produced on 333,000 ha or 8.3 % of grassland.

3.3 Results and discussion

3.3.1 BMP yields from mono- and co-digestion of grass silage and slurry

The biomethane yields obtained from the BMP assays are shown in Table 3.2. The 100:0 G:S treatment had the highest yield (400 L CH_4 kg^{-1} VS) which is comparable with studies on mono-digestion of grass previously discussed (Pakarinen *et al.,* 2008; Koch *et al.*, 2009; Nizami and Murphy, 2011). The sequential addition of slurry progressively reduced the biomethane yield observed. The 0:100 G:S treatment gave a biomethane yield of 239 L CH_4 kg^{-1} VS. Grass silage produced almost 7 times more methane per unit weight of substrate when compared to the slurry (107 versus 16 m^3 CH_4 t^{-1}). Cellulose was assessed as a standard for the BMP and attained a yield of 348 L CH_4 kg^{-1} VS which is 84 % of its theoretical potential. This suggests that there is potential to achieve a slightly higher yield with a fully acclimatised inoculum. For example, Thamsiriroj *et al.* (2012) found that long-term digestion of grass silage produced more biomethane than resulted from a BMP assay. Figure 3.1 and 3.2 show the cumulative specific methane production for all mono- and co-digestion assays, respectively, over the duration of the assay.

Table 3.2 Assessment of biomethane potential of substrates in mono- and co-digestion

Grass:Slurry VS basis g:g	BMP	Buswell [A]	Predicted yield [B]	Difference [C]	Bio-degradability Index [D]	Gas Production
	L CH_4 kg^{-1} VS	*L CH_4 kg^{-1} VS*	*L CH_4 kg^{-1} VS*	*%*		*m^3 CH_4 t^{-1} FW* [E]
100:0	400 ± 4	443	-	-	0.90	107.4 ± 1.1
80:20	345 ± 6	-	368	-7	0.80	78.7 ± 0.6
60:40	321 ± 3	-	336	-5	0.76	60.3 ± 1.4
50:50	308 ± 5	-	320	-4	0.74	51.6 ± 0.6
40:60	273 ± 5	-	303	-11	0.66	40.3 ± 0.8
20:80	250 ± 8	-	271	-8	0.63	26.8 ± 0.7
0:100	239 ± 9	389	-	-	0.61	16.0 ± 0.9
Cellulose	348 ± 3	415	-	-	0.84	-

[A] Methane predicted from the stoichiometric formula of the substrate
[B] Calculated in proportion to BMP values for Grass:Slurry 100:0 and 0:100
[C] Difference between BMP results and predicted yield results
[D] Biodegradability Index (BI) = BMP/Buswell
[E] FW – Fresh weight; ± standard deviation

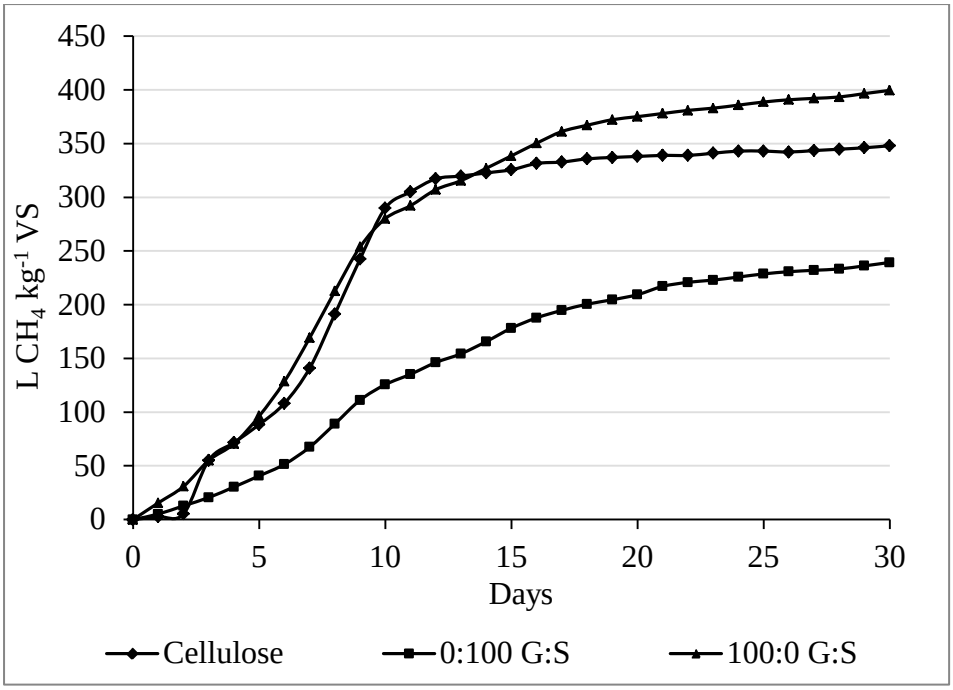


Figure 3.1 Specific methane yields for mono-digestion

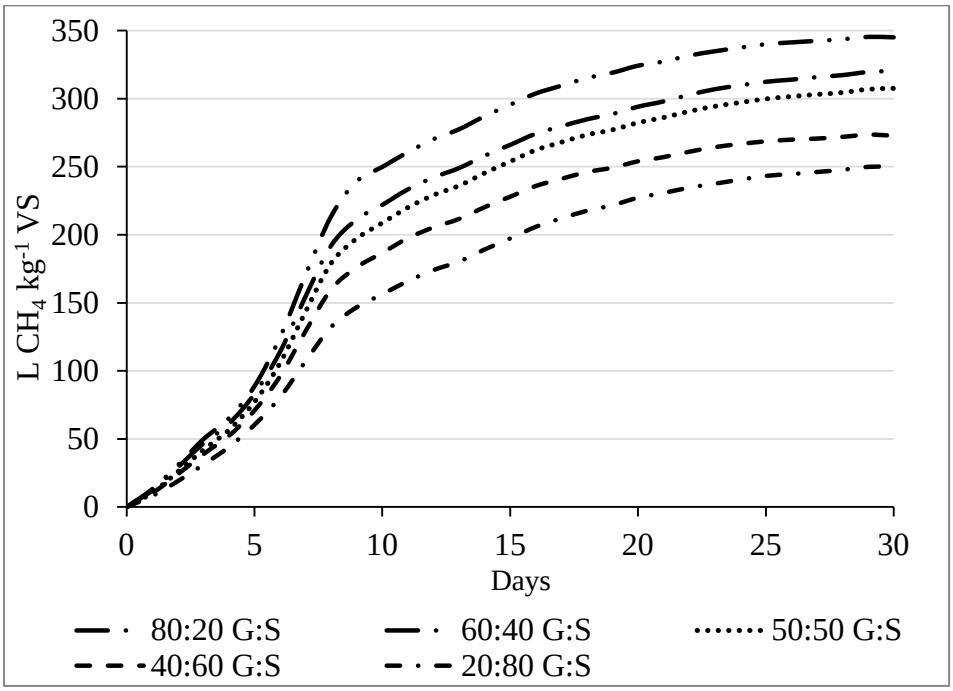


Figure 3.2 Specific methane yields for co-digestion

The calculated Buswell values, shown in Table 3.2, are maximum biomethane yields that should not be surpassed in the BMP. The BI for all G:S treatments was also determined with 100:0 G:S (Table 3.2) showing the highest digestibility (0.90) and 0:100 G:S having the lowest value (0.61).

When comparing the measured BMP yields to the corresponding predicted yields for the co-digested treatments (calculated proportionately from the 100:0 and 0:100 mono-digestion treatments), the co-digestion treatments yielded 4–11 % less than expected. Therefore, no synergistic effect was found in combining the grass silage and slurry. It is, however, difficult to say that the data indicates any significant antagonistic effects as pro-rata differences were small. Both the grass silage and slurry had C:N values close to the optimal range (26:1 and 19:1, respectively). Synergy in co-digestion tends to be associated with one substrate with a low C:N ratio coupled with a substrate of high C:N ratio (Allen *et al.*, 2013). There is little literature relating to synergies in grass/slurry combinations.

3.3.2 Results of kinetic analysis

First- and second-order kinetics were used to evaluate the decay constant (k-value), the half-life or T_{50} (number of days to produce 50 % of biomethane production) and the maximum specific biomethane production rate per day (u) for each G:S ratio (Table 3.3).

Co-digestion of grass and slurry and mono-digestion of grass displayed very similar k-values (0.097–0.113) and T_{50} values (7.14–8.33). This suggests that the retention time for the mono-digestion of grass and co-digestion of grass and slurry in an anaerobic digester is very similar. The value of u is found to drop linearly as the percentage of grass decreases in the substrate mix. This demonstrates that substrates

with higher proportions of grass will produce more biomethane within the same time period. The higher rate of degradation of grass silage in comparison to slurry may be explained by the liquors in the silage which have high levels of volatile fatty acids (Nizami and Murphy, 2011).

The k and u values for mono-digestion of slurry were found to be lowest for all treatments assessed. The T_{50} value was found to be highest. This would suggest that the addition of grass silage to a slurry digester should reduce the required retention time whilst increasing the biomethane yield.

Higher k-values and lower T_{50} values are indicative of substrates that biodegrade faster. The k-values presented in this study may be compared to those for food waste and dried seaweed of 0.433/d and 0.23/d respectively (Allen *et al.*, 2013), indicating that grass and slurry are slower to biodegrade.

The lag times (λ) for all G:S treatments in the BMP ranged from 1.34 to 2.45 days. This is low but would suggest that the inoculum is not fully acclimatised to the substrate. Cellulose had the longest lag time at 2.93 days, which may have resulted from the absence of soluble, immediately digestible, compounds in the substrate.

Table 3.3 First- and second order kinetic evaluation of substrates

Grass:Slurry VS basis	k day^{-1}	R^2	u mL CH_4 g^{-1} VS d^{-1}	λ days	T_{50} days
100:0	0.107	0.95	34.48	1.94	7.65
80:20	0.113	0.95	31.19	1.70	7.14
60:40	0.108	0.96	26.45	1.43	7.35
50:50	0.105	0.96	24.85	1.50	7.57
40:60	0.108	0.96	22.21	1.45	7.53
20:80	0.097	0.96	17.78	1.34	8.33
0:100	0.082	0.93	15.70	2.45	10.13
Cellulose	0.123	0.91	41.95	2.93	7.07

k is the first order decay constant (1/day), R^2 is the correlation coefficient. A good fit to the data is indicated by high R^2 values (above 0.95), *u* is the maximum specific biomethane production rate (mL CH_4 g^{-1} VS day^{-1}), λ is the lag phase (days), T_{50} is the half-life and is defined as the time taken (days) to produce 50 % of the CH_4

3.3.3 Bioresource of grass silage and slurry

Table 3.4 matches the resource of grass silage in Ireland with the required quantity of slurry to achieve the mixes assessed in the laboratory study. The quantity of grass silage in terms of t VS a^{-1} (column 3, Table 3.4) sets the required quantity of slurry in t VS a^{-1} (column 4, Table 3.4) depending on the mix ratio. Assuming the same ratio of VS/DS and DS content to wet weight as the slurry presented in Table 3.1, the quantity of slurry required is as expressed in column 5. Cells highlighted in bold with grey shading indicate instances where insufficient slurry from dairy cows is available in Ireland. The biomethane yield (column 7, Table 3.4) is derived from the results of the laboratory analysis for that mix (column 6, Table 3.4). The energy output is expressed in terms of energy in biomethane (column 8, Table 3.4) based on a Lower Heating Value (LHV) of 35.9 MJ m^{-3}.

The viable scenarios where slurry from dairy cows is available to match the supply of grass silage is as outlined in Table 3.5. The energy output is expressed as a percentage of expected energy in transport and as renewable energy supply in transport (RES-T) applying the double credit as allowed for in the Renewable Energy Directive (EC, 2009). The largest practicable resource equates to mono-digestion of grass silage at a level equivalent to 8.3 % of grassland. This can generate 25.6 % of energy in transport or over 50 % RES-T.

Table 3.4 Resource Scenario Matrix

Mix	Feedstock				Biomethane Output		
Grass: slurry VS basis	Grass resource *t DS a^{-1}*	Grass silage[A] *t VS a^{-1}*	Dairy slurry[B] *t VS a^{-1}*	Dairy Slurry[C] *t a^{-1}*	SMY[D] *L CH_4 kg VS*	Biomethane yield *M m^3 a^{-1}*	Energy in biomethane *PJ a^{-1}*
Scenario 1							
100:0	166,965	153,090	0	0	400	61.24	2.20
80:20	166,965	153,090	38,273	571,473	345	66.02	2.37
60:40	166,965	153,090	102,060	1,523,909	321	81.90	2.94
50:50	166,965	153,090	153,090	2,285,864	308	94.30	3.39
40:60	166,965	153,090	229,635	3,428,795	273	104.48	3.75
20:80	***166,965***	***153,090***	***612,360***	***9,143,454***	***250***	***191.36***	***6.87***
0:100	166,965	0	153,090	2,285,864	239	36.59	1.31
Scenario 2							
100:0	500,894	459,270	0	0	400	183.71	6.60
80:20	500,894	459,270	114,818	1,714,405	345	198.06	7.11
60:40	500,894	459,270	306,180	4,571,727	321	245.71	8.82
50:50	500,894	459,270	459,270	6,857,591	308	282.91	10.16
40:60	**500,894**	***459,270***	***688,905***	***10,286,386***	***273***	***313.45***	***11.25***
20:80	**500,894**	***459,270***	***1,837,080***	***27,430,363***	***250***	***574.09***	***20.61***
0:100	500,894	0	459,270	6,857,591	239	109.77	3.94

[A] Based on 917 kg VS t^{-1} DS
[B] Based on 765 kg VS t^{-1} DS
[C] Based on 87.5 kg DS t^{-1}
[D] Specific methane yield

Table 3.4 continued

Mix	Feedstock				Biomethane Output		
Grass: slurry VS basis	Grass resource *t DS a^{-1}*	Grass silage[A] *t VS a^{-1}*	Dairy slurry[B] *t VS a^{-1}*	Dairy Slurry[C] *t a^{-1}*	SMY[D] *L CH_4 kg VS*	Biomethane yield *M m^3 a^{-1}*	Energy in biomethane *PJ a^{-1}*
Scenario 3							
100:0	1,220,380	1,118,966	0	0	400	447.59	16.07
80:20	1,220,380	1,118,966	279,742	4,176,961	345	482.55	17.32
60:40	***1,220,380***	***1,118,966***	***745,977***	***11,138,562***	***321***	***598.65***	***21.49***
50:50	***1,220,380***	***1,118,966***	***1,118,966***	***16,707,843***	***308***	***689.28***	***24.75***
40:60	***1,220,380***	***1,118,966***	***1,678,449***	***25,061,764***	***273***	***763.69***	***27.42***
20:80	***1,220,380***	***1,118,966***	***4,475,864***	***66,831,371***	***250***	***1,398.71***	***50.21***
0:100	***1,220,380***	***0***	***1,118,966***	***16,707,843***	***239***	***267.43***	***9.60***
Scenario 4							
100:0	3,661,140	3,356,899	0	0	400	1,342.76	48.21
80:20	***3,661,140***	***3,356,899***	***839,225***	***12,530,886***	***345***	***1,447.66***	***51.97***
60:40	***3,661,140***	***3,356,899***	***2,237,933***	***33,415,695***	***321***	***1,795.94***	***64.47***
50:50	***3,661,140***	***3,356,899***	***3,356,899***	***50,123,543***	***308***	***2,067.85***	***74.24***
40:60	***3,661,140***	***3,356,899***	***5,035,349***	***75,185,315***	***273***	***2,291.08***	***82.25***
20:80	***3,661,140***	***3,356,899***	***13,427,596***	***200,494,173***	***250***	***4,196.12***	***150.64***
0:100	***3,661,140***	***0***	***3,356,899***	***50,123,543***	***239***	***802.30***	***28.80***

[A] Based on 917 kg VS t^{-1} DS
[B] Based on 765 kg VS t^{-1} DS
[C] Based on 87.5 kg DS t^{-1}
[D] Specific methane yield

Table 3.5 Potential mixes of grass silage and slurry with associated renewable energy production

Grass:Slurry VS basis	Energy in biomethane (PJ a^{-1})	% of expected energy in transport 2020 (%)	RES-T allowing for double credit (%)
Scenario 1 (equivalent to 0.4% of grass land)			
100:0	2.20	1.17	2.34
80:20	2.37	1.26	2.52
60:40	2.94	1.56	3.13
50:50	3.39	1.80	3.61
40:60	3.75	1.99	3.99
0:100	1.31	0.70	1.39
Scenario 2 (equivalent to 1.1% of grass land)			
100:0	6.60	3.51	7.02
80:20	7.11	3.78	7.56
60:40	8.82	4.69	9.38
50:50	10.16	5.40	10.81
0:100	3.94	2.10	4.19
Scenario 3 (equivalent to 2.8% of grass land)			
100:0	16.07	8.55	17.10
80:20	17.32	9.21	18.43
Scenario 4 (equivalent to 8.3% of grass land)			
100:0	48.21	25.64	51.29

To achieve 10 % RES-T (2020 EU target), 1.1 % of grassland at a 50:50 VS ratio with slurry from dairy cows is required. This would require practically all slurry in the country produced by dairy cows to be digested. There is, however, potential to supplement the digester with similar slurry from beef farms.

The total quantity of feedstock for digestion equates to 8.5 M t to achieve 10 % RES-T. This could be met with 170 anaerobic digestion facilities treating 10,000 t a^{-1} of grass silage and 40,000 t a^{-1} of slurry and producing of the order of 1.66 M m^3 a^{-1} of CH_4 (equivalent to a 0.75 MWe facility). This scale of industry may be compared with Austria (population of 8.2 million people as compared with 4.4 million in the Republic of Ireland). Austria has 350 agricultural biodigesters (Drosg, 2013), twice the number proposed here.

3.4 Conclusions

In BMP assays grass silage produced 400 L CH_4 kg^{-1} VS. Slurry, collected from a dairy farm, produced 239 L CH_4 kg^{-1} VS. On a fresh weight basis, grass silage produced almost 7 times more methane than slurry (107 m^3 CH_4 t^{-1} compared to 16 m^3 CH_4 t^{-1}). Co-digestion trials indicated that biomethane yields decreased by between 4 % and 11 % compared with predictions based on mono-digestion yields. The resource assessment suggested that grass silage from 1.1 % of grassland digested on a 1:1 VS basis with slurry would allow compliance with the 2020 RES-T target in Ireland.

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References

Allen, E., Browne, J., Hynes, S., Murphy, J.D. 2013. The potential of algae blooms to produce renewable gaseous fuel. *Waste Management, doi.org/10.1016/j.wasman.2013.06.017*.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology*, 59(5), 927-934.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater 21st ed. *American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA.*, 2540 B, E. Solids (Editorial revisions, 2011).

Buswell, A.M., Hatfield, W.D. 1936. Anaerobic Fermentations. *Bulletin No. 32. Available from: http://www.isws.illinois.edu/pubdoc/B/ISWSB-32.pdf*.

CSO. 2010. Census of Agriculture 2010. *Available from: http://www.cso.ie/en/releasesandpublications/agricultureandfishing/censusofagriculture2010-finalresults/*.

DAFF. 1994. Department of Agriculture, Food and Forestry (DAFF), "Revised quantities of farm wastes and storage requirements", Ireland.

DAFF. 2010. Food Harvest 2020 – A vision for Irish agri-food and fisheries. *http://www.agriculture.gov.ie/media/migration/agri-foodindustry/foodharvest2020/2020FoodHarvestEng240810.pdf Accessed 14th May 2013*.

Drosg, B. 2013. Austrian Country Report. *IEA Task 37 Meeting, Bern, April 17-19, 2013. Available in: http://www.iea-biogas.net/_download/publications/country-reports/may2013/Country_Report_Austria_April_2013.pdf accessed 10 October 2013*.

EC. 2009. Directive 2009/28/EC of The European Parliament and of The Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. *Official Journal of the European Union.*

EC. 2012. EU Commission tackles the climate impacts of biofuel production. Press release. . *Available In: http://ec.europa.eu/clima/policies/transport/fuel/documentation_en.htm accessed 30 October 2012*.

Jagadabhi, P., Lehtomäki, A., Rintala, J. 2008. Co-digestion of grass silage and cow manure in a CSTR by re-circulation of alkali treated solids of the digestate. *Environ Technol.*, Oct(29(10):1085-93.).

Jagadabhi, P.S., Kaparaju, P., Rintala, J. 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology*, 101(8), 2818-2824.

Kaparaju, P., Luostarinen, S., Kalmari, E., Kalmari, J., Rintala, J. 2002. Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Sci Technol*, 45(10), 275-80.

Koch, K., Wichern, M., Lübken, M., Horn, H. 2009. Mono fermentation of grass silage by means of loop reactors. *Bioresource Technology*, 100(23), 5934-5940.

Korres, N.E., Singh, A., Nizami, A.-S., Murphy, J.D. 2010. Is grass biomethane a sustainable transport biofuel? *Biofuels, Bioproducts and Biorefining*, 4(3), 310-325.

Lehtomäki, A., Huttunen, S., Lehtinen, T.M., Rintala, J.A. 2008a. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresource Technology*, 99(8), 3267-3278.

Lehtomäki, A., Huttunen, S., Rintala, J.A. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resour. Conserv. Recy.*, 51(3), 591-609.

Lehtomäki, A., Viinikainen, T.A., Rintala, J.A. 2008b. Screening boreal energy crops and crop residues for methane biofuel production. *Biomass and Bioenergy*, 32(6), 541-550.

Mahnert, P., Heiermann, M., Linke, B. 2005. Batch- and Semi-continuous Biogas Production from Different Grass Species. *CIGR E-Journal* Volume 7(1682-1130).

McEniry, J., Crosson, P., Finneran, E., McGee, M., Keady, T.W.J., O'Kiely, P. 2013. How much grassland biomass is available in Ireland in excess of livestock requirements? *Irish J Agr. Food. Res.*, 52: 67–80.

McEniry, J., O'Kiely, P. 2013. Anaerobic methane production from five common grassland species at sequential stages of maturity. *Bioresource Technology, 1*27 (2013) 143-150.

Murphy, J.D., Thamsiriroj, T. 2011. What will fuel transport systems of the future? *Materials Today*, 14(11), 518-524.

Nizami, A.-S., Murphy, J.D. 2011. Optimizing the Operation of a Two-Phase Anaerobic Digestion System Digesting Grass Silage. *Environmental Science & Technology*, 45(17), 7561-7569.

Nizami, A.-S., Singh, A., Murphy, J.D. 2011. Design, Commissioning, and Start-Up of a Sequentially Fed Leach Bed Reactor Complete with an Upflow Anaerobic Sludge Blanket Digesting Grass Silage. *Energy & Fuels*, 25(2), 823-834.

O'Donovan, M., Lewis, E., O'Kiely, P. 2011. Requirements of future grass based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research*, 50: 1-21.

Pakarinen, O., Lehtomäki, A., Rissanen, S., Rintala, J. 2008. Storing energy crops for methane production: Effects of solids content and biological additive. *Bioresource Technology*, 99(15), 7074-7082.

Prochnow, A., Heiermann, M., Drenckhan, A., Schelle, H. 2005. Seasonal Pattern of Biomethanisation of Grass from Landscape Management. *CIGR E-Journal*, Volume 7(1682-1130).

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J. 2009. Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource Technology*, 100(21), 4931-4944.

Seppälä, M., Paavola, T., Lehtomäki, A., Rintala, J. 2009. Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresource Technology*, 100(12), 2952-2958.

Smyth, B.M., Murphy, J.D., O'Brien, C.M. 2009. What is the energy balance of grass biomethane in Ireland and other temperate northern European climates? *Renewable and Sustainable Energy Reviews*, 13(9), 2349-2360.

Thamsiriroj, T., Murphy, J.D. 2010. Difficulties Associated with Monodigestion of Grass as Exemplified by Commissioning a Pilot-Scale Digester. *Energy & Fuels*, 24(8), 4459-4469.
Thamsiriroj, T., Nizami, A.S., Murphy, J.D. 2012. Why does mono-digestion of grass silage fail in long term operation? *Applied Energy*, 95(0), 64-76.
Weiland, P. 2003. Production and Energetic Use of Biogas from Energy Crops and Wastes in Germany. *Appl Biochem Biotechnol.*, Apr-Jun(109(1-3):263-74).
Xie, S., Lawlor, P.G., Frost, J.P., Hu, Z., Zhan, X. 2011. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource. Technology*, 102(10), 5728-5733.
Xie, S., Wu, G., Lawlor, P.G., Frost, J.P., Zhan, X. 2012. Methane production from anaerobic co-digestion of the separated solid fraction of pig manure with dried grass silage. *Bioresource Technology*, 104(0), 289-297.

4. Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry

Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry

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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.

Keywords: Grass silage, biomethane, dairy slurry

4.1 Introduction

4.1.1 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).

4.1.2 Review of grass digestion

Grass digestion literature integrates a variety of reactor designs (batch, continuous, leach bed, etc.) digesting numerous grass species (perennial ryegrass, cocksfoot, tall fescue, timothy, etc.) with an assortment of harvest dates, loading rates, pre-treatments and process conditions.

A previous work by the same authors examined the biomethane potential (BMP) assays for perennial ryegrass silage, dairy cow slurry and co-digestion of both substrates (Wall *et al.*, 2013). The grass silage gave a specific methane yield (SMY) of 400 L CH_4 kg^{-1} VS. A linear relationship was found to exist between the grass silage proportion in the substrate mix and the SMY. The highest SMY was achieved through mono-digestion of grass silage. The BMP for dairy slurry recorded the lowest SMY. As grass silage decreased as a proportion of the co-digestion mix, the SMY decreased.

The majority of analysis on grass digestion has been carried out at a similar batch scale (Seppälä *et al.*, 2009, 2013; Xie *et al.*, 2011) with limited literature reporting continuous digestion, particularly with regard to silage made from perennial ryegrass which is the dominant grass species sown in Ireland (DAFM, 2012). Thamsiriroj *et al.* (2012) reported up to 20 % losses in methane production when increasing the organic loading rate (OLR) from 2.0 to 2.5 kg VS m^{-3} d^{-1} for mono-digestion of perennial ryegrass silage and suggested that any increases in OLR should coincide with the addition of trace elements. Mechanical failure was also an issue due to the high solids content of the substrate. Recirculation of liquor was a recommended solution in order to control solids content and limit volatile fatty acid (VFA) accumulation (Thamsiriroj and Murphy, 2011). Mahnert *et al.* (2005) investigated biogas production from the mono-digestion of perennial ryegrass, cocksfoot and

meadow foxtail grass species at low feeding rates (0.7 and 1.4 kg VS m^{-3} d^{-1}). The resultant SMYs were in the range of 300–320 L CH_4 kg^{-1} VS for continuous trials.

A previous study by Lehtomäki *et al.* (2007) examined a similar concept to the work presented in this paper. However the focus of that paper was primarily on slurry acting as a base substrate to further optimise the C:N ratio when co-digesting with grass or other energy crops. The perennial ryegrass silage used in this present work had a C:N ratio of 26:1 and thus is deemed sufficient for mono-digestion purposes. The aim of this paper is to achieve the highest specific methane yields possible for grass silage whether mono-digested, or co-digested with slurry, in long term operation. Lehtomäki focused on a seed mixture grass (75 % timothy, 25 % meadow fescue) with a dry solids content of 259 g kg^{-1} that was chopped to a particle size of approximately 3 cm, harvested at a similar stage to the perennial ryegrass in this study but had a lower specific methane yield of 306 L CH_4 kg^{-1} VS. The highest specific methane yield reported by Lehtomäki in continuous trials was 268 L CH_4 kg^{-1} VS at a loading rate of 2.0 kg VS m^{-3} d^{-1} with a 30% proportion of grass (volatile solids (VS) basis) in the feedstock. Higher proportions of crop, up to 40% (VS basis), were said to decrease the specific methane yield while an increase in loading rate to 3.0 and 4.0 kg VS m^{-3} d^{-1} were also suggested to have an adverse effect.

Another study carried out by Jagadabhi *et al.* (2008) looked at the co-digestion of grass silage and cow manure at 30 % and 70 % of the substrates' VS content with an emphasis on the recirculation of alkali-treated and untreated solid fractions of digestate to the reactors. Once more, a 30 % share of grass (VS basis) was recommended in co-digestion, achieving methane yields in the range of 180–185 L CH_4 kg^{-1} VS, with recirculation of solids offering no enhancement to the process.

This study focuses on the return of separated liquor effluent rather than solid material. It has been previously reported that recirculating process liquid for alfalfa digestion can lead to an increase in the effective OLR (Nordberg *et al.*, 2007). This has been suggested to be a result of an increase in the availability of trace elements due to the added retention of the return liquor (Jarvis *et al.*, 1997).

4.1.3 Objectives

The objective of the trial was to highlight the potential process limitations for the production of biomethane as might occur on Irish farms. To effect this, a comprehensive study on the digestion of grass silage and co-digestion of grass silage with dairy slurry at different mix ratios on a VS basis was undertaken in a laboratory study using six continuously stirred tank reactors (CSTRs). The trial was run continuously for a period of 62 weeks. The OLR was increased incrementally from 2.0 to 4.0 kg VS m^{-3} d^{-1}. Recirculation of separated liquor effluent was undertaken for digesters with higher solids content to negate concerns of high viscosity (i.e. to reduce the dry solids (DS) content of the reactor to less than 100 g kg^{-1}). This also had the benefit of returning essential trace elements back to the digester, thereby increasing retention time for bioavailability.

4.2 Methods

4.2.1 Grass silage

A first-cut perennial ryegrass (*Lolium perenne*), harvested at an early inflorescence growth stage with relatively low lignified fibre content, was used as feedstock throughout the trial. The silage was initially field wilted for 24 h, and ensiled for 5 weeks in 1.2 m diameter cylindrical bales wrapped in polyethylene stretch-film.

Following this ensilage process, the herbage was re-ensiled in smaller 25 kg rectangular bales that were also wrapped in stretch-film. The bales were stored at approximately 18–20°C throughout the trial. To ensure a homogenous feedstock, the grass silage from a number of bales was cut to a particle size of approximately 1 cm using a heavy duty mincer. Aliquots of the cut grass were stored at -20°C until required. The characteristics of the grass silage are indicated in Table 4.1.

Table 4.1 Grass Silage Characteristics

Parameters	Grass Silage
pH	4.5
Dry solids (DS) (g kg^{-1})	293
Volatile solids (VS) (g kg^{-1})	268
VS/DS (g kg^{-1})	917
Neutral detergent fibre(g kg^{-1} DS)	627
Acid detergent fibre(g kg^{-1} DS)	373
Dry Solids Digestibility (DSD) (g kg^{-1})	653
Crude Protein (g kg^{-1} DS)	160
Water Soluble Carbohydrate (g kg^{-1} DS)	18
NH_3 (g kg^{-1} DS)[A]	4
NH_3-N (g kg^{-1} N)[B]	113
D+L Lactic Acid (g kg^{-1} DS)	77
D- Lactic Acid (g kg^{-1} DS)	46
L- Lactic Acid (g kg^{-1} DS)	31
Ethanol (g kg^{-1} DS)	3
Acetic Acid (g kg^{-1} DS)	3
Propionic Acid (g kg^{-1} DS)	0
Butyric Acid (g kg^{-1} DS)	0
C:N Ratio	26:1

[A] NH_3 concentration expressed as a proportion of dry solids in the grass silage; this is used as an indicator of substrate suitability for anaerobic digestion performance.
[B] Proportion of total N present in NH_3 this is used as a silage preservation quality indicator.

4.2.2 Dairy slurry

In total, approximately 200 kg of fresh dairy cow slurry was collected in separate 25 L heavy-plastic drums. Three collections (January, April and December) were made over a one year period. The source of slurry was a single farm with a herd of approximately 180 dairy cows. A mechanical scraper system forced faeces and urine into a reception channel from which the slurry samples were taken. Collected drums were stored at -20°C until required for anaerobic digestion. The DS content of the slurry varied from 57 to 96 g kg^{-1} depending on the time of year the sample was collected. These differences reflected the diet consumed by the animal pre-calving (fed grass silage with no concentrate) in December/ January as compared to post-calving (fed grass silage with up to 6 kg concentrate per animal) in April. The VS content was on average 750 g kg^{-1} DS. For the one year period of collection the C:N ratio for the dairy slurry remained relatively consistent, with an average yearly value at 20:1. Slurry from individual drums were mixed thoroughly prior to use in the trial ensuring a homogeneous sample.

4.2.3 Inoculum source and commissioning phase

The inoculum was sourced as digestate from two existing digesters in Ireland – one operating on food waste, the second running on a mix of poultry and cattle manure. An equal amount of inoculum from each source was mixed together thoroughly and used in the start-up of the trial. The DS and VS of the mixed inoculum was 29.4 g kg^{-1} and 18.5 g kg^{-1}, respectively. In total, 24 L of mixed inoculum was used (4 L per reactor). The reactors were given a four week commissioning period to reach the desired temperature while daily checks were made to ensure the system was completely anaerobic. The mixing system was also examined throughout this

commissioning phase and was operating identically in each reactor. A further four weeks was taken to drip-feed each reactor (2–3 times per week) with their respective substrate feed mixes of grass silage and dairy slurry, thereby allowing acclimatisation of the microbial culture to be established. Over this period, parameters such as DS, VS, chemical oxygen demand (COD), total ammonical nitrogen (TAN) and pH were carefully monitored to ensure each reactor's performance was stable. Minimal variations between reactor conditions were observed prior to start-up.

4.2.4 Semi-continuous trials

Six continuously stirred tank reactors (CSTRs) were run simultaneously over a period of 62 weeks. Each reactor was fabricated from thick plastic pipe with a total volume of 5 L and a working volume of 4 L. A vertically mounted stirrer provided mixing for the system. The temperature of the reactors was kept constant at 37 ± 1°C for the duration of the trial. This was achieved by a thermo-circulator which pumped hot water through copper pipes that were coiled around the reactors. To reduce heat loss an insulated cover was positioned over the reactors. Biogas produced in the reactor passed out through an outlet discharge pipe and continued to a bespoke wet tip gas meter for gas measurement. Individual tip meters were calibrated for each reactor (52–57 mL/tip). The wet tip gas meters were connected to a stand-alone computer which recorded the number of tips from each CSTR. Gas samples were collected twice-a-week in 1 L Tedlar gas bags from the outlet of the wet tip gas meters and used for biogas composition analysis.

4.2.5 Loading rates and feeding

Each reactor began with a low OLR of 2.0 kg VS m^{-3} d^{-1}. This equated to a total of 8 g VS per day per reactor regardless of the substrate mix. As this was the first loading rate of the trial, three hydraulic retention times (HRTs) were run at the first OLR to allow stable operation. The OLR was then increased in a stepwise fashion from 2.0 to 2.5 (10 g VS per day per reactor), 2.5 to 3.0 (12 g VS per day per reactor), and finally 3.0 to 3.5 (14 g VS per day per reactor). All subsequent OLRs were run for at least two HRTs. The two reactors with the highest proportion of grass silage were run at a further loading rate of 4.0 kg VS m^{-3} d^{-1} in an effort to maximise the biomethane potential from the substrates under examination. The reactors were fed for five days each week (Saturdays and Sundays unfed); this approach is as carried out by other authors in feeding CSTRs (Allen *et al.*, 2014; Seppälä *et al.*, 2013). The HRT was accounted for only on the days in which the reactors were fed. The DS content in all six reactors was kept below 100 g kg^{-1} for the duration of the trial. Where the incoming substrate feed of a reactor had a high solids content, a calculated quantity of separated effluent liquor (pressed from the digestate of that reactor) was returned to the system with the incoming daily feed. This lowered the DS content within the reactor (< 100 g kg^{-1}) and also shortened the relative HRT.

4.2.6 Reactor mixes

Six reactors (R1–R6) were run for a continuous period of 62 weeks and consisted of different compositions of grass silage and dairy slurry (G:S). R1 consisted of 100 % dairy slurry (0:100 G:S) and acted as a baseline for the trial. R6 constituted the second baseline comprising of 100 % grass silage (100:0 G:S). Reactors R2–R5

operated in co-digestion with 20:80, 40:60, 60:40 and 80:20 mixes (G:S) on a VS basis, respectively.

4.2.7 Analytical and chemical methods

4.2.7.1 Digestate

Analysis of pH was performed daily using a Jenway 3510 pH meter. FOS/TAC, a measure of a reactor's acid concentration relative to its buffering capacity (i.e. volatile organic acids as a ratio of alkalinity), was measured weekly via the Nordmann-method using 0.1 N sulphuric acid. This is a two point titration method (endpoints 5.0 pH and 4.4 pH) used as a guide in assessing the stability of the reactors microbial community. A Titronic Universal Automatic Titrator was used for the analysis. Results below 0.300 are indicative of a stable process (Drosg, 2013). All DS and VS analyses were determined according to Standard Methods 2540 G (APHA, 2005). Chemical oxygen demand (COD) and total ammonical nitrogen (TAN) were analysed weekly using Hach Lange cuvette tests (LCK 914 and LCK 313, respectively) and evaluated using a DR3900 Hach Lange Spectrophotometer.

4.2.7.2 Biogas

The biogas was evaluated for methane (CH_4) composition via two devices, a handheld Ntron biogas analyser and an Agilent 6890 GC with thermal conductivity detector. The GC method could account for quantities of additional gases such as carbon dioxide, hydrogen and nitrogen. The handheld monitor provided more frequent reference checks on methane composition.

4.2.7.3 Grass silage

For parameters shown in Table 4.1, samples were dried at 40°C for 48 h and subsequently ground using a Wiley hammer mill with a 1 mm pore screen. In vitro dry solids digestibility (DSD) was evaluated via the Tilley and Terry (1963) method but with final residue isolated by filtration (Whatman GF/A 55 mm, pore size 1.6 µm, Whatman International) instead of centrifugation. Quantities of neutral detergent fibre (NDF), assayed with a heat stable amylase and sodium sulphite, and acid detergent fibre, were evaluated using the ANKOM filter bag technique (ANKOM, 2006a, b) with reference to the analytical method of Van Soest *et al.* (1991), and expressed on an ash-free basis. Crude protein (total nitrogen (N) × 6.25) was measured with a LECO FP-528N analyser by measuring the thermal conductivity of N present in a sample following total combustion at 900°C, based on the methods from the Association of Analytical Chemists (Association of Official Analytical Chemists, 1990). The water soluble carbohydrates content was determined using the automated anthrone method (Thomas, 1977). Aqueous extract pressed from the silage sample was used for the remainder of the analysis. pH was analysed using a Hanna Instruments pH meter. Ammonia (NH_3) concentrations were evaluated using the SP-Ace Clinical Chemical Analyser and the Thermo Electron Infinity ammonia liquid stable reagent kinetic method. Lactic acid quantities were measured using the L-lactic acid UV method on an SP-Ace Clinical Chemical Analyser, while D-lactate was determined after using the enzyme D-lactate dehydrogenase. Both volatile fatty acids (acetic, propionic and butyric) and ethanol concentrations were measured using a GC (Shimadzu GC 17-A) with a flame ionisation detector with a chromopack glass column using the method of Ranfft (1973).

4.2.7.4 Dairy slurry

Analysis of pH, DS and VS was carried out on all collected slurry samples via the methods as described for digestate samples.

4.2.7.5 C:N (carbon-to-nitrogen) ratio

Both the grass silage and dairy slurry were analysed in triplicate for C, H, N and O with an elemental analyser using a thermal conductivity detector (Exeter Analytical, CE 440 Model).

4.3 Results and discussion

Table 4.2 illustrates the results of important process characteristics such as pH, DS/VS, COD, TAN and methane composition for R1–R6. All data displayed represents average values determined over the duration of the final HRT episode at the relative OLR. The initial HRTs at each OLR served as an acclimatisation period for the microbial community in each reactor; this allowed the systems to reach steady state conditions and to achieve consistent gas yields. Due to the five day feeding regime (Monday to Friday), a slight decline in gas production was evident on Saturday and Sunday. There was no evidence of reduced microbial activity during the five day week as very stable daily CH_4 yields were exhibited. Table 4.3 indicates the SMYs and biomethane efficiencies for each reactor over the various OLRs. The biomethane efficiency is defined as the SMY from continuous digestion divided by the SMY obtained from a BMP test of the same substrate under optimum conditions.

Table 4.2 Operational parameters for R1-R6 over the final HRT for each OLR

Reactor	R1	R2	R3	R4	R5	R6
			OLR 2.0 kg VS m^{-3} d^{-1}			
(G:S)	(0:100)	(20:80)	(40:60)	(60:40)	(80:20)	(100:0)
pH	7.64	7.63	7.63	7.70	7.70	7.64
FOS/TAC	0.168	0.181	0.181	0.186	0.188	0.184
DS (g kg^{-1})	49.3	48.9	56.7	59.1	57.2	47.1
VS (g kg^{-1})	37.3	34.3	37.9	40.6	40.8	34.7
COD (g L^{-1})	36.7	42.7	43.9	51.0	49.8	46.9
TAN (g L^{-1})	1.6	1.6	1.9	2.2	2.2	2.0
CH_4 % (v/v)	50.1	49.5	51.6	53.6	52.3	52.8
HRT	28	28	38	40	39	37
			OLR 2.5 kg VS m^{-3} d^{-1}			
pH	7.62	7.71	7.74	7.76	7.76	7.70
FOS/TAC	0.186	0.201	0.203	0.199	0.189	0.228
DS (g kg^{-1})	55.6	70.2	71.8	73.3	67.9	54.8
VS (g kg^{-1})	39.3	43.9	46.0	48.8	46.8	41.1
COD (g L^{-1})	43.7	55.0	56.0	60.9	58.5	53.0
TAN (g L^{-1})	1.8	2.2	2.4	2.5	2.5	2.0
CH_4 % (v/v)	54.2	54.7	54.9	54.2	53.3	51.2
HRT	25	30	30	30	30	29
			OLR 3.0 kg VS m^{-3} d^{-1}			
pH	7.70	7.70	7.71	7.71	7.74	7.71
FOS/TAC	0.197	0.220	0.217	0.198	0.190	0.361
DS (g kg^{-1})	73.6	81.6	76.4	69.3	64.7	71.1
VS (g kg^{-1})	45.6	52.0	50.6	47.5	45.7	55.2
COD (g L^{-1})	50.0	58.4	62.3	60.2	62.0	77.7
TAN (g L^{-1})	2.2	2.3	2.2	2.3	2.3	2.2
CH_4 % (v/v)	54.9	55.0	55.0	55.1	56.7	52.7
HRT	18	26	25	25	26	25
			OLR 3.5 kg VS m^{-3} d^{-1}			
pH	-	7.65	7.78	7.69	7.74	7.78
FOS/TAC	-	0.206	0.210	0.202	0.209	0.275
DS (g kg^{-1})	-	78.3	75.8	75.2	70.3	71.1
VS (g kg^{-1})	-	48.2	50.3	53.3	51.1	54.5
COD (g L^{-1})	-	56.8	60.2	66.6	69.9	80.7
TAN (g L^{-1})	-	2.1	2.3	2.4	2.4	2.3
CH_4 % (v/v)	-	54.9	54.6	53.2	55.2	52.9
HRT	-	21	20	25	25	21
			OLR 4.0 kg VS m^{-3} d^{-1}			
pH	-	-	-	-	7.74	7.66
FOS/TAC	-	-	-	-	0.240	0.298
DS (g kg^{-1})	-	-	-	-	76.8	66.5
VS (g kg^{-1})	-	-	-	-	57.4	51.9
COD (g L^{-1})	-	-	-	-	71.8	71.7
TAN (g L^{-1})	-	-	-	-	2.5	2.0
CH_4 % (v/v)	-	-	-	-	52.5	49.5
HRT	-	-	-	-	21	19

Table 4.3 Specific methane yields (SMY[A]) and biomethane efficiency (B.Ef)

OLR[B]	R1 (0:100)		R2 (20:80)		R3 (40:60)		R4 (60:40)		R5 (80:20)		R6 (100:0)	
	SMY	B.Ef	SMY	B.Ef	SMY	B.Ef	SMY	B.Ef	SMY	B.Ef	SMY	B.Ef
2.0	112	0.59	220	0.88	233	0.85	328	1.02	352	1.02	414	1.04
2.5	143	0.75	198	0.79	239	0.88	316	0.98	343	0.99	398	1.00
3.0	65	0.34	207	0.83	253	0.93	331	1.03	355	1.03	409	1.02
3.5	-	-	217	0.87	266	0.97	321	1.00	366	1.06	398	1.00
4.0	-	-	-	-	-	-	-	-	349	1.01	360	0.90

[A] SMY in L CH_4 kg^{-1} VS
[B] OLR in kg VS m^{-3} d^{-1}

4.3.1 Organic loading rate 2.0 kg VS m^{-3} d^{-1}

All reactors maintained a similar pH range (7.63–7.70), deemed sufficient for digestion. The FOS/TAC value was low for R1 (100 % dairy slurry) at 0.168, while R2–R6 displayed very similar FOS/TAC results (0.181–0.188). As the FOS/TAC values were below 0.200 for all reactors, it was assumed the microbial communities were somewhat underfed and hence the OLR could be increased. The DS content was comparable for all reactors and the higher concentrations of grass silage in R5 and R6 were offset by the recirculation of separated liquor effluent (<25 g DS kg^{-1}). COD values were lower in R5 and R6 as compared to R4, which did not have any effluent return. R1, as expected, had a lower COD value. Co-digestion with grass silage supplemented the accumulation of COD. This is potentially due to COD rich liquors associated with the grass silage. TAN concentrations were deemed adequate for digestion as all reactors, being below 2.5 g L^{-1}. The methane concentration in the biogas was similar for all reactors regardless of the grass-slurry composition (49.5–53.6 % v/v).

R4–R6 performed best in terms of SMYs at 328, 352 and 414 L CH_4 kg^{-1} VS and these values represented ceiling values with regards to biomethane efficiency at 1.02, 1.02 and 1.04 respectively. A reduction in grass silage (and subsequent increase in dairy slurry) in the substrate mix decreased the biomethane efficiency. R1 had the lowest efficiency at 0.59, while R2 and R3, with low grass silage contributions in co-digestion, gave efficiencies of 0.88 and 0.85, respectively.

4.3.2 Organic loading rate 2.5 kg VS m^{-3} d^{-1}

The increase in OLR had negligible effect on pH for all reactors and values remained in a similar range (7.62–7.76). The FOS/TAC remained low for R1, although

marginally increased at 0.186. R2–R5 showed minor rises in FOS/TAC values as compared to an OLR of 2.0 kg VS m^{-3} d^{-1}. R6, containing 100 % grass silage, showed the sharpest jump in FOS/TAC climbing to 0.228 (24 % increase) but was still considered in a suitable range in terms of microbial stability. DS values increased significantly at this loading rate. R2 saw the highest increase at 44 %, while R3 also saw a substantial increase of 27 %. The reason for this rise in DS was twofold, higher volumes of grass silage being added to the reactors and absence of dilution (no separated liquor effluent return). With the higher loading rate, R4 had a small volume of separated liquor effluent returned in order to maintain the reactor's DS content below 100 g kg^{-1}. However, R4 still saw an increase in DS of 24 %. R1, R5 and R6 saw increases in DS of less than 20 %. Similar increases were seen in the COD concentrations of all reactors, ranging from 13–29 %. R2–R6 had COD concentrations in the range of 50–60 g L^{-1} while R1 was lower at approximately 43 g L^{-1}. The effect of the COD rich liquors present in the grass silage was again apparent in raising COD levels. All mixes containing slurry had TAN values increase with higher OLR. Increases were in the range of 10–43 %. R6 had very slightly reduced TAN in comparison to the previous OLR of 2.0 kg VS m^{-3} d^{-1}. The methane concentration did not change significantly and was again similar for all reactors (51.2–54.9 % v/v). Concentrations of methane in the range of 50–55 % were deemed adequate for the digestion of such agricultural substrates.

R4–R6 again performed best in terms of SMYs at 316, 343 and 398 L CH_4 kg^{-1} VS obtaining maximum biomethane efficiency values at 0.98, 0.99 and 1.00 respectively. A similar pattern emerged as with that of an OLR of 2.0 kg VS m^{-3} d^{-1} as the mixes with higher dairy slurry content decreased the biomethane efficiency. However, R1 did improve its efficiency to 0.75, while R2 and R3 reported

efficiencies of 0.79 and 0.88, respectively. It was evident that high proportions of dairy slurry, a residue where much of its available energy had already been removed through the digestive system of the animal, hindered the biomethane efficiency as compared to reactors that had higher additions of the energy rich grass silage.

4.3.3 Organic loading rate 3.0 kg VS m^{-3} d^{-1}

R1, containing 100 % dairy slurry, struggled significantly at this OLR. No problems were evident with pH or COD levels. The FOS/TAC value remained low despite the increased loading rate and TAN concentrations increased by 23 % on the previous OLR of 2.5 kg VS m^{-3} d^{-1}. As the dairy slurry had a very high water content (over 900 g kg^{-1}) it is postulated that the HRT (18 days) became too short for the microbial community. Thus, methane yields were seen to drop dramatically as bacterial washout occurred. This was most likely associated with hydrolytic bacteria as the methane content in the reactor remained at a high level. Therefore, it is advised that the HRT for dairy slurry digestion should be greater than 20 days in order to establish and maintain a sufficient microbial consortium. A yield of 65 L CH_4 kg^{-1} VS was reported for R1 with biomethane efficiency of 0.34 after 3 HRTs. To counteract the poor performance of R1, the OLR was reduced back to 2.5 kg VS m^{-3} d^{-1} (data not shown) with an effective HRT of 25 days. The SMY picked up significantly to approximately 180 L CH_4 kg^{-1} VS while the TAN accumulation subsided with levels reducing below 2.0 g L^{-1}. The OLR of R1 was not increased any further and the reactor was shut down.

The pH values for R2–R6 were consistent with lower OLRs and remained in a satisfactory range (7.71–7.74). The FOS/TAC value spiked for R6 at a level of 0.361, representing a 58 % increase on the previous OLR (2.5 kg VS m^{-3} d^{-1}). This

illustrated the first sign of stress (due to potential over-feeding) for mono-digestion of grass silage as it surpassed the FOS/TAC stability limit. All other reactors remained steady at the lower FOS/TAC limit. R6 also showed a sharp rise in solids content (30 % increase) although the reactor remained comfortably under the 100 g DS kg^{-1} target as a higher quantity of separated liquor effluent was returned. R2–R5 did not see as much of a change in DS content at this OLR. A vast difference in COD values was evident when contrasting R2–R5 with R6. Reactors with dairy slurry (R1–R5) averaged 60 g L^{-1} whereas the grass silage reactor (R6) displayed values close to 78 g L^{-1}. The increase in OLR corresponded to an increased COD release from the grass silage. The TAN levels in reactors containing dairy slurry all dropped slightly at this OLR while R6 saw an 11 % increase although this value still remained lower overall. Overall, TAN concentrations did not become a limiting process parameter as recommended threshold values were never breached despite increases in OLR. At 3.0 kg VS m^{-3} d^{-1}, it became apparent that the methane composition in the biogas was low for the mono-digestion of grass silage (52 % v/v CH_4) as compared to any reactor that contained dairy slurry (averaging 55 % v/v CH_4).

Once more the SMYs were most efficacious for R4–R6 with yields of 331, 355 and 409 L CH_4 kg^{-1} VS, coupled with maximum biomethane efficiencies of 1.03, 1.03 and 1.02, respectively. R2 and R3 showed minor progressions with yields of 207 and 253 L CH_4 kg^{-1} VS signifying 0.83 and 0.93 biomethane efficiencies, respectively.

4.3.4 Organic loading rate 3.5 kg VS m^{-3} d^{-1}

Optimal levels of pH were once again maintained for all reactors (7.65–7.79). FOS/TAC values remained generally consistent with the levels of the previous OLR

(3.0 kg VS m^{-3} d^{-1}), however, R6 seemed to have acclimatised better to the higher feeding rate as its value decreased by 24 % although this still remained considerably higher than any other reactor at 0.275. No significant effect on DS was evident by the increase in loading rate. Similar consistencies were apparent when examining COD as values fluctuated by ±3 % for R2–R6, however, R5 containing the minimum contribution of dairy slurry did see a 12 % rise on the previous OLR. Fluctuations in TAN levels were limited to less than 10 % for all reactors. The trend of lower methane concentrations in the mono-digestion of grass silage continued though the range of results was comparable with previous studies.

In terms of SMYs, R5 was the highest performer in terms of biomethane efficiency (1.06), equating to a yield of 366 L CH_4 kg^{-1} VS. R4 and R6 were again at maximum efficiency with SMYs of 321 and 398 L CH_4 kg^{-1} VS respectively. R3 showed further improvement with a SMY of 266 L CH_4 kg^{-1} VS and approached its relative maximum biomethane efficiency. The SMY for R2 also improved (217 L CH_4 kg^{-1} VS) but with a higher proportion of dairy slurry, still struggled to reach its maximum potential.

R2–R4 were subsequently shutdown as they could not compete with the SMYs attainable from R5 to R6.

4.3.5 Organic loading rate 4.0 kg VS m^{-3} d^{-1}

Two reactors, R5 and R6, were operated at this loading rate. The pH for both was deemed adequate for digestion and displayed no significant change to any of the previous OLRs. The FOS/TAC for both reactors increased in comparison to the previous OLR, with R6 showing a higher value of 0.298 – bordering on the upper limit threshold for process stability. Interestingly, COD for both reactors were almost

identical at approximately 72 g L^{-1}. TAN levels increased slightly for R5 and reached 2.5 g L^{-1} for the first time. Any accumulation above this threshold may cause inhibition. R6 saw a considerable decrease in TAN levels as values remained below 2.0 g L^{-1}. A reduction in methane content was evident for the both reactors. For the first time in the 62 weeks of operation methane composition fell below 50 % (v/v) for mono-digestion of grass. R5 had a methane composition of 52.5 % (v/v) but this was lower than the value recorded at the previous OLR (55.2 % v/v).

At a loading rate of 4.0 kg VS m^{-3} d^{-1}, the SMY for R6 decreased by approximately 12 % to 360 L CH_4 kg^{-1} VS in comparison to the other OLRs tested throughout the trial. R6 reported a biomethane efficiency of 0.90. R5, containing 20 % dairy slurry and 80 % grass silage (VS basis), reported a SMY of 349 L CH_4 kg^{-1} VS which again equated to a maximum biomethane efficiency (1.00) and hence did not suffer negatively with respect to the higher OLR. Figures 4.1 and 4.2 show the SMYs of R6 and R5, respectively, over their operational lifetime.

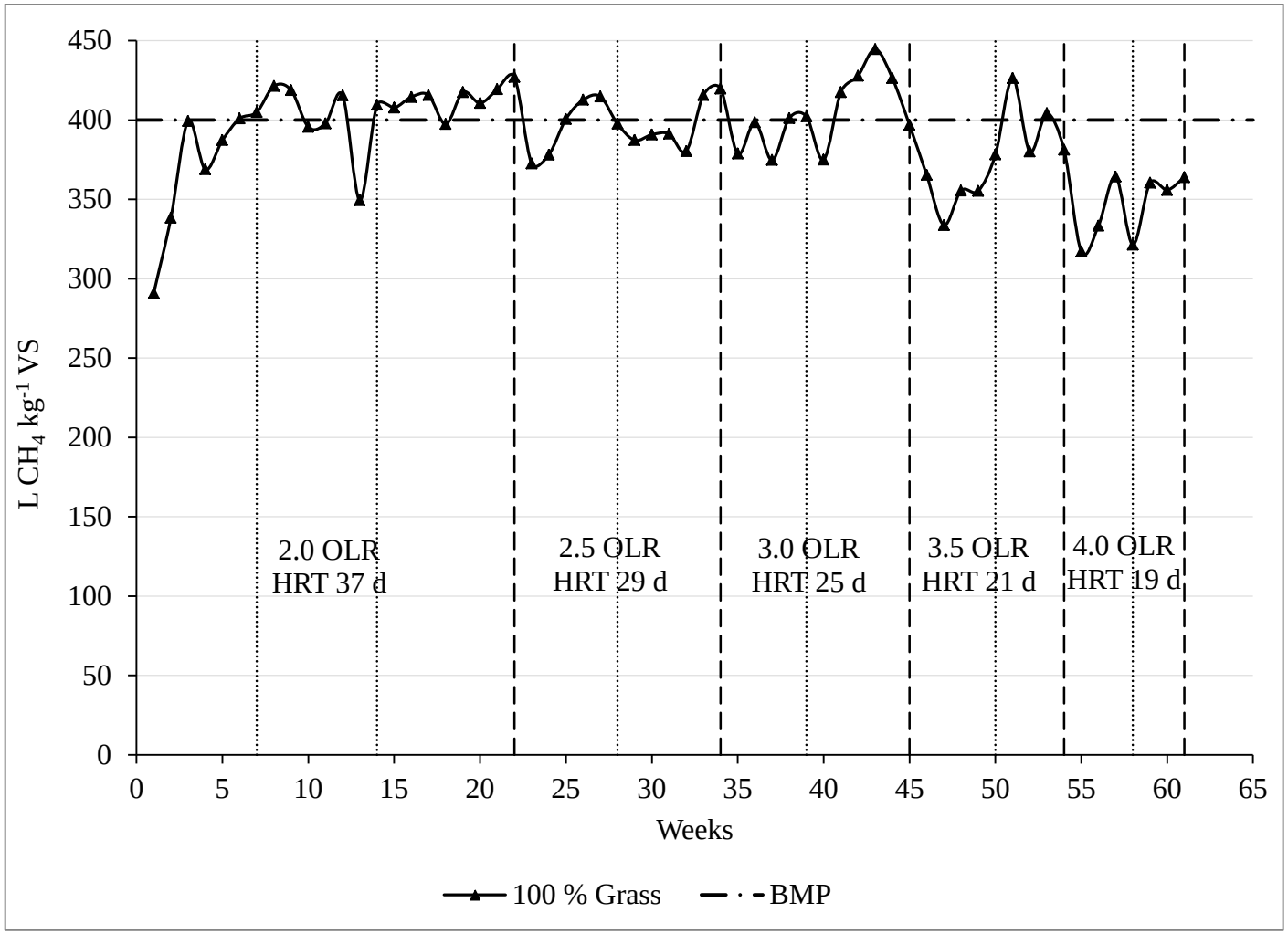


Figure 4.1 Specific methane yield for R6 (mono-digestion of grass)

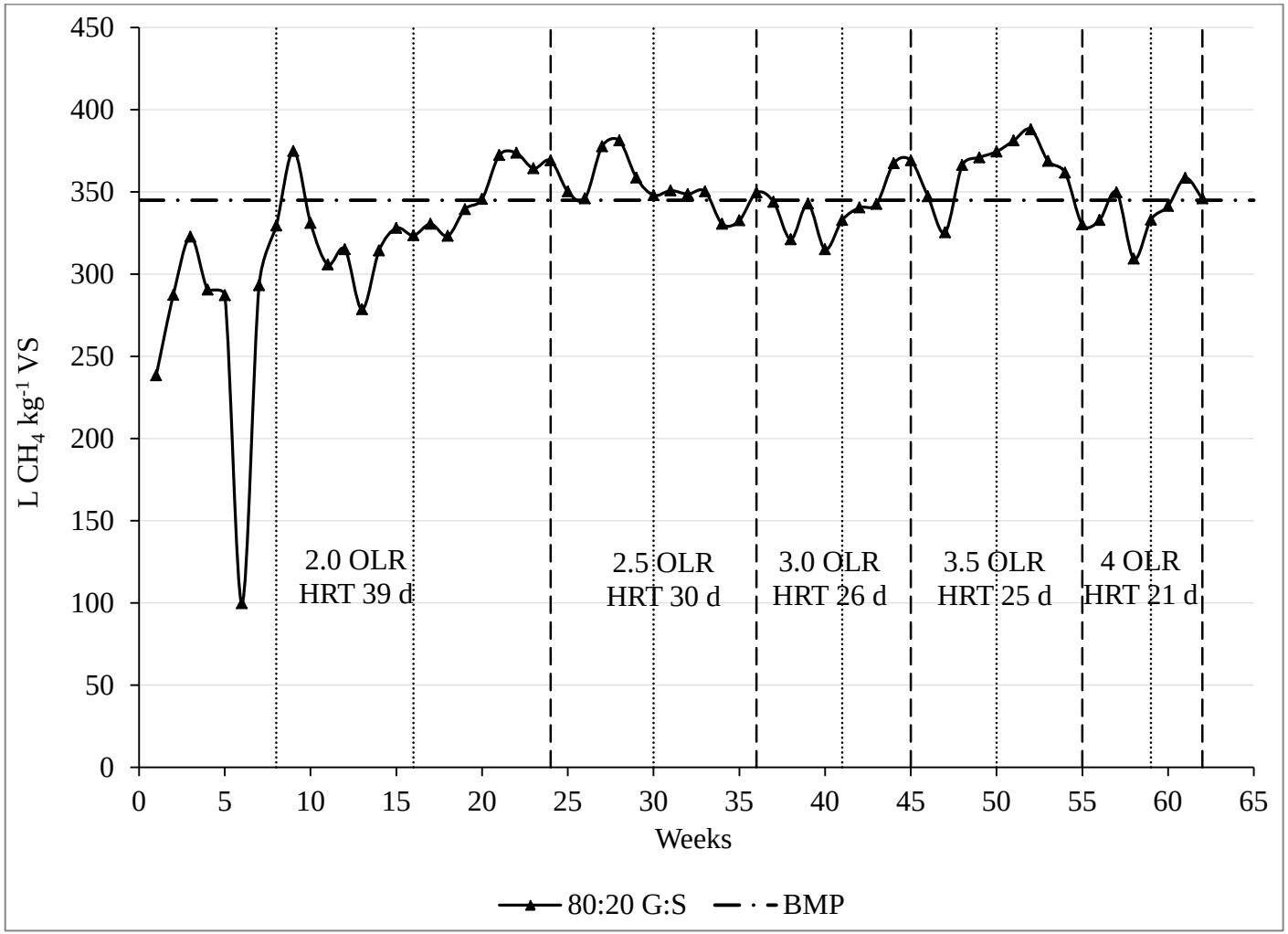


Figure 4.2 Specific methane yield for R5 (80 % VS grass, 20% VS slurry)

4.3.6 Mono-digestion of slurry and high slurry proportions in co-digestion (R1−R4)

R1, representing the mono-digestion of dairy slurry, performed best at an OLR of 2.5 kg VS m^{-3} d^{-1}. Increasing the increase of OLR to 3.0 kg VS m^{-3} d^{-1} severely inhibited the process (Table 4.3). It is thought the HRT (18 days) became too short to for the microbial community and hence washout of hydrolytic bacteria occurred.

At a loading rate of 3.5 kg VS m^{-3} d^{-1} it was evident that the SMYs from reactors with higher slurry proportions (R2–R4) could not realistically compete with that of reactors containing higher grass silage proportions (R5 and R6). This contrasts to previous studies where slurry acted as the base substrate component of the digester and high grass additions were not recommended (Lehtomäki *et al.*, 2007). Results from this trial show that higher additions of dairy slurry to a digester contributes to decreased efficiencies with respect to potential biomethane yields.

4.3.7 Low dairy slurry additions (20 %) in co-digestion (R5)

From a farmer's perspective, the addition of dairy slurry to a grass-fed digester may be beneficial. The SMYs achievable at OLRs of 3.0, 3.5 and 4.0 kg VS m^{-3} d^{-1}, maintaining maximum biomethane production efficiencies, closely correspond to that of R6 at 4.0 kg VS m^{-3} d^{-1} with reduced biomethane efficiency. This scenario would be advantageous to farmers who wish to reduce the feedstock cost of grass silage purchase through addition of dairy slurry to the digester. This may facilitate high biomethane returns at high loading rates in smaller digesters with minimum drop off in biomethane production.

4.3.8 Mono-digestion of grass silage (R6)

The OLRs of 3.0, 3.5 and 4.0 kg VS m^{-3} d^{-1} were higher than previously reported in literature for mono-digestion of grass silage. It is thought the higher OLRs were achievable through substantial recirculation of separated liquor effluent which, in general, kept the FOS/TAC values below the threshold of 0.300. It is postulated that the deterioration in SMY and corresponding drop in biomethane efficiency suffered by R6 at 4.0 kg VS m^{-3} d^{-1} was a consequence of the HRT becoming too short at 19 days. Maximum effective degradation of such a high lignocellulosic content substrate was not possible in such a short time.

4.4 Conclusions

Continuous long term digestion trials illustrated the requirement for high grass silage input to maximise the potential biomethane output. 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 at a biomethane efficiency of 1.0. Increasing this to 4.0 kg VS m^{-3} d^{-1} caused a drop in SMY of 12 %. With addition of 20 % dairy slurry the optimum condition was at an OLR of 4.0 kg VS m^{-3} d^{-1} generating a SMY of 349 L CH_4 kg^{-1} VS at a biomethane efficiency of 1.01.

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References

Allen, E., Wall, D.M., Herrmann, C., Murphy, J.D. 2014. Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel. *Bioresouce Technoogy.*, 170(0), 436-444.

ANKOM. 2006a. Acid detergent fiber in feeds filter bag technique. *Macedon, NY, USA: Ankom Inc.*

ANKOM. 2006b. Neutral detergent fiber in feeds filter bag technique. *Macedon, NY, USA: Ankom Inc.*

APHA. 2005. Standard Methods for the Examination of Water and Wastewater 21st ed. *American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA.*, 2540 B, E. Solids (Editorial revisions, 2011).

Association of Official Analytical Chemists. 1990. Official Methods and Analysis. *First supplement (1990) to 15th edition, method 990-03,* Arlington, VA: AOAC.

DAFM. 2012. Grass and Clover Recommended List Varieties for Ireland. *Department of Agriculture Food and the Marine*, Available In: http://www.agriculture.gov.ie/media/migration/farmingsectors/crops/seedcertification/cropvarietyevaluationcvepublications/2012/GrassCloverRecommendedList2012.pdf (accessed June 2014).

Drosg, B. 2013. IEA Task 37 - Process monitoring in biogas plants.

Gerin, P.A., Vliegen, F., Jossart, J.-M. 2008. Energy and CO2 balance of maize and grass as energy crops for anaerobic digestion. *Bioresouce. Technology.*, 99(7), 2620-2627.

IEA. 2014. Task 37 Biogas Country Overview. *http://www.iea-biogas.net/country-reports.html [Accessed 29 July, 2014]*.

Jagadabhi, P., Lehtomäki, A., Rintala, J. 2008. Co-digestion of grass silage and cow manure in a CSTR by re-circulation of alkali treated solids of the digestate. *Environ Technol.*, Oct(29(10):1085-93.).

Jarvis, Å., Nordberg, Å., Jarlsvik, T., Mathisen, B., Svensson, B.H. 1997. Improvement of a grass-clover silage-fed biogas process by the addition of cobalt. *Biomass. Bioenerg.*, 12(6), 453-460.

Lehtomäki, A., Huttunen, S., Rintala, J.A. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resour. Conserv. Recy.*, 51(3), 591-609.

Mahnert, P., Heiermann, M., Linke, B. 2005. Batch- and Semi-continuous Biogas Production from Different Grass Species. *CIGR E-Journal* Volume 7(1682-1130).

McEniry, J., Crosson, P., Finneran, E., McGee, M., Keady, T.W.J., O'Kiely, P. 2013. How much grassland biomass is available in Ireland in excess of livestock requirements? *Irish J Agr. Food. Res.*, 52: 67–80.

Nordberg, Å., Jarvis, Å., Stenberg, B., Mathisen, B., Svensson, B.H. 2007. Anaerobic digestion of alfalfa silage with recirculation of process liquid. *Bioresource Technology*, 98(1), 104-111.

O'Mara, F. 2008. Country pasture/forage resource profile – Ireland. *www.fao.org/ag/AGP/agpc/doc/Counprof/Ireland/Ireland.htm [Accessed 28 July, 2014]*.

O'Donovan, M., Lewis, E., O'Kiely, P. 2011. Requirements of future grass based ruminant production systems in Ireland. *Irish J Agr. Food. Res. 50: 1–21*.
Ranfft, K. 1973. Determination by gas chromatography of short-chain volatile fatty acids in ruminal fluids. *Arch. Tierernahr*, 23:343–352.
Seppälä, M., Paavola, T., Lehtomäki, A., Rintala, J. 2009. Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresource Technology*, 100(12), 2952-2958.
Seppälä, M., Pyykkönen, V., Väisänen, A., Rintala, J. 2013. Biomethane production from maize and liquid cow manure – Effect of share of maize, post-methanation potential and digestate characteristics. *Fuel*, 107(0), 209-216.
Thamsiriroj, T., Murphy, J.D. 2011. Modelling mono-digestion of grass silage in a 2-stage CSTR anaerobic digester using ADM1. *Bioresource Technology*, 102(2), 948-959.
Thamsiriroj, T., Nizami, A.S., Murphy, J.D. 2012. Why does mono-digestion of grass silage fail in long term operation? *Appl. Energ.*, 95(0), 64-76.
Thomas, T.A. 1977. An automated procedure for the determination of soluble carbohydrates in herbage. *J. Sci. Food Agric.*, 28(7), 639-642.
Tilley, J.M.A., Terry, R.A. 1963. A TWO-STAGE TECHNIQUE FOR THE IN VITRO DIGESTION OF FORAGE CROPS. *Grass. Forage. Sci.*, 18(2), 104-111.
Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy. Sci.*, 74(10), 3583-3597.
Wall, D.M., O'Kiely, P., Murphy, J.D. 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. *Bioresource Technology*, 149(0), 425-431.
Xie, S., Lawlor, P.G., Frost, J.P., Hu, Z., Zhan, X. 2011. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technoogy.*, 102(10), 5728-5733.

5. The effect of trace element addition to mono-digestion of grass silage at high organic loading rates

The effect of trace element addition to mono-digestion of grass silage at high organic loading rates

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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.

Keywords: Trace elements, biogas, grass silage, slurry

5.1 Introduction

5.1.1 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.

5.1.2 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 (on a VS basis) to a grass silage fed digester (termed R5 in the paper) was shown to ensure maximum biomethane efficiency at an OLR of 4.0 kg VS m^{-3} d^{-1} (Wall *et al.*, 2014). Biomethane efficiency is defined as the SMY in continuous digestion divided by the SMY from a biomethane potential (BMP) test. Thus in co-digestion, the SMY obtained from continuous trials matched the yields from a BMP test under optimum conditions.

It is postulated that TEs present in the grass-slurry mixture (R5) allowed a higher biomethane efficiency (1.01) to be achieved than mono-digestion of grass silage (R6) which achieved an efficiency of 0.90. However, as a potential anaerobic digestion co-substrate, slurry is high in water content, takes up a large proportion of reactor volume and at high concentrations can dilute the SMYof the digester.

5.1.3 Review of trace element additions in anaerobic digestion

Successful digestion of all biomass involves a sufficient concentration of both macronutrients and TEs (Takashima and Speece, 1989). Past literature has shown certain TEs, more specifically cobalt, nickel, molybdenum and selenium, are reported to be critical to process performance and any deficiency in such nutrients can inhibit methanogenesis (Schattauer *et al.*, 2011). Other micronutrients such as cadmium, manganese, iron, zinc and copper are also accounted for in the digestion process but are generally thought to be abundant in most feedstocks (Schattauer *et al.*, 2011). TEs in a digester serve as co-factors in enzymes directly involved with the degradation of the feedstock and in the formation of methane (Pobeheim *et al.*, 2010, 2011). The unavailability of essential TEs in a reactor can upset digester stability and

performance even when other process conditions remain under control (Demirel and Scherer, 2011).

The digestion process involves a complex matrix of both organic and inorganic matter and thus the bioavailability of certain TEs is often difficult to assess (Gustavsson *et al.*, 2013). In general, the bioavailable nutrients represent only a fraction of the total amount measured in the medium (Oleszkiewicz and Sharma, 1990). Most of these TEs are present in the solid fraction of the substrate, whereas alkalinity and ammonia are more closely associated with the liquid phase (Zhang *et al.*, 2011). Microbial communities in mono-digestion systems are said to have more issues with TE bioavailability than systems operating in co-digestion (Pobeheim *et al.*, 2011). It is recommended that the process liquid effluent should be recirculated within the system as this can potentially increase the availability of the TEs (Jarvis *et al.*, 1997). The addition of slurry residues to a digester has been recommended to alleviate concerns of TE deficiencies in a mono-substrate reactor (Braun *et al.*, 2003; Seppälä *et al.*, 2013). However, contrasting studies have suggested that slurry alone may not be enough to overcome such shortfalls (Schattauer *et al.*, 2011). The addition of a low methane-yielding substrate such as slurry (<20 m^3 CH_4 m^{-3}) must be carefully balanced with the economic viability of the digester (Angelidaki and Ellegaard, 2003). Likewise, the supplementation of a digester with TEs needs to be done with care. Facchin *et al.* (2013) suggested that the addition of unneeded metals can have an adverse effect on methanogenesis. In a study examining a number of digesters across Europe, Schattauer *et al.* (2011) found great variations in TE concentrations ranging from 1–2 orders of magnitude.

The majority of past literature examining the role of TEs has focused on the digestion of food waste. This can be seen in the work of Zhang *et al.* (2011, 2012),

Zhang and Jahng (2012), Banks *et al.* (2012), Jiang *et al.* (2012) and Facchin *et al.* (2013). These particular studies indicate a deficiency in TE for food waste digestion although this can potentially be rectified by the addition of element- rich supplements. However, recommended guidelines for concentrations of TE additions are generally not applicable to the digestion of crops since crops have quite different TE contents (Hinken *et al.*, 2008). Previous literature on the availability of TEs in crops is limited, particularly when considering mono-digestion of grass. Jarvis *et al.* (1997) examined grass clover digestion and the requirements of methanogenic archaea for different TEs. It was reported that an increase in TE availability could potentially be achieved through recirculated process effluent. The same study indicated a critical cobalt concentration of 0.02 mg L^{-1}. Additions of cobalt provided a stimulatory effect on methanogenesis and thus higher methane yields were achieved. Another study, focused on the digestion of napier grass, showed that the addition of nickel, cobalt, molybdenum, selenium and sulphate solution (0.25 mg L^{-1}, 0.19 mg L^{-1}, 0.30 mg L^{-1}, 0.062 mg L^{-1} and 1.6 mg L^{-1}, respectively) enhanced methane yields by 40 % and prevented volatile fatty acid (VFA) accumulation (Wilkie *et al.*, 1986).

Although grass silage is a key feedstock in establishing an anaerobic digestion industry in Ireland, maize silage is the predominant substrate in central Europe. Pobeheim *et al.* (2010) looked at a synthetic model substrate for maize silage and the impact of essential TEs in mesophilic batch reactors. The study showed that the addition of a TE solution boosted methane yields by up to 30 %, with nickel and cobalt the most significant components. Molybdenum was not found to have any significant effect. TE additions in semi-continuous reactors were also examined, again with a defined model substrate for maize (Pobeheim *et al.*, 2011). Once more,

nickel and cobalt were found to be limiting and deficiencies in these TEs caused an accumulation of organic acids. Concentrations of 0.6 and 0.05 mg kg^{-1} (fresh weight) of nickel and cobalt were recommended and allowed an OLR of 4.3 kg VS m^{-3} d^{-1} to be achieved.

Methanogenesis has two principal pathways – acetoclastic and hydrogenotrophic. The acetoclastic pathway refers to the conversion of acetic acid to methane and carbon dioxide while the hydrogenotrophic pathway refers to the formation of methane from hydrogen and carbon dioxide (Schattauer *et al.*, 2011). TE non-availability is growth limiting to methanogens (Karlsson *et al.*, 2012). Previous studies have shown that when the concentrations of both nickel and cobalt are sufficient, the microbial community will be dominated by acetogenic methanogens while hydrogenotrophic methanogens will thrive under a deficiency in these elements, resulting in VFA accumulation (Gustavsson *et al.*, 2013).

5.1.4 Objectives

This study expands upon previous work undertaken by the authors (Wall *et al.*, 2014) in assessing mono-digestion of grass silage and co-digestion of grass silage with dairy cow slurry in continuously stirred tank reactors (CSTR). Mono-digestion of grass silage (R6) saw a decreased SMY and reduced biomethane efficiency (0.90) at an OLR of 4.0 kg VS m^{-3} d^{-1}. The objective of this study was to develop comprehensive TE profiles to pinpoint specific TEs that may be supplemented to mono-digestion of grass silage to boost efficiency at high OLRs. This was assessed by comparing TE concentrations of R6 (100 % grass silage) and R5 (co-digestion of grass silage with 20 % slurry addition on a VS basis) at the same OLR over an operational timeframe of 70 weeks.

5.2 Methods

5.2.1 Grass silage

The grass silage used for the CSTR trial was a first-cut perennial ryegrass (*Lolium perenne*), harvested at an early inflorescence growth stage, and was obtained from the Animal and Grassland Research and Innovation Centre (Teagasc, Grange) in Dunsany, Co. Meath, Ireland. A period of 24 h was allowed for field wilting post-mowing. The silage was then ensiled in 1.2 m diameter × 1.2 m wide cylindrical bales wrapped in polyethylene stretch-film for 5 weeks. For storage purposes, the silage was then segmented into smaller 25 kg rectangular bales which were again wrapped in stretch-film and stored at approximately 18–20°C. All grass silage used was shredded to a particle size of less than 1 cm (approximately) using a heavy duty mincer and was stored at -20°C until required for experimental use. The dry solids (DS) and volatile solids (VS) content of the grass silage was 293 g kg^{-1} and 920 g kg^{-1} DS, respectively.

5.2.2 Dairy slurry

Three collections of slurry were made over a one year period (January, April and December) with approximately 200 kg obtained in total from a single farm in Co. Cork, Ireland, with a herd of approximately 180 dairy cows. The slurry samples were taken from a reception channel that had faeces and urine forced into it by means of a mechanical scraper. Slurry samples were stored at -20°C until required. The DS content of the slurry varied from 57.2 to 95.7 g kg^{-1} depending on the time of year the sample was collected. This was attributable to the animals feed at the time of collection, with higher concentrate diets supplied post-calving (April) as compared to pre-calving (January, December). The VS content was on average 750 g kg^{-1} DS.

5.2.3 Semi-continuous reactor operation

Two CSTRs were used in this trial. R5 operated in co-digestion, containing 80 % grass silage with 20 % dairy slurry on a VS basis, while R6 represented mono-digestion of grass silage. Both reactors were fabricated from thick plastic pipe, each having a total volume of 5 L and a working volume of 4 L. A vertically mounted stirrer provided identical mixing for both systems. The temperature of the reactors was kept constant at 37 ± 1°C by means of a thermo-circulator which pumped hot water through copper pipes that were coiled around the reactors. Biogas produced in each reactor was measured by a calibrated wet tip gas meter. Each tip was recorded by a stand-alone computer.

To avoid mixing difficulties and the formation of grass layers at the top of the liquid level in both reactors, the incoming feed was reduced to less than 100 g DS kg^{-1} wet weight (ww) through dilution. This was achieved by recirculating a calculated quantity of effluent liquor (<25 g DS kg^{-1}) separated from the digestate of each respective reactor. This allowed the HRT to be assessed in two ways – the HRT from a solely substrate perspective and also the HRT including recirculated liquor (Box 5.1). For the purposes of this paper, the HRT associated with the return of separated effluent liquor was used as a counter for the phases of these trials. This approach was also used in a previous work (Wall *et al.*, 2014).

Box 5.1 Organic loading rate and hydraulic retention time of grass digestion (R6)

To explain the calculation of the OLR and HRT the following example is highlighted

At an OLR of 4.0 kg VS m^{-3} d^{-1} and an effective reactor volume of 4 L an addition of 16 g VS d^{-1} is added.

Grass has 270 g VS kg^{-1}; hence 16 g VS = 60 g wet weight (ww) addition per day

The HRT of grass is therefore 67 days (4000 g / 60 g per day).

Recirculation of liquor is effected to reduce the dry solids to 100 g DS kg^{-1}

The liquor had a DS content of 25 g DS kg^{-1}; thus a mass balance indicates an influent of 213 g ww d^{-1}

(153 g * 25 g DS kg^{-1}) + (60 g * 293 g DS kg^{-1}) = 213 g * 100 g DS kg^{-1}

HRT with recirculation is 4000 g / 213 g per day = 19 days.

5.2.4 Analytical methods

A Titronic Universal Automatic Titrator was used for weekly FOS/TAC (flüchtige organische säuren/totales anorganisches carbonat) analysis – measured via the Nordmann-method using 0.1 N sulphuric acid (Nordmann, 1977; Weiland, 2008). FOS/TAC is a measure of a reactor's acid concentration relative to its buffering capacity (or alkalinity) and assesses the stability of the microbial community. Results below 0.300 are indicative of a stable process (Drosg, 2013). VFA concentrations were measured using an Agilent 6890 GC with flame ionisation detector (Allen *et al.*, 2014). This was performed on a weekly basis from week 12 until the end of the trial. Analysed samples were centrifuged for 10 min at 15,000 rpm with 0.2 mL HPO_3 added to remove any particulate matter. For all TE analysis, samples of reactor digestate were collected 2–3 times per week over the final HRT of the relative OLR and were stored at -20°C. To ensure enough material for testing at each OLR, a number of week's samples were mixed together. The mixed samples were then dried at 40°C for 48 h and comminuted (particle size reduction) using a

heavy-duty pestle and mortar. Analysis of cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) were carried out using a Thermo Fisher Scientific X Series II ICP-MS.

5.2.5 Trace element addition

Whilst investigating TE deficiencies in the trial, three TEs were added to R6 commencing from week 65. The TE additions of Co, Fe and Ni were prepared as a single mixture by dissolving $CoCl_2.6H_20$ (CAS 7791-13-1), $FeCl_3.6H_20$ (CAS 10025-71-1) and $NiCl_2.6H_20$ (CAS 7791-20-0) in deionised water. This equated to the addition of 0.13 mg Co L^{-1}, 74.40 mg Fe L^{-1} and 2.48 mg Ni L^{-1} to the reactor.

5.3 Results and discussion

5.3.1 VFA profile for R5 (80 % grass silage: 20 % dairy slurry)

Figure 5.1 shows the VFA profile for R5 with corresponding FOS/TAC values. Acetic acid and propionic acid were the only VFAs detected throughout the analysis. Overall, R5 exhibited stable conditions over the duration of the trial. FOS/TAC remained relatively steady, with gradual increases corresponding to increases in OLR, in the range of approximately 0.190–0.240. At lower loading rates of 2.0, 2.5 and 3.0 kg VS m^{-3} d^{-1}, propionic acid was, for the most part, negligible and the medium was dominated by acetic acid. This illustrated the supremacy of the acetoclastic pathway in methanogenesis. As the OLR was increased to 3.5 and 4.0 kg VS m^{-3} d^{-1}, minor propionic acid accumulation was evident and at times exceeded acetic levels but in general remained at low quantities. Acetic acid levels remained particularly constant throughout the trial. The total concentration of VFAs in R5

resided well below the recommended upper safety limits of operation – approximately 1 g L^{-1} as stated in the IEA monitoring brochure (Drosg, 2013).

5.3.2 VFA profile for R6 (100 % grass silage)

Figure 5.2 shows the VFA profile for R6 with corresponding FOS/TAC values. In contrast to R5, there were evident spikes in FOS/TAC and corresponding spikes in acetic and propionic acid concentrations. This suggests that at times the acetoclastic pathway regressed and the microbial community shifted towards the hydrogenotrophic pathway, particularly at OLRs of 3.0 and 3.5 kg VS m^{-3} d^{-1}, where the SMYs remained at a maximum output. R6 had a much more erratic trend in comparison to R5. However, similar to R5, there was negligible propionic acid at lower loading rates, in this case up to 2.5 kg VS m^{-3} d^{-1}. At OLRs in the range of 3.0– 4.0 kg VS m^{-3} d^{-1}, FOS/TAC values were elevated and bordered the recommended upper threshold limit of 0.300. This suggests the microbial community were somewhat stressed. However, in terms of total concentrations of VFAs, the reactor rarely exceeded the recommended upper safety threshold. This suggests that although a highly erratic trend was evident, there was not significant accumulation of VFAs that would cause significant inhibition to the reactor.

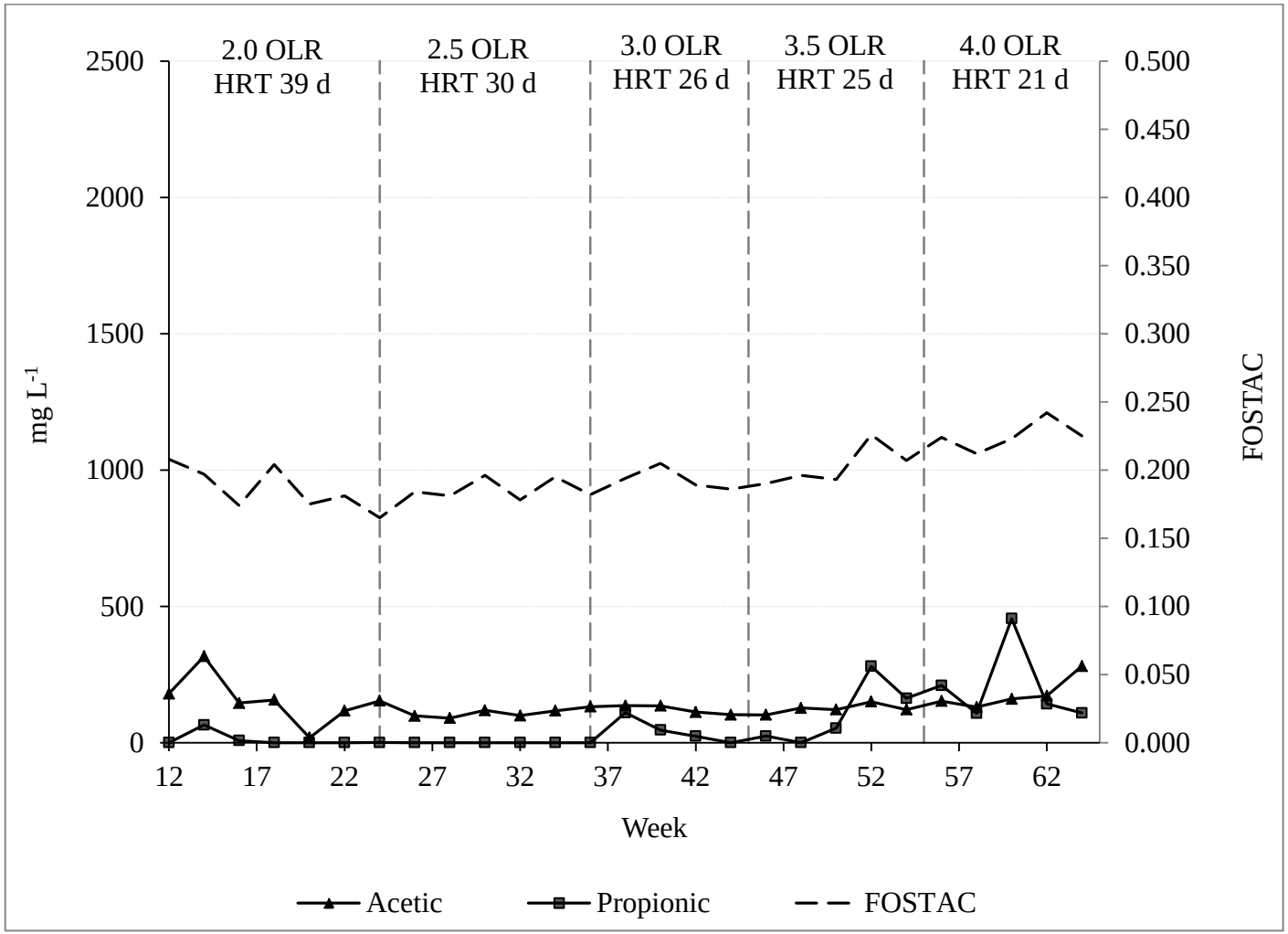


Figure 5.1 VFA profile for R5 digestate samples

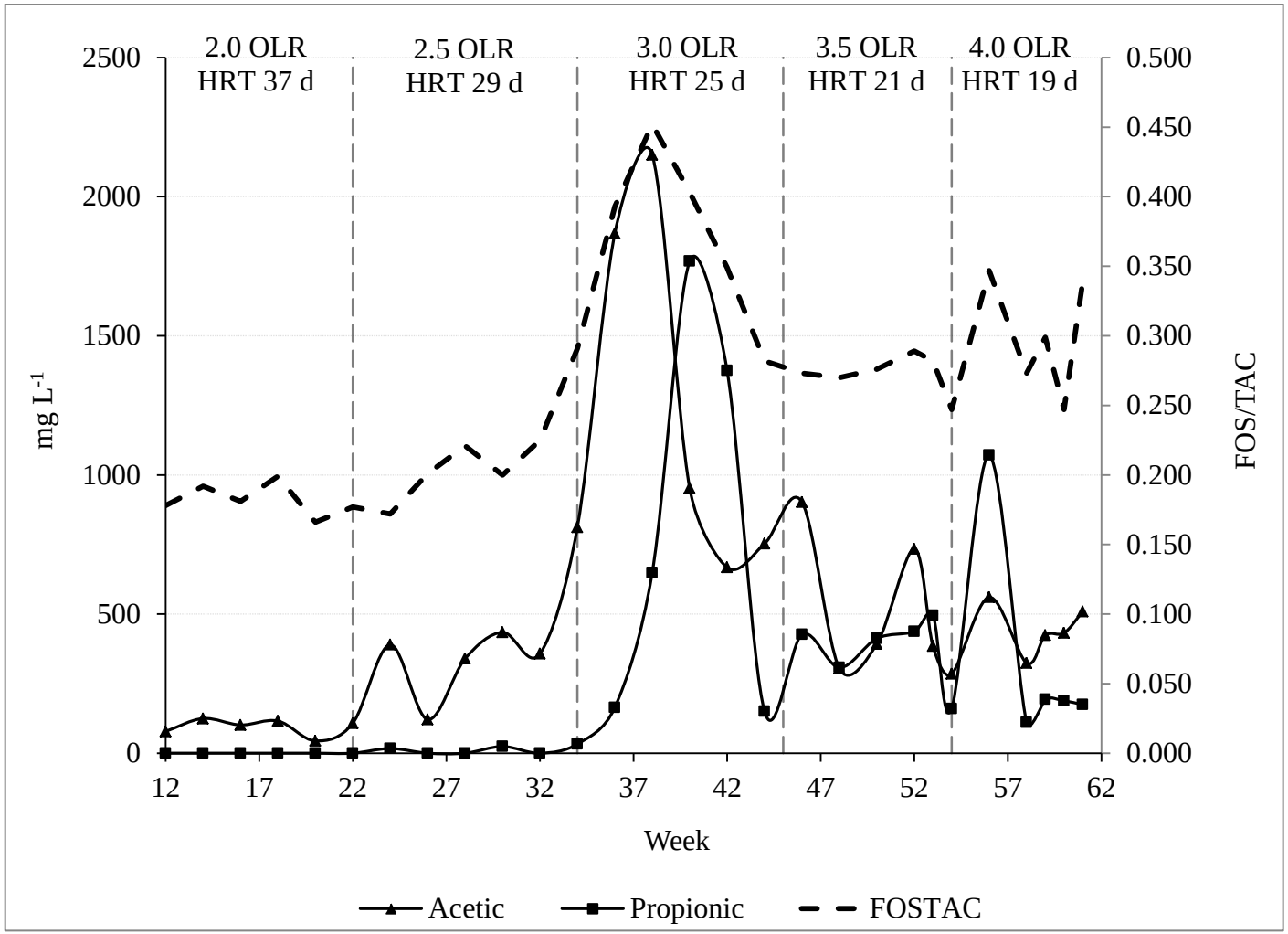


Figure 5.2 VFA profile for R6 digestate samples

5.3.3 Trends in SMY for R5 and R6

Upon further investigation, an interesting trend was found in relation to the SMYs recorded for R5 and R6. Figure 5.3 shows the SMY trend for R5 and R6 over the range of OLRs tested. All data shown represent the final retention time at each respective OLR. At OLRs of 2.0 and 2.5 kg VS m^{-3} d^{-1} (A and B), a distinct difference can be seen between the methane yields from both reactors. As the feeding rate is increased to 3.0 kg VS m^{-3} d^{-1} (C), the yields from both reactors begin to converge. A further increase in loading rate to 3.5 kg VS m^{-3} d^{-1} (D) causes the SMYs to crossover at certain points. When both reactors are fed at a rate of 4.0 kg VS m^{-3} d^{-1} (E), it can be seen that the SMYs are essentially overlapping. This is a very significant observation, whereby, it may be beneficial for a farmer to add low proportions of slurry to a grass digester at an OLR of 4.0 kg VS m^{-3} d^{-1} as the attainable SMY is similar to that of the mono-digestion of grass silage. From the VFA profiles discussed earlier it is also evident that there is greater reactor stability achieved with 20 % additions of dairy slurry (VS basis). Ireland has an abundance of slurry and therefore a potential use for such agricultural residues would be advantageous.

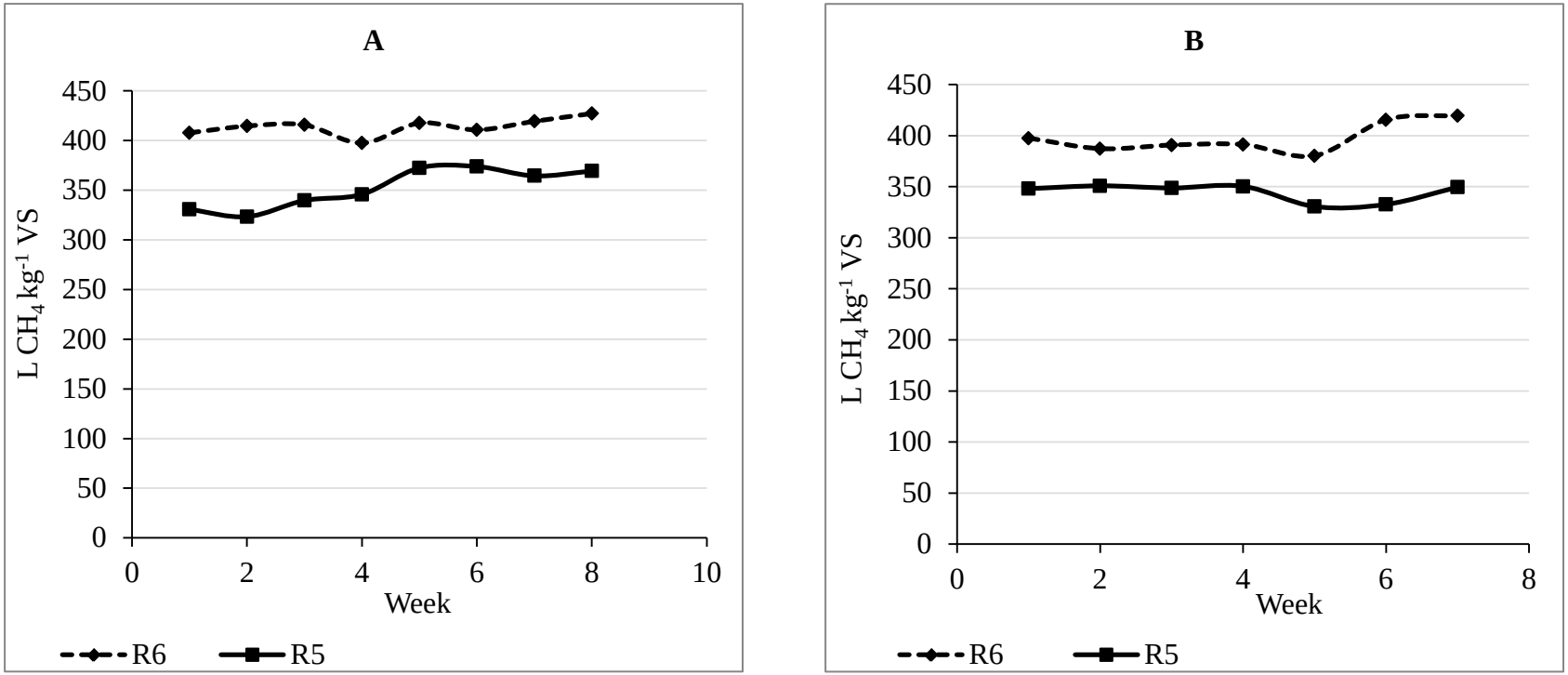
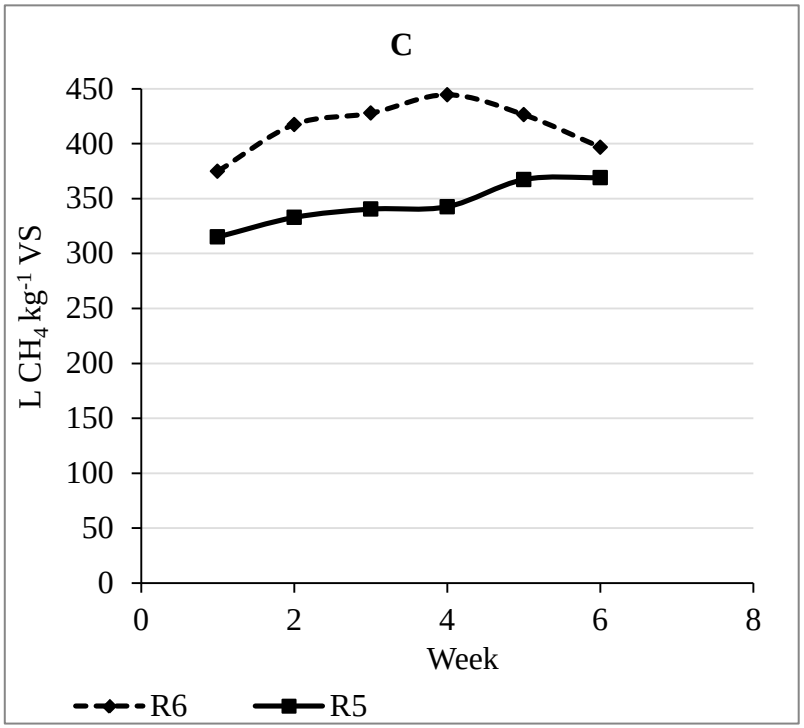


Figure 5.3 SMYs for R5 and R6: A: 2.0 kg VS m^{-3} d^{-1}; B: 2.5 kg VS m^{-3} d^{-1};



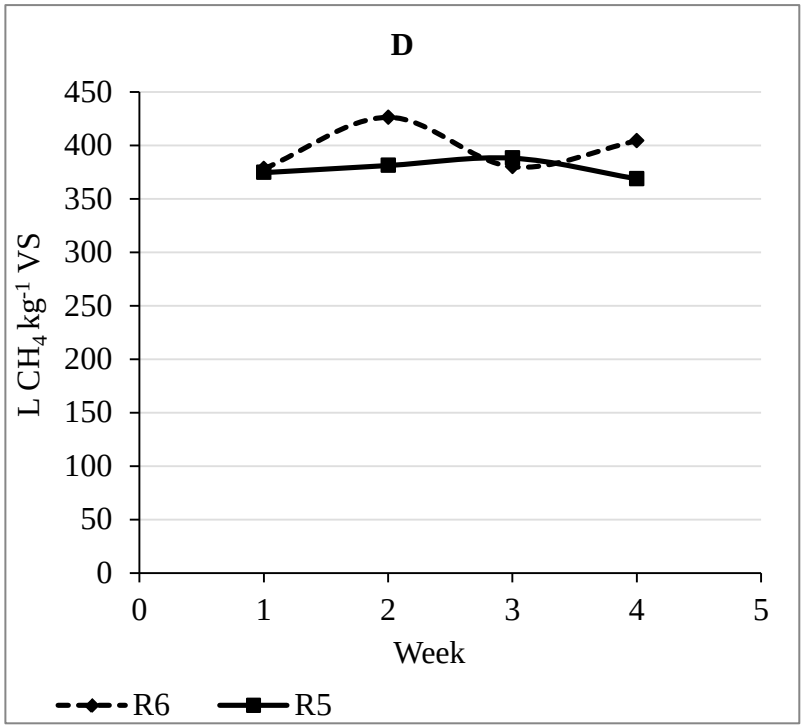


Figure 5.3 continued SMYs for R5 and R6: C: 3.0 kg VS m^{-3} d^{-1}; D: 3.5 kg VS m^{-3} d^{-1};

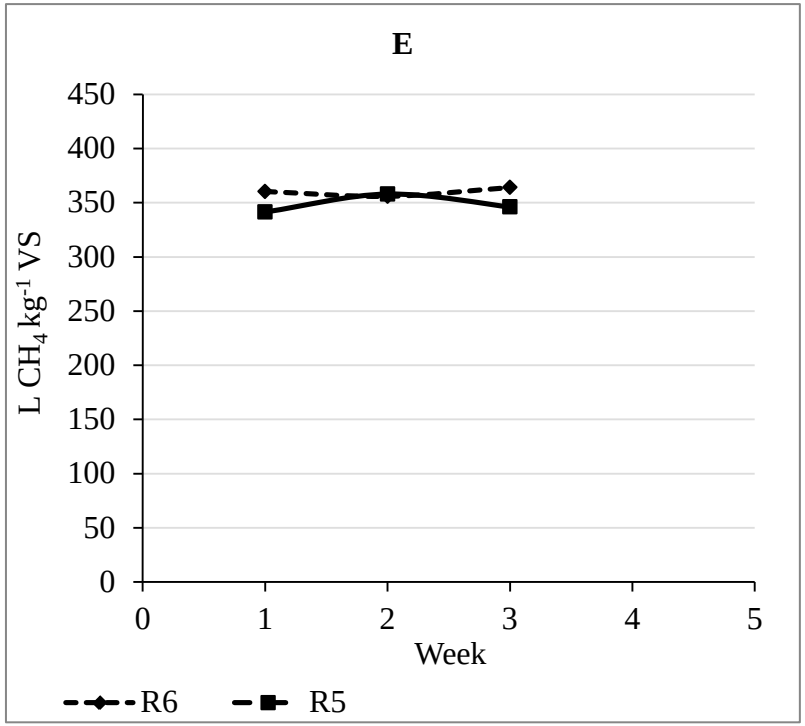


Figure 5.3 continued SMYs for R5 and R6: E: 4.0 kg VS m^{-3} d^{-1}

5.3.4 Analysis of trace elements in fresh samples of grass silage and dairy slurry

TE analysis was performed on fresh samples of both grass silage and dairy slurry before reactor start up (Table 5.1). Two samples of dairy slurry were examined in order to test the variability of TEs at different collection times of the slurry. The concentration of Co for grass silage was low, recording a value of less than 0.25 mg kg^{-1}. This corresponded to concerns raised in previous literature (Jarvis *et al.*, 1997) regarding Co deficiency in grass digestion. Both slurry samples showed relatively consistent values for Co, averaging 2.81 mg kg^{-1}. Dairy slurry samples also had higher concentrations of Ni (averaging 5.89 mg kg^{-1}) in comparison to the grass silage (3.63 mg kg^{-1}). Interestingly, concentrations of Mo and Se were over 6 and 7 times greater, respectively, in the fresh grass silage sample compared to the dairy slurry samples. For this reason, it was postulated that deficiencies in Se and Mo were less likely to be a factor in mono digestion of this grass silage. As expected, the remaining TEs were more abundant in the dairy slurry than in the grass silage with large variations evident in concentrations of Cu, Fe, Mn, Zn.

Table 5.1 Trace element analysis of fresh substrates

Element	Unit	Grass Silage	Slurry A	Slurry B
Cadmium	mg kg^{-1}	<0.25	<0.25	<0.25
Cobalt	mg kg^{-1}	<0.25	2.5	3.2
Copper	mg kg^{-1}	5.7	59.9	55.9
Iron	mg kg^{-1}	277	3228	4270
Manganese	mg kg^{-1}	38.4	250	261
Molybdenum	mg kg^{-1}	13.6	2.0	2.3
Nickel	mg kg^{-1}	3.6	5.3	6.5
Selenium	mg kg^{-1}	10.3	1.6	1.1
Zinc	mg kg^{-1}	23.5	304	154

5.3.5 Analysis of trace elements from digestate of R5 and R6 over trial period

Comprehensive TE analyses were carried out on both R5 and R6. This was performed over the lifetime of the trial for the range of OLRs with the objective of finding what compositional differences were generated through 20 % dairy slurry addition. The concentration of each TE assessed, for R5 and R6, are shown in Table 5.2 and Table 5.3, respectively. As the SMYs for R6 were found to be satisfactory up to an OLR of 3.5 kg VS m^{-3} d^{-1}, attaining maximum biomethane efficiencies, it could not be said that the mono-digestion of grass silage showed any TE deficiencies. This was most likely due to the recirculation of effluent liquor which is recommended in increasing bioavailability and retention of such nutrients (Jarvis *et al.*, 1997). Compared to previous literature on grass digestion, R6 remained above the recommended critical concentration for Co of 0.02 mg L^{-1}.

The potential to supplement specific TEs to R6 to maximise the biomethane efficiency (and thereby negate effects of short HRT) at high loading rates (4.0 kg VS m^{-3} d^{-1}) was explored. In examining the TE profile, for an OLR range of 2.0–3.5 kg VS m^{-3} d^{-1}, Co, Ni and Fe were three TEs identified as being undersupplied in R6. This was based on the overall concentrations of these three specific TEs in R6 relative to the concentrations present in R5 at the same timescale of operation. Figure 5.4 illustrates the comparison between concentrations of Co, Fe and Ni between R5 and R6 and the range of OLRs tested. As R5 maintained maximum biomethane efficiencies at each OLR, it was assumed the reactor had a sufficient quantity of these and other nutrients for effective digestion of the substrates. The premise was then to match these conditions within R6 and establish if TE supplementation could improve the performance, at an OLR of 4.0 kg VS m^{-3} d^{-1}, compared to the same reactor prior to supplementation of TEs.

Table 5.2 Trace element analysis for R5

		Organic Loading Rate (kg VS m^{-3} d^{-1})				
Element	Unit	2.0	2.5	3.0	3.5	4.0
Cadmium	mg kg^{-1}	0.2	0.2	<0.25	<0.25	0.4
Cobalt	mg kg^{-1}	3.2	2.8	3.2	2.9	2.3
Copper	mg kg^{-1}	51.3	46.4	44.2	41.2	40.4
Iron	mg kg^{-1}	1843	2581	3408	2066	1491
Manganese	mg kg^{-1}	207	193	220	226	236
Molybdenum	mg kg^{-1}	35.3	33.7	39.8	36.0	36.0
Nickel	mg kg^{-1}	64.3	40.0	88.6	73.0	67.9
Selenium	mg kg^{-1}	27.5	26.1	12.3	7.2	7.4
Zinc	mg kg^{-1}	174	143	104	107	125

Table 5.3 Trace element analysis for R6

		Organic Loading Rate (kg VS m^{-3} d^{-1})				
Element	Unit	2.0	2.5	3.0	3.5	4.0
Cadmium	mg kg^{-1}	0.2	0.2	<0.25	<0.25	0.3
Cobalt	mg kg^{-1}	1.7	1.9	0.6	0.9	1.0
Copper	mg kg^{-1}	46.2	30.9	22.5	24.8	25.3
Iron	mg kg^{-1}	1469	614	749	967	1020
Manganese	mg kg^{-1}	152	144	203	192	212
Molybdenum	mg kg^{-1}	42.6	50.6	44.3	49.7	42.6
Nickel	mg kg^{-1}	38.5	21.3	20.3	36.4	46.3
Selenium	mg kg^{-1}	33.9	35.1	9.4	11.2	8.3
Zinc	mg kg^{-1}	78.3	67.1	54.4	54.2	88.0

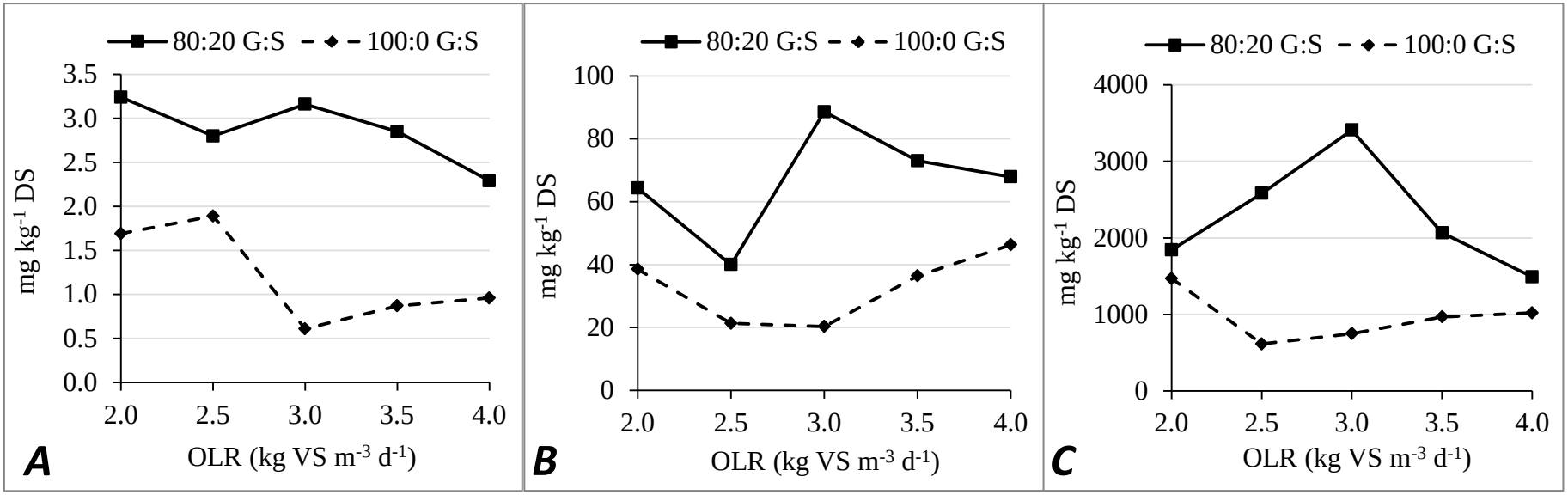


Figure 5.4 Trace element concentrations for R5 and R6: A: Cobalt; B: Nickel; C: Iron

5.3.6 Supplementation of undersupplied trace elements

Mono-digestion of grass silage (R6) was supplemented daily with Co, Ni and Fe from week 65, which commenced the fourth HRT at an OLR of 4.0 kg VS m^{-3} d^{-1}. The exact concentrations added to the reactor were 0.13 mg Co L^{-1} reactor, 2.48 mg Ni L^{-1} and 74.40 mg Fe L^{-1}. This addition allowed R6 to match the concentrations available in R5 at 3.5 kg VS m^{-3} d^{-1} for these specific TEs. All other TEs were assumed to be at an adequate level for R6. The supplementation of Co, Fe and Ni to R6 was carried out over a full HRT.

The supplementation of TEs to R6 effected an increase in the SMY for R6 from circa. 360 L CH_4 kg^{-1} VS to 404 L CH_4 kg^{-1} VS. Thus the biomethane efficiency of the reactor improved from 0.90 to 1.01. Figure 5.5 shows the SMY for R6 at an OLR of 4.0 kg VS m^{-3} d^{-1} WTE (with TE) with the corresponding bounce in SMY as the TEs were added. Figure 5.6 shows the VFA profile for R6 at an OLR of 4.0 kg VS m^{-3} d^{-1} WTE. The accumulation of propionic acid that was apparent before TE supplementation was eliminated. Furthermore, acetic acid concentrations declined to low values after adding Co, Ni and Fe, illustrating a stable and efficient process. This suggests the acetoclastic pathway once again dominated methanogenesis.

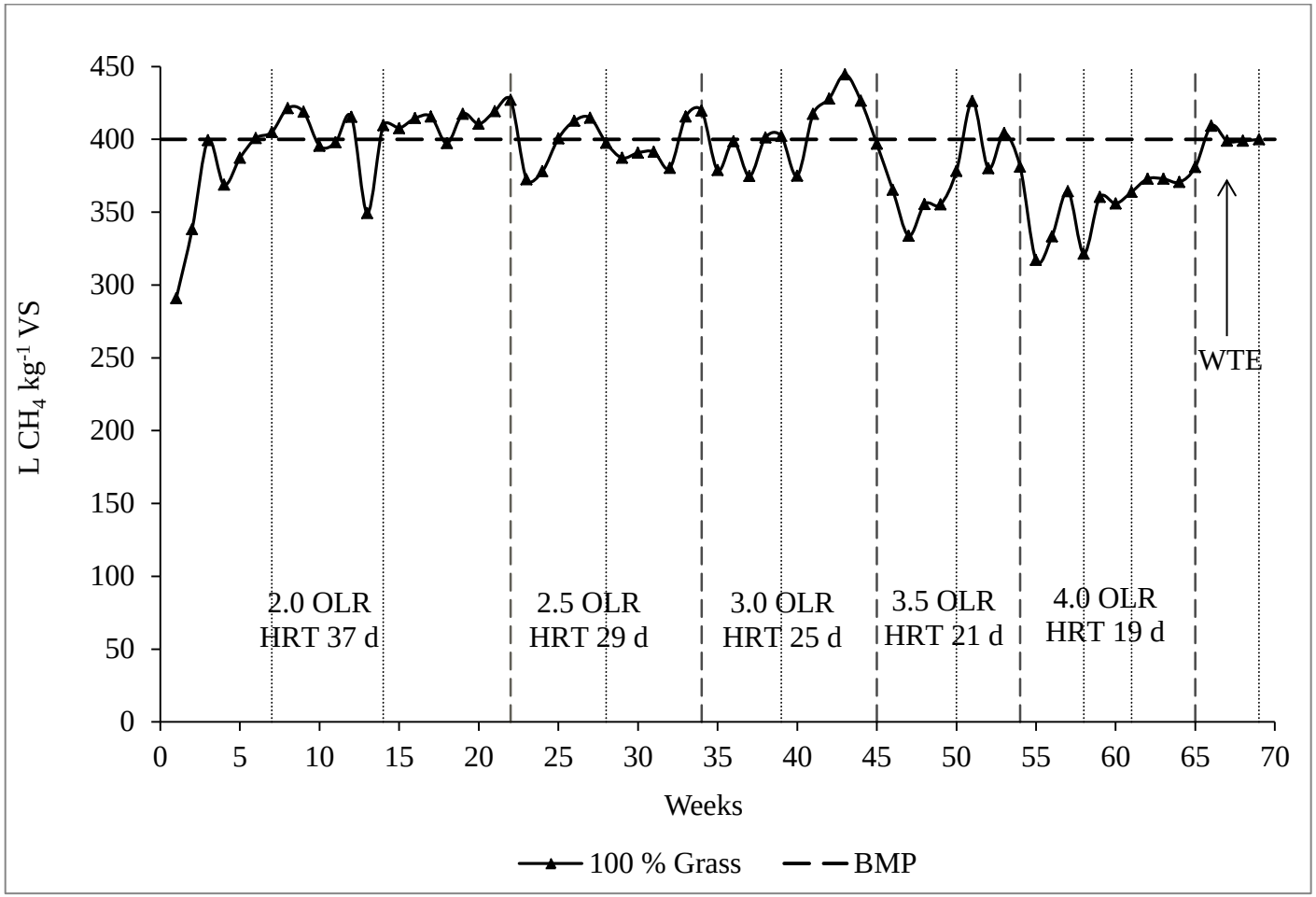


Figure 5.5 SMY for R6 without and with TE supplementation (WTE) in the reactor medium

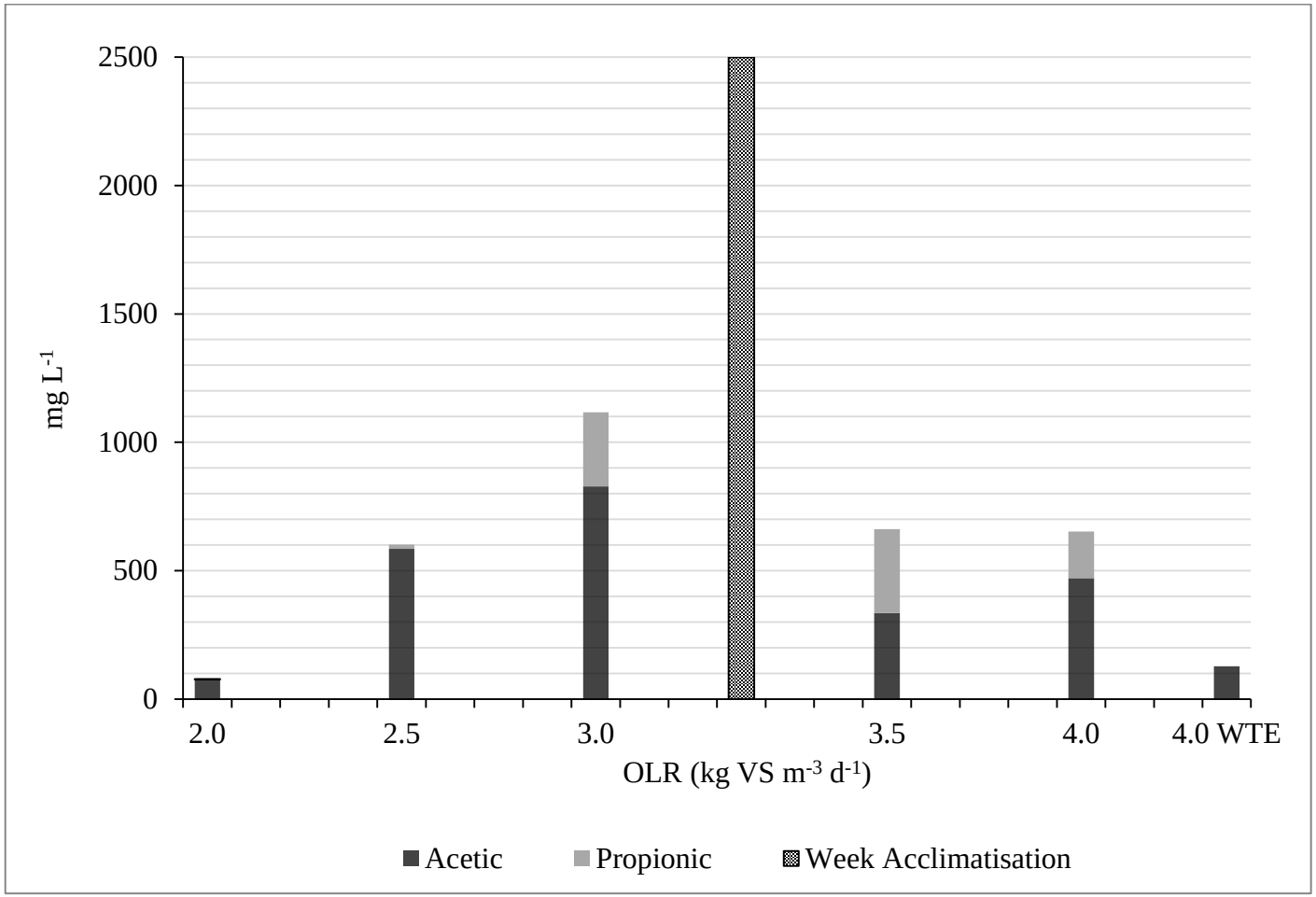


Figure 5.6 VFA Profile for R6 digestate samples without and with TE supplementation (WTE) in the reactor medium

5.3.7 Optimal long term digestion of grass silage and dairy slurry

Although the addition of Co, Ni and Fe (in calculated quantities) alleviated restrictions in mono-digestion of this ensiled perennial ryegrass, different ensiled grasses and different slurries will have different TE profiles. The mineral composition of grass silages depends on grass species, time of harvest, soil type and fertiliser input. The mineral composition of slurry depends on the diet fed to livestock and the method of collection (for example with and without straw bedding). The availability of TEs will be influenced by their solubility and the associated ease of being 'washed out' of the digester. Thus, the specific TEs that could limit anaerobic digestion, and the extent to which any of them will be 'in deficit' will logically vary. For this reason, the findings of this study are specific to this particular grass silage and this particular dairy slurry.

The following recommendations can be made for the operation of farm digesters based on the perennial ryegrass silage used in this study:

- Recirculation of separated liquor effluent maintained the dry solids content of the reactor at less than 100 g kg^{-1} and allowed organic loading rates of 4.0 kg VS m^{-3} d^{-1} be achieved.
- Co-digesting grass silage with dairy slurry at a mixture of 80:20 by VS content provided a stable process which did not require addition of trace elements.
- Mono-digestion of this grass silage required addition of cobalt, nickel and iron to allow stable digestion.

5.4 Conclusions

Biomethane efficiency was higher for co-digestion of grass silage and dairy slurry (80:20 VS basis) than for mono-digestion of grass silage at an OLR of 4.0 kg VS m^{-3} d^{-1} prior to addition of trace elements (1.01 versus 0.90 respectively). The VFA profile for mono-digestion also displayed significantly higher levels of propionic acid. Supplementation of three identified trace elements (cobalt, nickel and iron) increased the SMY in mono-digestion of grass silage to 404 L CH_4 kg^{-1} VS (12 % increase). The VFA profile after addition of TEs improved with negligible concentrations of propionic acid.

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References

Allen, E., Wall, D.M., Herrmann, C., Murphy, J.D. 2014. Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel. *Bioresour. Technol.*, 170(0), 436-444.

Angelidaki, I., Ellegaard, L. 2003. Codigestion of manure and organic wastes in centralized biogas plants. *Applied Biochemistry and Biotechnology*, 109(1-3), 95-105.

Banks, C.J., Zhang, Y., Jiang, Y., Heaven, S. 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresource Technology*, 104(0), 127-135.

Braun, R., Brachtl, E., Grasmug, M. 2003. Codigestion of proteinaceous industrial waste. *Applied Biochemistry and Biotechnology*, 109(1-3), 139-153.

Demirel, B., Scherer, P. 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass and Bioenergy*, 35(3), 992-998.

Drosg, B. 2013. IEA Task 37 - Process monitoring in biogas plants.

Facchin, V., Cavinato, C., Fatone, F., Pavan, P., Cecchi, F., Bolzonella, D. 2013. Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: The influence of inoculum origin. *Biochemical Engineering Journal*, 70(0), 71-77.

Gustavsson, J., Shakeri Yekta, S., Sundberg, C., Karlsson, A., Ejlertsson, J., Skyllberg, U., Svensson, B.H. 2013. Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. *Applied Energy*, 112(0), 473-477.

Hinken, L., Urban, I., Haun, E., Weichgrebe, D., Rosenwinkel, K.H. 2008. The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests. *Water Science & Technology*, 58(7), 1453-1459.

Jarvis, Å., Nordberg, Å., Jarlsvik, T., Mathisen, B., Svensson, B.H. 1997. Improvement of a grass-clover silage-fed biogas process by the addition of cobalt. *Biomass. Bioenerg.*, 12(6), 453-460.

Jiang, Y., Heaven, S., Banks, C.J. 2012. Strategies for stable anaerobic digestion of vegetable waste. *Renewable Energy*, 44(0), 206-214.

Karlsson, A., Einarsson, P., Schnürer, A., Sundberg, C., Ejlertsson, J., Svensson, B.H. 2012. Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. *Journal of Bioscience and Bioengineering*, 114(4), 446-452.

Nordmann, W. 1977. Die Überwachung der Schlammfaulung. KA-Informationen für das Betriebspersonal. *Beilage zur Korrespondenz Abwasser, 3/77.*

Oleszkiewicz, J.A., Sharma, V.K. 1990. Stimulation and inhibition of anaerobic processes by heavy metals—A review. *Biological Wastes*, 31(1), 45-67.

Pobeheim, H., Munk, B., Johansson, J., Guebitz, G.M. 2010. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresource Technology*, 101(2), 836-839.

Pobeheim, H., Munk, B., Lindorfer, H., Guebitz, G.M. 2011. Impact of nickel and cobalt on biogas production and process stability during semi-continuous anaerobic fermentation of a model substrate for maize silage. *Water Research*, 45(2), 781-787.

Schattauer, A., Abdoun, E., Weiland, P., Plöchl, M., Heiermann, M. 2011. Abundance of trace elements in demonstration biogas plants. *Biosystems Engineering*, 108(1), 57-65.

Seppälä, M., Pyykkönen, V., Väisänen, A., Rintala, J. 2013. Biomethane production from maize and liquid cow manure – Effect of share of maize, post-methanation potential and digestate characteristics. *Fuel*, 107(0), 209-216.

Takashima, M., Speece, R.E. 1989. Mineral nutrient requirements for high-rate methane fermentation of acetate at low SRT. *Research Journal of the Water Pollution Control Federation*, 61(11/12), 1645-1650.

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D. 2014. Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry. *Bioresource Technology, 173(2014) 422-428*.

Weiland, P. 2008. Wichtige Messdaten für den Prozessablauf und Stand der Technik in der Praxis. *Fachagentur Nachwachsende Rohstoffe e.V. (FNR).*

Wilkie, A., Goto, M., Bordeaux, F.M., Smith, P.H. 1986. Enhancement of anaerobic methanogenesis from napiergrass by addition of micronutrients. *Biomass*, 11(2), 135-146.

Zhang, L., Jahng, D. 2012. Long-term anaerobic digestion of food waste stabilized by trace elements. *Waste Management*, 32(8), 1509-1515.

Zhang, L., Lee, Y.-W., Jahng, D. 2011. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresource Technology*, 102(8), 5048-5059.

Zhang, L., Ouyang, W., Lia, A. 2012. Essential Role of Trace Elements in Continuous Anaerobic Digestion of Food Waste. *Procedia Environmental Sciences*, 16(0), 102-111.

6. Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage

Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage

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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.

Keywords: grass silage, anaerobic digestion, rumen fluid, particle size, biomethane.

6.1 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 & 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 & 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 substrates, and hence were recommended in maximising methane yields. Intensive chopping (to a particle size below 7−8 mm) was not

recommended for such substrates as the potential energy output gain did not merit the associated additional energy input costs (Herrmann *et al.*, 2012b). A range of particle sizes for grass digestion are discussed in the scientific literature. For batch biomethane potential (BMP) assays, particle sizes of 1 cm have been widely reported (Lehtomäki *et al.*, 2008; Xie *et al.*, 2011). Previous studies at continuous scale have investigated macerated grass silage with particle size of approximately 1 cm (Wall *et al.*, 2014b), however longer particle lengths of 2−3 cm have also been reported in co-digestion of grass silage with cow manure (Jagadabhi *et al.*, 2008; Lehtomäki *et al.*, 2007).

Rumen fluid, containing archaea, bacteria, protists and fungi (Yue *et al.*, 2013), is found in the first compartment of a ruminant's stomach (reticulo-rumen) and possesses high cellulosic-degrading properties (Gijzen *et al.*, 1990; Hu *et al.*, 2004). Rumen fluid has been sought to potentially enhance the digestion of lignocellulosic biomass by hydrolysing the linkages between cellulose, hemicellulose and lignin (Yue *et al.*, 2013). A pH range of 6.8−7.3 has been suggested as the optimum range for rumen microorganisms to hydrolyse such structures (Hu *et al.*, 2004) that can constitute up to 75 % of grass silage dry matter (Xie *et al.*, 2011). Hydrolysis is the rate-limiting step of the anaerobic digestion process for these lignocellulosic materials (Lynd *et al.*, 2002).

Rumen fluid has primarily been used as an inoculum source and is widely indicated in literature to have a beneficial impact. It has been reported that rumen fluid can potentially breakdown cellulose structures in such materials faster than other inoculum sources (Yue *et al.*, 2013). In semi-continuous digestion of corn stover, the use of strained rumen fluid inoculum effected rapid destruction of VS and generated a high production of volatile fatty acids (VFAs) (Hu & Yu, 2005). A study

comparing two inoculum sources, digester sludge and rumen fluid, for the digestion of aquatic plants indicated that rumen fluid increased product formation rate in terms of g chemical oxygen demand (COD) per g total solids (Yue *et al.*, 2012). The digestion of bagasse and maize bran with strained rumen fluid exhibited VFA production within 3 days, indicating effective hydrolytic conversion to acids (Kivaisi & Eliapenda, 1995). However according to Sawatdeenarunat *et al.*, (2015) the use of rumen fluid as an inoculum source has restrictions as it would be difficult to direct such large quantities to full-scale commercial biogas plants.

The use of rumen fluid as a co-substrate has also been investigated. Co-digestion of palm oil mill effluent with small quantities of rumen fluid (5−10 % by volume) in semi-continuous reactors was examined at different hydraulic retention times (HRTs) and organic loading rates (OLRs) (Alrawi *et al.*, 2011). High COD removal efficiencies and high methane content were observed in co-digestion. Studies on the influence of rumen fluid in treating municipal solid waste also highlighted that higher proportions of rumen fluid gave higher destruction rates of organic matter (Lopes *et al.*, 2004). Continuous systems operating with rumen fluid were also shown to be more efficient than batch cultures due to a more stable pH environment. The objective of this study was to examine the effect of physical (particle size reduction) and biological (rumen fluid addition) treatments, to stimulate hydrolysis, on the digestion of grass silage with low DSD for the production of biomethane.

6.2 Methods

6.2.1 Grass silage

The grass silage, a first-cut perennial ryegrass *(Lolium perenne),* was harvested on June 24th at an advanced growth stage (grass was stemmy, had fully headed-out and

was after flowering) when it had a relatively high lignified fibre content. The dry solids (DS) and volatile solids content of the grass silage was 217 g kg^{-1} and 907 g VS kg^{-1} DS, respectively. The measured neutral detergent fibre (NDF) was 716 g kg^{-1} DS while the DSD was low at 555 g kg^{-1}. The grass silage represented a much less digestible crop substrate for anaerobic digestion than examined in previous grass silage digestion studies where the DSD was higher at 653 g kg^{-1} (Wall *et al.*, 2014a; Wall *et al.*, 2014b). Once harvested, the grass was initially wilted for 48 hours and subsequently baled and stretch-wrapped in polyethylene film. For storage and handling purposes the silage was then subdivided into smaller rectangular bales of approximately 25 kg, again wrapped in stretch film, and stored at ambient room temperature (18−20°C). To represent the different particle sizes the grass silage was comminuted by two methods. The "<1 cm" particle size was achieved using a heavy duty mincer (Buffalo Heavy Duty Mincer, 250 kg hr^{-1}) which macerated the grass silage. To achieve the ">3 cm" particle size the grass silage was chopped with a scissors by hand. This meant that the silage, with two different methods of chopping, not only varied in particle size but also differed in the extent of physical shredding, tearing and disruption. Subsamples of the two grass silages were stored at -20°C until required for experimental use.

6.2.2 Rumen fluid

To collect a sufficient quantity of rumen fluid, six fattened beef heifers were offered hay (made from stemmy grass) ad libitum as their sole dietary ingredient for 7−10 days prior to collection. Post-mortem, rumen contents were retrieved, mixed to ensure a homogenous sample and squeezed through muslin cloth and a large sieve to leave only the strained rumen fluid. Approximately 70 L of this liquor was decanted

into 50 mL and 4 mL vials and immediately frozen in liquid N and stored at -20°C until required. The pooled rumen fluid had a pH of 7.22 and a DS and VS content of 22 g kg^{-1} and 636 g VS kg^{-1} DS, respectively. At both batch and continuous scale, rumen fluid additions were made at a rate of 50 mL per kg grass silage added. This was based on the grass silage (217 g DS kg^{-1}) being able to retain the liquid without excess seeping out and removing soluble substrates with it. The frozen rumen fluid was thawed and heated to approximately 39°C immediately prior to application. It has been shown (Prates *et al.*, 2010) that freezing small volumes of rumen fluid in liquid N, and implementing a quick thawing process, provided a negligible effect on the microbial diversity.

6.2.3 Biomethane potential (BMP) assay

The Bioprocess™ automatic methane potential test system (AMPTS) was used to carry out BMP assays in triplicate on the selected substrates, as well as a cellulose standard (Sigma Aldrich, CAS Number: 9004-34-6) and an inoculum control. Each bottle had a 400 mL working volume with 250 mL of headspace. The bottle contents were individually mixed by stirrers at 30 rpm and operated every other minute. Temperature for all bottles was held constant at 37°C by means of a large heated water bath. A calculated quantity of each substrate and inoculum was initially added to the bottles corresponding to a 2:1 inoculum-to-substrate ratio which is recommended to overcome any problems with process inhibition (Chynoweth *et al.*, 1993). Distilled water was added to bring the content level in the bottle to 400 mL and the headspace was flushed with nitrogen prior to start-up to ensure anaerobic conditions. A 3 M sodium hydroxide (NaOH) solution was used to remove carbon dioxide and other trace gases from the biogas produced. The resultant methane was

sent to a flow measurement device which measured gas through water displacement. Pressure and temperature were recorded continuously and gas yields were logged on a bespoke software package. Seven BMP assays (in triplicate) ran for a period of 30 days and included for: (1) mono-digestion of >3 cm grass silage (25 g wwt.); (2) mono-digestion of <1 cm grass silage (25 g wwt.); (3) inoculum; (4) mono-digestion of >3 cm grass silage (25 g wwt.) with rumen fluid addition (1.25 mL); (5) mono-digestion of <1 cm grass silage (25 g wwt.) with rumen fluid addition (1.25 mL); (6) Inoculum with rumen fluid (1.25 mL) (corresponding to 50 mL per kg silage as in 4 and 5); (7) cellulose.

6.2.4 Semi-continuous trials

Two identical continuously stirred tank reactors (CSTRs) were run for a period of 30 weeks. The cylindrical PVC pipe reactors operated with a 4 L working volume and a 1 L headspace. Temperature was controlled at mesophilic range (37 ± 1°C) by a heated water circulator which pumped water through brass coils around both reactors. Mixing was provided by vertically mounted stirrers attached to 24 V direct-current motors. Feeding was performed through an inlet port at the top of the reactor that was otherwise sealed by a rubber bung. The removal of digestate was achieved through an outlet port at the bottom of the reactor. The production of biogas was measured by wet-tip gas meters that were individually calibrated (50-55 mL/tip). The counting of gas tips was recorded by a data logger. All gas data were corrected for standard pressure and temperature.

The CSTRs operated with four permutations in total. These permutations included for both particle sizes and rumen fluid addition. Reactor R1 was used in mono-digestion of >3 cm grass silage (Week 1–18) and mono-digestion of <1 cm grass

silage (Week 19–30). Reactor R2 was used in mono-digestion of >3 cm grass silage with rumen fluid addition (Week 1–18) and mono-digestion of <1 cm grass silage with rumen fluid addition (Week 19–30). The organic loading rate (OLR) was held constant at 2.5 kg VS m^{-3} d^{-1} for the duration of the trial (30 weeks) for both R1 and R2. This equated to an effective HRT of 31 days allowing for daily recirculation of effluent liquor (< 25 g DS kg^{-1}) separated from the digestate. The addition of rumen fluid was executed by adding ca. 3 mL daily to the grass silage input feed, which corresponded to the daily input equivalent of 50 mL per kg silage.

The inocula for the BMP and CSTR trials were sourced from laboratory scale digesters operating on grass silage co-digested with dairy slurry. The inoculum was sieved through a 1 mm mesh to remove any larger particles and acclimatised by heating at 39°C for the week prior to start-up.

6.2.5 Analytical methods

For weekly calculations of FOS/TAC (volatile organic acids/total inorganic carbon), the Nordmann-method was used with 0.1 N sulphuric acid (Nordmann, 1977). A Titronic Universal Automatic titrator was used to perform a two-point titration (endpoints at pH 5.0 and pH 4.4). FOS/TAC values of below 0.300 indicate stable fermentation (Drosg, 2013). pH was measured daily using a Jenway 3510 pH meter. DS and VS analyses were determined according to Standard Methods 2540 G (APHA, 2005). Chemical oxygen demand (COD) and total ammonical nitrogen (TAN) were determined weekly using Hach Lange cuvette tests (LCK 914 and LCK 313, respectively) and evaluated by a DR3900 Hach Lange Spectrophotometer. Biogas samples from the continuous trials were collected three times per week from the outlet of the wet-tip gas meters using 1 L Tedlar gas bags. Analysis of the biogas

composition (% CH_4 v/v) was measured by an Agilent 6890 GC with thermal conductivity detector. For evaluation of DSD and NDF of the grass silage, samples were dried at 40°C for 48 h and subsequently ground using a Wiley hammer mill with a 1 mm pore screen. DSD was evaluated by the Tilley and Terry (1963) method but with final residue isolated by filtration (Whatman GF/A 55 mm, pore size 1.6 µm, Whatman International) instead of centrifugation. NDF, assayed with a heat stable amylase and sodium sulphite, concentrations were evaluated using the ANKOM filter bag technique (ANKOM, 2006a; ANKOM, 2006b) with reference to the analytical method of Van Soest *et al.* (1991), and expressed on an ash-free basis. VFA concentrations were measured using an Agilent 6890 GC with flame ionisation detector as described in Allen *et al.* (2014). VFA analysis was performed on an almost weekly basis for the CSTR trials, with samples prepared by centrifuging for 10 minutes at 15,000 rpm with 0.2 mL HPO_3 added to remove any particulate matter.

6.2.6 Kinetics and statistical analyses

The k-values (decay constants) for the BMP assays were determined using first-order kinetics:

$$y(t) = y_m \times \left(1 - e^{(-kt)}\right)$$

Where y(t) is the cumulative specific methane yield (SMY) at time t (mL CH_4 g^{-1} VS), y_m is the maximum SMY of the substrate (mL CH_4 g^{-1} VS), t is the time in days and k is the decay constant (1/days). The first-order kinetics were run using Matlab R2009a software.

Analysis of variance was conducted to determine the significance of effects of particle size reduction and rumen fluid addition on SMYs generated in the BMP

assays. The statistical analyses were carried out with SAS 9.4 using the procedure PROC GLM.

6.3 Results and discussion

6.3.1 Batch trials

6.3.1.1 Results of specific methane yields and kinetics analysis from the BMP trial

The results of the BMP assays for the grass silage are shown in Table 6.1. The values in the table include for removal of the effects of the inoculum. An increase of +7 % (or +4 L CH_4 kg^{-1} $VS_{inoculum}$) was found when comparing the mean SMY value of 'Inoculum with rumen fluid (1.25 mL)' treatment to the 'Inoculum' treatment (section 6.2.3: case 6 and 3).

Table 6.1 Mean SMYs (±standard deviation) and k-values of grass silage (>3 cm, <1 cm) with and without rumen fluid addition

Grass particle size	Rumen fluid	SMY (L CH_4 kg^{-1} VS)	k-value	$R^{2\,A}$
>3 cm	None	340 ± 9.2	0.12	0.94
>3 cm	Added	343 ± 12.5	0.10	0.93
<1 cm	None	343 ± 2.8	0.13	0.94
<1 cm	Added	350 ± 1.0	0.14	0.94
Level of significance		*(P value)*		
Particle size		0.322^{B}		
Rumen fluid		0.311^{B}		
Particle size*Rumen fluid		0.600^{B}		

[A] Coefficient of determination
[B] Not significant

6.3.1.2 Particle size and rumen fluid addition at batch BMP scale

BMP assays of the grass silage were investigated using the same inoculum and under identical conditions. Without rumen fluid, the particle size of <1 cm produced a

mean SMY similar to that of grass silage particle size of >3 cm (343 ± 2.8 v. 340 ± 9.2 L CH_4 kg^{-1} VS). However, these yields represented a drop in SMY of approximately 14−15 % on previous batch digestion studies investigating macerated (<1 cm) grass silage of lower lignocellulosic concentration and higher DSD (Wall *et al.*, 2014a; Wall *et al.*, 2014b).

The addition of rumen fluid increased the mean SMY values for both <1 cm (+7 L CH_4 kg^{-1} VS) and >3 cm (+3 L CH_4 kg^{-1} VS) grass silage as compared to their corresponding BMPs with no rumen fluid addition. These increases were in the range of that reported when comparing the treatment of 'Inoculum' with 'Inoculum with rumen fluid (1.25 mL)'. Thus, although the rumen fluid addition instigated a minor improvement in the methane yield, no obvious synergistic effects were evident by amalgamating the rumen fluid with grass silage in the BMP. A statistical analysis showed no significant difference between the SMYs with and without rumen fluid addition ($P = 0.311$) and for the different particle sizes of >3 and < 1 cm ($P = 0.322$). The effectiveness of the inoculum in the trial was examined using a cellulose control; this yielded 341 L CH_4 kg^{-1} VS, which equated to 82 % of the maximum theoretical value. Thus the yields obtainable from such batch digestion tests may not always be indicative of the SMYs produced at fully optimal conditions. The SMYs can be affected by inoculum that is not fully acclimatised to the substrate. Higher SMYs can be expected in continuous digestion involving substrates with high grass silage proportions with more acclimatised inoculum (Wall *et al.*, 2014b).

6.3.1.3 Kinetics analysis from BMP assays

First-order kinetics were used to evaluate the k-values (decay constants) at batch scale. The k-value obtained for grass silage of particle size <1 cm with rumen fluid

addition was 0.14 and was numerically the highest of the treatments assessed. All other treatments, regardless of particle size or rumen fluid addition, had marginally lower k-values in the range of 0.10–0.13. The k-values calculated were similar to the range previously reported for grass silage and can be compared to k-values for food waste and sea lettuce of 0.43 and 0.23, respectively (Allen *et al.*, 2013; Wall *et al.*, 2013). The coefficient of determination (R^2) was at a satisfactory range.

6.3.2 Continuous trials

6.3.2.1 Results of continuous digestion

Table 6.2 illustrates the measured process parameters in continuous digestion of grass silage at the two particle sizes (>3 cm and <1 cm) with and without the addition of rumen fluid. The concentrations presented reflect averaged values taken from the final HRT when the reactors were at steady state.

Table 6.2 Operational parameters for R1 (no rumen fluid addition) and R2 (rumen fluid addition) at steady state

Grass silage particle size	>3 cm		<1 cm	
Reactor	R1	R2	R1	R2
pH	7.78	7.81	7.63	7.72
FOS/TAC	0.164	0.163	0.436	0.181
Dry solids (g kg^{-1})	55	53	49	47
Volatile solids (g kg^{-1})	40	37	36	34
COD (g L^{-1})	43.4	35.5	46.2	42.3
TAN (g L^{-1})	2.1	1.9	1.9	1.8
Total VFA (g L^{-1})	0.1	0.2	2.9	0.1
CH_4 % (v/v)	47.7	45.5	48.7	51.2

6.3.2.2 Continuous digestion of grass silage (>3 cm) with (R2) and without (R1) rumen fluid addition

Grass silage, of particle size >3 cm, was digested for a total of 3 HRTs at an OLR of 2.5 kg VS m^{-3} d^{-1} (Figure 6.1) with the first and second HRTs acting as acclimatisation periods for the reactors to reach steady state conditions. The data reported in Table 6.2 was taken from the third and final HRT. The pH for both R1 and R2 remained stable and at an adequate range, effective for digestion. FOS/TAC values were maintained below the upper threshold of 0.300 and hence there was no evidence of stress by the microbial consortium or build-up of VFAs within the reactor. The DS content of the digestate from both reactors represented approximately 75 % destruction of solids from the process although this was somewhat misleading due to the mechanical problems associated with the reactors (detailed below). The COD concentration for R2 was lower than that in R1 at steady state conditions and may have been an effect of the rumen fluid providing a more proactive consortium of microorganisms in converting COD to gas. Concentrations of TAN and VFAs were adequate for both reactors. Previous grass digestion studies have indicated a methane content in the range of 50−53 % (v/v) (Wall *et al.*, 2014b) however the methane concentrations for this study were lower.

The particle size of >3 cm caused substantial mechanical problems for the duration of the trial for both R1 and R2. Grass silage tended to float on the surface of the reactor. This in turn caused only minor quantities of the grass silage to be removed from the outlet/effluent port at the bottom of the digester during the daily feeding-and-removal process. Longer grass silage particles also tended to wrap around the mixing blades in the reactor. This put excess strain on the motor driving the blades. As a result, numerous electrical shutdowns occurred in the 3 HRTs in replacing

damaged motors. In the first two weeks of the third HRT, approximately 447 g of grass silage was removed from R1 to alleviate the persistent mechanical failures; this equated to ca. 7 % of total grass silage fed to the reactor. Approximately 638 g of grass silage (or 10 % of total grass silage fed to reactor) was removed from R2 in the same period. All grass silage removed was immediately squeezed in a hydraulic press to remove the liquor incorporated within the substrate. This liquor was returned to the digester and feeding of the >3 cm grass silage continued. The digester substrate levels were inspected closely to ensure the correct volume was kept in each reactor. In effect, grass silage digestate that should have been removed from the bottom of the digesters was now being removed from the top due to floating material. Both R1 and R2 remained extremely difficult to operate with the longer grass silage particle size as a result of blockages/clogging in the outlet port. For the final two weeks of the third HRT, the electrical mixing system was shut off and both reactors were manually mixed with a lever by hand three times per day. The average SMYs for R1 and R2 in the final HRT were 343 and 342 L CH_4 kg^{-1} VS respectively. The daily addition of rumen fluid was not found to have an impact on the SMYs for the grass silage at >3 cm length.

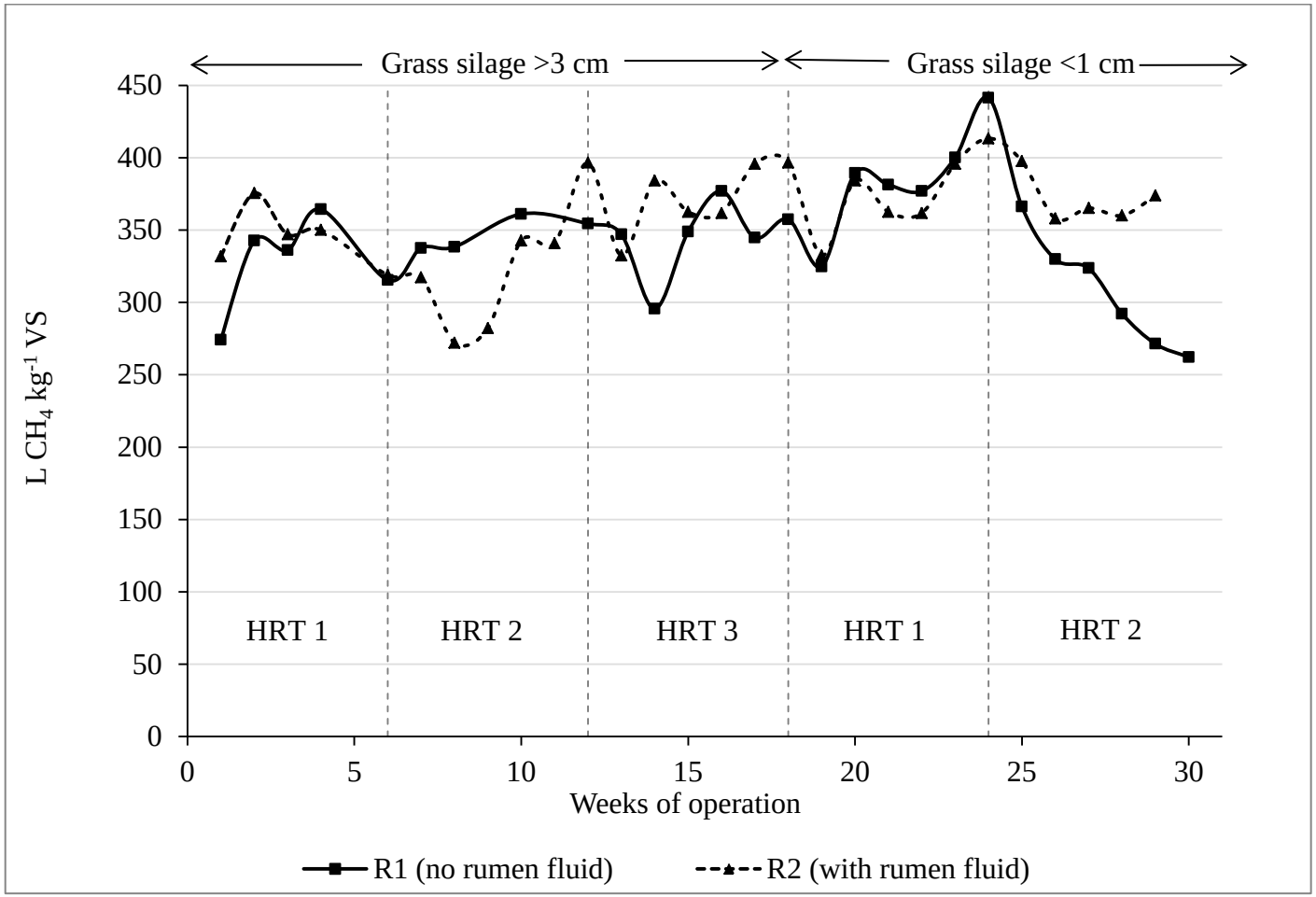


Figure 6.1 Specific methane yields for R1 (no rumen fluid addition) and R2 (rumen fluid addition)

6.3.2.3 Continuous digestion of grass silage (<1 cm) with (R2) and without (R1) rumen fluid addition

Grass silage, of length of <1 cm, was digested for 2 HRTs at an OLR of 2.5 kg VS m^{-3} d^{-1} immediately following the completion of the >3 cm grass silage digestion (Figure 6.1). The first HRT acted as an acclimatisation period for the reactors to reach steady state conditions. Data reported in Table 6.2 was taken from the second and final HRT. The disparity in performance between R1 and R2 was significantly more pronounced at this shorter grass silage particle size. R1, with no rumen fluid addition, had a substantial build-up of VFAs, reaching over 5 g L^{-1} on the final day of operation. This corresponded to a FOS/TAC value of 0.878 – far beyond the recommended threshold (0.300) for stable fermentation. This is illustrated in Figure 6.2. Consequently, pH values for R1 dropped and the reactor performance diminished with the SMY falling to as low as 271 L CH_4 kg^{-1} VS. As previously indicated, the grass silage investigated in this study represented a low-quality crop with high fibre content. R1 struggled to maintain stable fermentation and failed to digest the high fibre substrate effectively; this may be due to a lack of micronutrients (Wall *et al.*, 2014a) or deficiency in microbial diversity. At the time of trial shutdown, reactor R1 was digressing to a state of failure (see Figure 6.1 HRT 2 and Figure 6.2 weeks 25-30).

Reactor R2, with daily rumen fluid addition, performed much more efficiently. No accumulation of VFAs occurred and the pH remained stable throughout. It is believed that the addition of rumen fluid acted as a buffer, continuously helping to provide more favourable conditions for the bacterial consortium within the reactor. This provided a synergistic effect in terms of lignocellulosic breakdown and process efficiency. The average yield over the final HRT was 371 L CH_4 kg^{-1} VS. This

significantly out-performed the SMYs of the BMP for grass silage cut at <1 cm with rumen fluid addition (350 L CH_4 kg^{-1} VS) and can be attributed to a more efficient acclimatised inoculum with continuous supplementation from the rumen fluid addition. FOS/TAC remained in a healthy state with no evidence of stress on the microbes. Concentrations of TAN did not exceed 2.5 g L^{-1} for both reactors and thus there were no associated inhibitory effects. The methane composition in the biogas increased to expected concentrations (50–55 %) for R2 at the <1 cm particle size. However, R1 remained low and did not reach over 50 % (v/v).

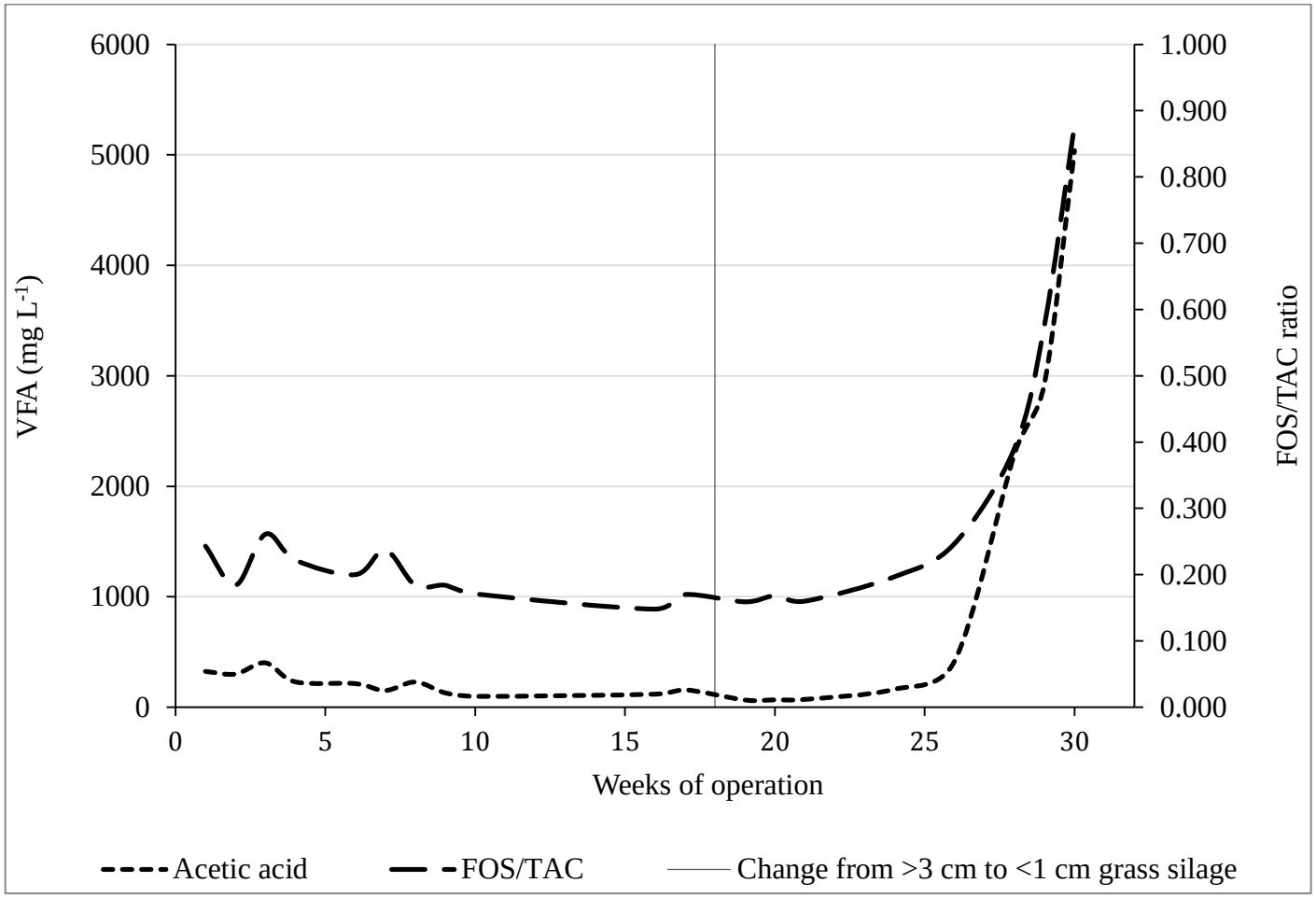


Figure 6.2 Acetic acid profile with corresponding FOS/TAC values for R1 (no rumen fluid addition)

6.3.3 Effect of particle size and rumen fluid treatment in BMP assays and CSTRs

The BMP assays did not illustrate the intricacy of the effects of variables such as particle size and addition of rumen fluid on digestion of high fibre, low DSD grass silage. Only small differences in SMY were exhibited when comparing the >3 cm particle size to the <1 cm particle size. However, continuous trials highlighted some substantial differences in digestion. Particle size was found to be crucial for efficient continuous digester operation. Grass silage, with particle size >3 cm suffered in terms of digester operation. The methane yields achieved in continuous digestion for >3 cm particle size were almost identical to those indicated from the BMP assay but the operation of the CSTR reactors was extremely problematic. The issues leading to mechanical problems within the digester were all derived from the longer grass silage particle size of >3 cm. Reactor downtime through such mechanical issues inevitably leads to economic losses and hence it is imperative that such disruptions are avoided. The issue of particle size is relative to the digester in question. However, it is recommended that shorter grass silage particle sizes of 1 cm length are better for mixing and obtaining higher SMYs in continuous digestion.

The addition of rumen fluid as a form of fibrolytic treatment to challenging substrates, such as high-fibre content grass silages, presents a valuable opportunity. The results indicated that at a rate of 50 mL rumen fluid per kg silage, a more stable process could be achieved in continuous digestion at grass silage particle sizes of <1 cm. This is indicative of previous literature which suggested that rumen fluid can stimulate the rate of hydrolysis within a reactor given the right conditions (pH and temperature) (Yue *et al.*, 2013). The application of rumen fluid must be carefully managed. For effective use, the rumen fluid was first heated to 39°C, representing

the core body temperature of cattle. The quantities of rumen fluid available to a digester within economically feasible transport distances must be assessed prior to initiation of such a strategy. Cost of storage, heating and application should also be considered.

6.4 Conclusions

The digestion of grass silage with low DSD was investigated for biomethane production. In BMP assays, physical reduction in particle size had little impact on the obtainable methane yields. Likewise, rumen fluid addition reported insignificant gains at BMP scale. However in CSTRs, shorter particles sizes of <1 cm were more suitable than >3 cm particle sizes. Furthermore, the addition of rumen fluid facilitated higher SMYs in CSTRs with <1 cm grass silage. The maximum SMY achieved was 371 L CH_4 kg^{-1} VS. Thus, both treatments can be utilised to enhance biomethane production from the grass silage with low DSD.

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References

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

Allen, E., Wall, D.M., Herrmann, C., Murphy, J.D. 2014. Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel. *Bioresour. Technol.*, 170(0), 436-444.

Alrawi, R.A., Ahmad, A., Ismail, N., Kadir, M.O.A. 2011. Anaerobic co-digestion of palm oil mill effluent with rumen fluid as a co-substrate. *Desalination*, 269(1–3), 50-57.

ANKOM. 2006a. Acid detergent fiber in feeds filter bag technique. *Macedon, NY, USA: Ankom Inc.*

ANKOM. 2006b. Neutral detergent fiber in feeds filter bag technique. *Macedon, NY, USA: Ankom Inc.*

APHA. 2005. Standard Methods for the Examination of Water and Wastewater 21st ed. *American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA.*, 2540 B, E. Solids (Editorial revisions, 2011).

Browne, J.D., Murphy, J.D. 2013. Assessment of the resource associated with biomethane from food waste. *Applied Energy*, 104(0), 170-177.

Chynoweth, D.P., Turick, C.E., Owens, J.M., Jerger, D.E., Peck, M.W. 1993. Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy*, 5(1), 95-111.

Drosg, B. 2013. IEA Task 37 - Process monitoring in biogas plants.

EC. 2009. Directive 2009/28/EC of The European Parliament and of The Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. *Official Journal of the European Union.*

Gijzen, H.J., Derikx, P.J.L., Vogels, G.D. 1990. Application of rumen microorganisms for a high rate anaerobic digestion of papermill sludge. *Biological Wastes*, 32(3), 169-179.

Herrmann, C., Heiermann, M., Idler, C., Prochnow, A. 2012a. Particle Size Reduction during Harvesting of Crop Feedstock for Biogas Production I: Effects on Ensiling Process and Methane Yields. *BioEnergy Research*, 5(4), 926-936.

Herrmann, C., Prochnow, A., Heiermann, M., Idler, C. 2012b. Particle Size Reduction During Harvesting of Crop Feedstock for Biogas Production II: Effects on Energy Balance, Greenhouse Gas Emissions and Profitability. *BioEnergy Research*, 5(4), 937-948.

Hu, Z.-H., Wang, G., Yu, H.-Q. 2004. Anaerobic degradation of cellulose by rumen microorganisms at various pH values. *Biochemical Engineering Journal*, 21(1), 59-62.

Hu, Z.-H., Yu, H.-Q. 2005. Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. *Process Biochemistry*, 40(7), 2371-2377.

Jagadabhi, P., Lehtomäki, A., Rintala, J. 2008. Co-digestion of grass silage and cow manure in a CSTR by re-circulation of alkali treated solids of the digestate. *Environ Technol.*, Oct(29(10):1085-93.).

Kaparaju, P., Luostarinen, S., Kalmari, E., Kalmari, J., Rintala, J. 2002. Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Sci Technol*, 45(10), 275-80.

Kivaisi, A.K., Eliapenda, S. 1995. Application of rumen microorganisms for enhanced anaerobic degradation of bagasse and maize bran. *Biomass and Bioenergy*, 8(1), 45-50.

Lehtomäki, A., Huttunen, S., Rintala, J.A. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resour. Conserv. Recy.*, 51(3), 591-609.

Lehtomäki, A., Viinikainen, T.A., Rintala, J.A. 2008. Screening boreal energy crops and crop residues for methane biofuel production. *Biomass and Bioenergy*, 32(6), 541-550.

Lopes, W.S., Leite, V.D., Prasad, S. 2004. Influence of inoculum on performance of anaerobic reactors for treating municipal solid waste. *Bioresource Technology*, 94(3), 261-266.

Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S. 2002. Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506-577.

McEniry, J., Crosson, P., Finneran, E., McGee, M., Keady, T.W.J., O'Kiely, P. 2013. How much grassland biomass is available in Ireland in excess of livestock requirements? *Irish J Agr. Food. Res.*, 52: 67–80.

McEniry, J., O'Kiely, P. 2013. Anaerobic methane production from five common grassland species at sequential stages of maturity. *Bioresource Technology*, 127(0), 143-150.

Nordmann, W. 1977. Die Überwachung der Schlammfaulung. KA-Informationen für das Betriebspersonal. *Beilage zur Korrespondenz Abwasser, 3/77.*

Prates, A., de Oliveira, J.A., Abecia, L., Fondevila, M. 2010. Effects of preservation procedures of rumen inoculum on in vitro microbial diversity and fermentation. *Animal Feed Science and Technology*, 155(2–4), 186-193.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology,* 178 (2015) 178-186.

Tilley, J.M.A., Terry, R.A. 1963. A TWO-STAGE TECHNIQUE FOR THE IN VITRO DIGESTION OF FORAGE CROPS. *Grass. Forage. Sci.*, 18(2), 104-111.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy. Sci.*, 74(10), 3583-3597.

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D. 2014a. 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.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D. 2014b. 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.M., O'Kiely, P., Murphy, J.D. 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. *Bioresour. Technol.*, 149(0), 425-431.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., Zhan, X. 2011. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. *Bioresource Technology*, 102(19), 8748-8755.

Yue, Z.-B., Li, W.-W., Yu, H.-Q. 2013. Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. *Bioresource Technology*, 128(0), 738-744.
Yue, Z.-B., Wang, J., Liu, X.-M., Yu, H.-Q. 2012. Comparison of rumen microorganism and digester sludge dominated anaerobic digestion processes for aquatic plants. *Renewable Energy*, 46(0), 255-258.

7. Investigating two-phase digestion of grass silage for demand-driven biogas applications: effect of particle size and rumen fluid addition

Investigating two-phase digestion of grass silage for demand-driven biogas applications: effect of particle size and rumen fluid addition

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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.

7.1 Introduction

7.1.1 Single-phase digestion of lignocellulosic grass silage

Grass can be used for the production of biomethane through anaerobic digestion. With a typical dry solids (DS) content of greater than 200 g kg^{-1}, grass silage benefits from dilution with a co-substrate (such as dairy slurry) in a continuously stirred tank reactor (CSTR) (Wall *et al.*, 2014). Such co-substrates allow for efficient mixing and prevent solids accumulation, thus, ensuring a better interaction between the microorganisms and the substrate. Alternatively liquor separated from the digestate may be recirculated to the reactor, maintaining the DS content at a required level, typically less than 120 g kg^{-1}. A previous study has shown that mono-digestion of low dry solids digestibility (DSD) silage, at greater than 3 cm particle size (>3 cm), caused significant operational problems in one-phase digestion (Wall *et al.*, 2015). Even at shorter particle sizes, of less than 1 cm (<1 cm), mono-digestion of the same grass silage failed without continuous addition of a rumen fluid supplement. Challenging crop substrates, such as high lignocellulose content grass silage, are potentially more suited to two-phase digestion (Nizami & Murphy, 2011).

7.1.2 Two-phase digestion of lignocellulosic grass silage

Two-phase digestion systems split the anaerobic digestion process into two parts. One such system would involve hydrolysis and acidogenesis, which takes place in leach bed reactors (LBRs), and acetogenesis and methanogenesis in a high-rate methanogenic reactor, such as an upflow anaerobic sludge blanket (UASB). Good performance in the leaching phase is critical, and is demonstrated by high volatile solids (VS) destruction rates, high soluble chemical oxygen demand (SCOD) concentrations and significant accumulation of volatile fatty acids (VFA). Hydrolysis

of the grass silage substrate is achieved by recirculation of leachate, which repeatedly percolates through the crop. When the leachate is of sufficient quality it can be directed to the UASB for gas production. Traditionally leach-bed reactors are sequentially-fed to ensure a continuous supply of SCOD/VFA to the UASB for biogas production (see SLBR-UASB, Figure 7.1). Two-phase systems also facilitate higher organic loading rates than conventional CSTRs (Aslanzadeh *et al.*, 2014).

7.1.3 Application of two-phase digestion to demand-driven biogas

The SLBR-UASB can support the concept of demand-driven biogas production. This is an approach whereby biogas production is enhanced at times of peak electricity demand and reduced when demand is low. For the SLBR-UASB system, leachate could potentially be recirculated over the grass silage feedstock until a leachate rich in VFAs and with high SCOD concentrations is produced. At times of peak electricity demand, the leachate could be sent to the UASB for biogas production, and the biogas to CHP units to produce electricity. At times of low electricity demand the leachate can be disconnected from the UASB. Such flexibility in energy output from biogas systems can facilitate increased levels of intermittent variable renewable electricity in the energy system; such as from wind turbines (Persson *et al.*, 2014). When the wind is not blowing, biogas systems can come on line and produce electricity; when the wind is high biogas systems can go off line.

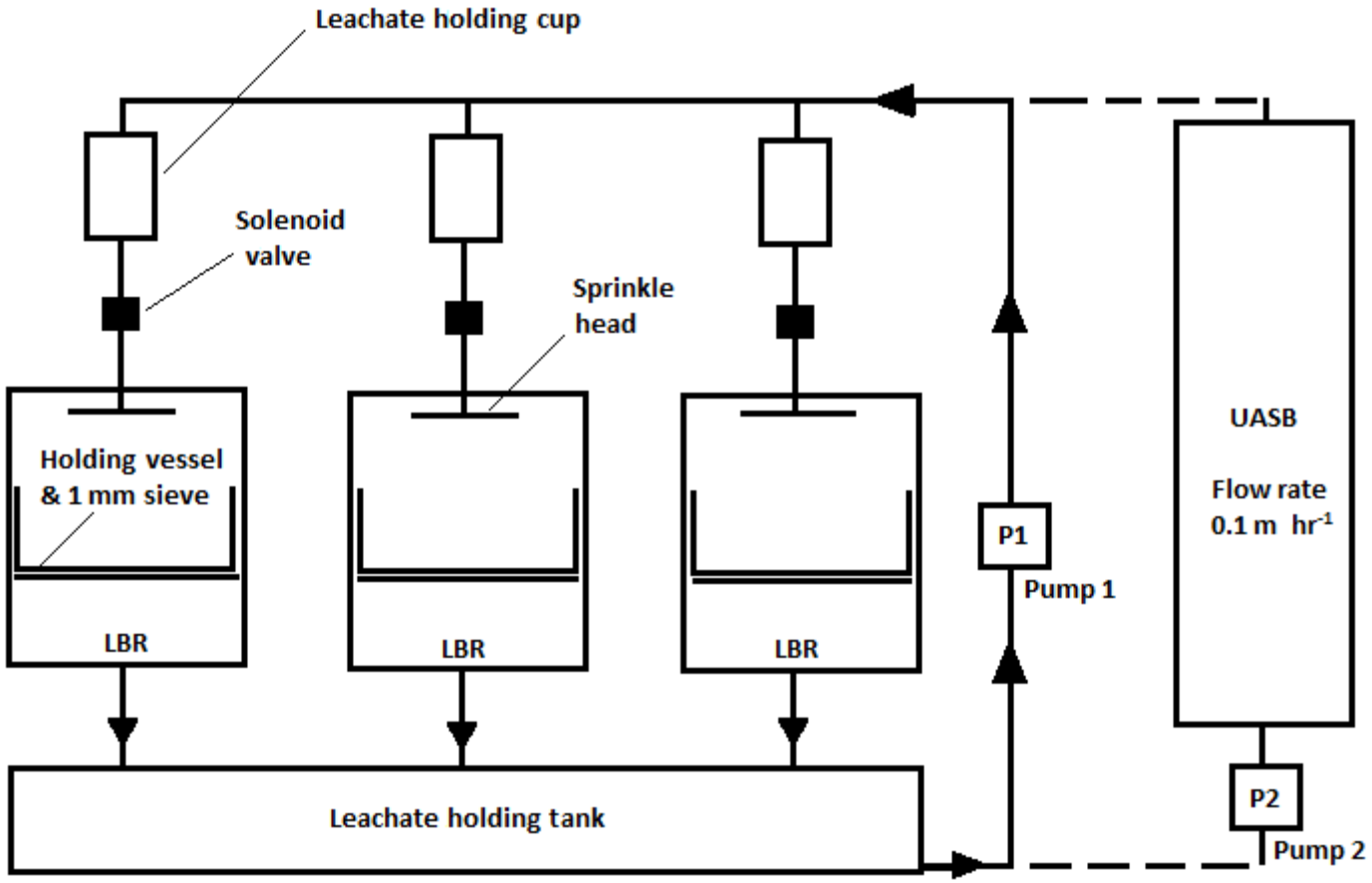


Figure 7.1 SLBR-UASB set-up

7.1.4 Effect of particle size and rumen fluid addition in grass silage digestion

The digestibility of grass silage can vary depending mainly on its phenological growth stage at harvest time. Properties such as DSD, neutral detergent fibre (NDF) and C:N ratio give an indication as to the crops' suitability for anaerobic digestion. If the grass is of high-fibre content, specific treatments can be employed to target the breakdown of lignocellulosic structures. The effectiveness of a specific treatment can be expressed by the extent of SCOD production (Teghammar *et al.*, 2010). Particle size reduction (physical) and rumen fluid addition (biological) are two such treatments. Literature reporting the influence of particle size reduction for grass silage is limited, however, it is suggested that shorter particle sizes can stimulate hydrolysis by increasing the surface area with which the microorganisms can access (Hu *et al.*, 2005; Sanders *et al.*, 2000). A previous study by the authors in single-phase digestion illustrated that reduction in particle size to <1 cm allowed for digestion of low DSD grass silage in a CSTR, at a loading rate of 2.5 kg VS m^{-3} d^{-1}, when coupled with rumen fluid addition at a rate of 50 mL per kg silage (Wall *et al.*, 2015). CSTRs fed with grass silage of longer particle size (>3 cm), proved extremely difficult to operate due to mechanical difficulties, irrespective of rumen fluid addition. Implementing two-phase digestion systems may potentially alleviate the need for particle size reduction and, hence, reduce the onsite energy input costs. For batch leach-bed processes, Lehtomäki *et al.* (2008) reported chopping the grass to a particle size of 3 cm. In a similar system, Xie *et al.* (2012) reduced the particle size to 2−3 cm length.

The role of fibrolytic treatments, specifically rumen fluid addition, in two-phase systems reported in literature is scarce. A study investigating the degradation of municipal solid waste in leaching trials was performed whilst adding rumen fluid

daily and on alternating days (Ganesh *et al.*, 2010). The addition of rumen fluid was found to enhance the hydrolysis efficiency. The leaching trials exhibited an initial drop in pH to 4.09 as a result of increased VFA production, but subsequently increased to 5.86. Characteristics of leaching (such as dry solids (DS), chemical oxygen demand (COD) and VFAs) were reported to show a first-order rate pattern, that is, high production on the first day followed by a period of levelling off. For two-phase digestion systems, the addition of rumen fluid may provide a valuable opportunity to improve hydrolysis efficiency.

7.1.5 Relationship between pH and VFAs in the leaching phase

The first phase of the SLBR-UASB system is the leaching process. During this phase a high-strength leachate is produced with a sizeable concentration of VFAs. This causes the pH to drop in the system. The VFAs produced in digestion using rumen microorganisms have been reported to be pH-dependent. At a pH of less than 5.5, acetic acid is predominant. Propionic acid is more pronounced at pH values greater than 6.0 (Hu *et al.*, 2004). Quantities of butyric, iso-butyric and valeric acid have been produced at batch scale at a pH range higher than 6.0 (Hu & Yu, 2006; Hu & Yu, 2005). However, a pH value of 6.8−7.3 has been suggested as the optimum range for rumen microorganisms to degrade cellulose structures (Hu *et al.*, 2004). The degradation of cellulose using rumen microorganisms has been reported to be inhibited at a pH of less than 5.5 and was not easily remedied through adjustment of pH (Hu *et al.*, 2005). Digestion of cattail (*Typha latifolia linn*), a wetland plant, using rumen microorganisms also showed a decrease in substrate degradation and VFA production when the pH dropped to below 5.8 when compared to digestion in a pH range of 6.7−7.6 (Hu & Yu, 2006).

7.1.6 Objectives

The objective of this study was to investigate two-phase digestion of low DSD grass silage as would occur with a demand-driven biogas production system. Specifically, four leaching trials (LT1–LT4) were evaluated to uncover potentially favourable conditions for biogas production. To stimulate the hydrolysis and acidogenesis phase, particle size (<1 cm and >3 cm) and rumen fluid addition treatments were investigated. Profiles were developed of process parameters such as pH, VFAs, SCOD and VS destruction. One further trial was conducted by sequentially feeding the LBRs in tandem with a UASB to compare VS destruction rates in a traditionally-fed two-phase system. This was carried out without rumen fluid addition on the >3 cm particle size (rationale for this explained in section 7.3.2.1).

7.2 Methods

7.2.1 Grass silage

A first-cut perennial ryegrass (*Lolium perenne*) harvested at an advanced growth stage (stemmy grass, fully headed-out, after flowering) with relatively high lignified fibre content (NDF = 716 g kg^{-1} DS) was used for the leaching trials. This represented a grass silage of low digestibility and a challenging feedstock for anaerobic digestion. The grass was initially field-wilted for 48 hours post-harvest and subsequently baled (1.2 m wide x 1.2 m diameter) and stretch-wrapped in polyethylene film. Smaller rectangular bales of approximately 25 kg were subsequently manufactured for storage and handling purposes, and stored at ambient room temperature (18−20°C). Two particle sizes were employed. The <1 cm particle size was achieved using a heavy duty mincer (Buffalo Heavy Duty Mincer, 250 kg/hr) which macerated the grass silage. The >3 cm particle size was chopped with a

scissors by hand. The different chopping techniques meant that the silage not only varied in particle size but also by the extent of shredding and physical disruption. Aliquots of the chopped grass silage were stored at -20°C until required for experimental use. The DS content of the grass silage was 217 g kg^{-1}. VS content was 908 g VS kg^{-1} DS. The DSD of the grass was 555 g kg^{-1}.

7.2.2 Grass silage energy content

The evaluation of the energy content of the grass silage (MJ kg^{-1} VS) used in this study and its theoretical conversion of VS to COD is described below. Using the modified Dulong Formula allows assessment of energy content of biomass (Tchobanoglous *et al.*, 1993):

$$\text{Energy Value (kJ/kg)} = 337\text{C} + 1419\left(\text{H} - \frac{1}{8}\text{O}\right) + 93\text{S} + 23.26\text{N},$$

where carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) are proportioned as a percentage of the dry solids. In this case, C is 43.8 %, H is 5.91 %, O is 44.21 % and N is 1.08 %. Contribution of S was assumed to be negligible. Expressed as a percentage of the volatile solids, C is 48.24 %, H is 6.51 %, O is 48.69 % and N is 1.19 % (908 g VS kg^{-1} DS for the grass silage).

$$\text{Energy Value (kJ/kg)} = 337(48.24) + 1419\left(6.51 - \frac{48.69}{8}\right) + 23.26(1.19)$$

The energy value on a VS basis is evaluated as 16.89 MJ kg^{-1} VS. Knowing the energy content of methane (37.78 MJ m^{-3}), the energy content of the grass silage (16.89 MJ kg^{-1} VS) and that 1 kg of COD produces 0.350 m^3 CH_4 (Nizami *et al.*, 2009; Sperling & Chernicharo, 2005), it may be shown that the energy content of 1 kg COD destroyed generates 13.22 MJ (37.78 MJ m^{-3} x 0.35 m^3 CH_4). Therefore the

relationship between VS destroyed and COD produced is 1.28 kg COD kg^{-1} VS (16.89 MJ kg^{-1} VS/13.22 MJ kg^{-1} COD).

This is, as expected, lower than the conversion rate (1.42 kg COD kg^{-1} VS) reported by Nizami *et al.* (2009) for an early growth stage perennial ryegrass. The grass silage investigated in this paper is of lower digestibility (harvested at advanced growth stage).

7.2.3 Rumen fluid

Approximately 70 L of rumen fluid was obtained from six fattened beef heifers that were offered a hay (made from stemmy grass) diet for 7−10 days prior to slaughter. The retrieved rumen contents were filtered through a muslin cloth and sieved to remove any solid particles. The strained rumen fluid was thoroughly mixed and transferred into 50 mL and 4 mL samples vials that were immediately frozen in liquid N and stored at -20°C. Additions of rumen fluid in the leaching trials were made at a rate of 50 mL per kg grass silage added. This was based on the grass silage (DS = 217 g kg^{-1}) being able to retain the liquid in the added rumen fluid without excess seeping out and removing soluble substrates with it. The frozen rumen fluid was thawed and heated to approximately 39°C immediately prior to application. The rumen fluid had a DS content of 22 g kg^{-1}, a VS content of 612 g VS kg^{-1} DS and a pH value of 7.22.

7.2.4 Leaching trials

LBRs were run in triplicate for all leaching trials. Stainless steel holding vessels, with cylindrical 3 mm mesh outlet, held 3.5 kg samples of the grass silage within each LBR. Figure 7.2 illustrates the >3 cm particle size, while Figure 7.3 shows the

<1 cm grass silage in the holding vessels. Beneath each vessel was situated a 1 mm mesh to prevent wash-through of solid grass particles. The LBRs were loaded identically for each trial. Leachate (initially 25 kg of water added) was recirculated from a leachate holding tank (25 L total capacity), positioned below the LBRs (Figure 7.1), to three leachate holding cups (each of approximately 2 L total capacity), held directly above the LBRs, via a peristaltic pump (Watson Marlow 323S). The recirculation pump operated at a constant rate of 40 rpm. The leachate holding cups operated with solenoid valves, which opened for 1 minute periods at 20 minute intervals. Once the timed solenoid valves were opened, approximately 1.2 L of leachate was dispersed over the grass silage in each LBR through custom-made sprinkle heads. The dilution rate per LBR was approximately 87 L d^{-1}, with approximately 260 L d^{-1} of leachate recirculated throughout the entire system per day. The leachate percolated through the grass silage and returned to the leachate storage tank where the process was repeated. Temperature in the leachate holding tank and LBRs was kept constant at 37 ± 1°C for each trial by means of a heating coil with all surrounding sections insulated. Each leaching trial ran for 30 days, and when completed, the system was emptied and cleaned. Fresh leachate (water) and grass silage were then added to the system for the next trial. A similar setup for this system has been previously described for digestion of early growth stage grass silage (higher DSD) and food waste in studies by Nizami *et al.* (2009) and Browne *et al.* (2013), respectively.



Figure 7.2 Grass silage of particle size >3 cm



Figure 7.3 Grass silage of particle size <1 cm

7.2.5 Sequential LBRs with upflow anaerobic sludge blanket (SLBR-UASB)

The SLBR-UASB system was comprised of 6 LBRs (as described in section 7.2.4 and shown in Figure 7.1) operating in tandem with a UASB reactor. The LBRs were sequentially fed with 3.5 kg of grass silage on every fifth day (LBR1 fed on day 0, LBR2 fed on day 5, LBR3 fed on day 10 etc.). This effected a total retention time of 30 days (6 LBRs x 5 days). The working volume of the UASB was approximately 32 L with the temperature held constant at 37 ± 1°C by a heating element within the reactor. Similar to the leaching trials, a peristaltic pump recirculated leachate from the holding tank to the leachate holding cups, which was subsequently sprinkled over the grass silage feedstock. A second peristaltic pump was also utilised in the SLBR-UASB system. This second pump (Watson Marlow 323S) supplied leachate from the leachate holding tank to feed the UASB. The UASB was seeded with inoculum (section 7.2.6) and commissioned for a period of 8 weeks. This ensured that the system could reach its desired temperature output and examination of leachate pump speeds/rates and scrutiny of overall system effectiveness could be undertaken. In the period of data collection the hydraulic retention time (HRT) for the UASB was 10 hours – equivalent to a 0.1 m hr^{-1} up flow rate. This was built up slowly over the commissioning period from a HRT of 5 days to 10 hours to prevent granule wash-out at an early stage. The recirculation pump rate to the leachate holding cups was set at 100 rpm (a high dilution rate) with the solenoid valves timed to open every 20 minutes for 1 minute periods. In total, over 600 L of leachate per day was recirculated through the entire system via the two pumps operating continuously. This higher recirculation rate accounted for the operation of 6 LBRs rather than 3 LBRs as in the leaching trials.

7.2.6 Granular sludge inoculum for UASB

Approximately 80 L of granular sludge inoculum was collected from an industrial scale UASB reactor operating in Co. Cork, Ireland, prior to experimental start-up in the SLBR-UASB system. The DS and VS content of the granular sludge were 112 g kg^{-1} and 795 g VS kg^{-1} DS, respectively. Upon collection, the granular sludge was sieved (by a 150 µm sieve) which separated the sludge (granules) from the liquor component. Approximately 16 L of separated sludge was placed in the UASB while a further 16 L of the liquor was added to fill the remainder of the UASB volume.

7.2.7 Analytical methods

The pH was measured daily using a Jenway 3510 pH meter. DS and VS analyses were determined according to Standard Methods 2540 G (APHA, 2005). COD and total ammonical nitrogen (TAN) were determined weekly using Hach Lange cuvette tests (LCK 914 and LCK 313, respectively) and evaluated by a DR3900 Hach Lange Spectrophotometer. SCOD was evaluated via the same procedure as COD however samples were centrifuged at 15,000 rpm for 10 minutes prior to testing. The biogas produced from the SLBR-UASB system was evaluated for methane composition (% CH_4 v/v) by an Agilent 6890 GC with thermal conductivity detector. VFA concentrations were measured using an Agilent 6890 GC with flame ionisation detector as described in Allen *et al.* (2014). For the leaching trials, two samples per week were analysed over the 30 days of operation for each individual leaching trial. VFA samples were prepared by centrifuging for 10 minutes at 15,000 rpm with 0.2 mL HPO_3 added to remove any particulate matter.

7.2.8 Experimental design

Four leaching trials (first phase) were carried out in triplicate:

LT1: Grass silage of >3 cm particle size

LT2: Grass silage of >3 cm particle size with rumen fluid addition

LT3: Grass silage of <1 cm particle size

LT4: Grass silage of <1 cm particle size with rumen fluid addition

Rumen fluid addition was undertaken by adding 175 mL to the 3.5 kg grass silage in each batch. This corresponded to the loading rate of 50 mL rumen fluid per kg silage. The rumen fluid was poured evenly over the batch, which was immediately sealed and left to sit for 4 hours to avoid immediate wash-out. The temperature of the batch was kept at 37 ± 1°C. Following this 4 hour period, the leaching recirculation pump was switched on and the trial commenced.

The SLBR-UASB system was also investigated with grass silage of >3 cm particle size with no rumen fluid addition (rationale explained in section 7.3.2.1). The system was operated for a total of 3 HRTs.

7.3 Results and discussion

7.3.1 Leaching trials

7.3.1.1 Trends in pH

Figure 7.4 shows the operative pH range for the leaching trials (LT1−LT4). LT1 and LT2 (with grass particle size >3 cm) saw an immediate drop in pH to values of 4.37 and 4.51 respectively, on day 1. A similar fall in pH was observed for LT3 and LT4 (with particle size of <1 cm) with values of 4.43 – 4.44, respectively, on day 1. The immediate fall in pH can be attributed to the acidic liquors incorporated in the grass silage which were quickly washed through the system as the leachate recirculated.

The general trend for pH in all four leaching trials was to initially drop and gradually increase over the 30 day trial period. Similar results were evident in studies investigating the hydrolysis of the organic fraction of municipal solid waste in LBRs (Dogan *et al.*, 2009). In the trials with no rumen fluid addition, the pH seemed to self-buffer more effectively. Both LT1 and LT3 typically demonstrated higher pH values over the 30 days than their corresponding trials with rumen fluid addition (LT2 and LT4). Since the LBR vessels were not strictly anaerobic, the rise in pH may potentially be attributed to the oxidation of VFAs. This was previously reported for leaching trials of food waste (Browne *et al.*, 2013). It has also been suggested that the pH can vary during the acidogenesis phase, and with no pH control, the system can subsequently self-buffer towards a higher pH range (Dogan *et al.*, 2009; Guerrero *et al.*, 1999). The optimum range of pH reported for acidogenic bacteria is 4.0-6.5 (Speece, 1996; Yu *et al.*, 2002). However previous studies have indicated that efficient hydrolysis may not always be achieved at such a low pH range (Babel *et al.*, 2004; Browne *et al.*, 2013).

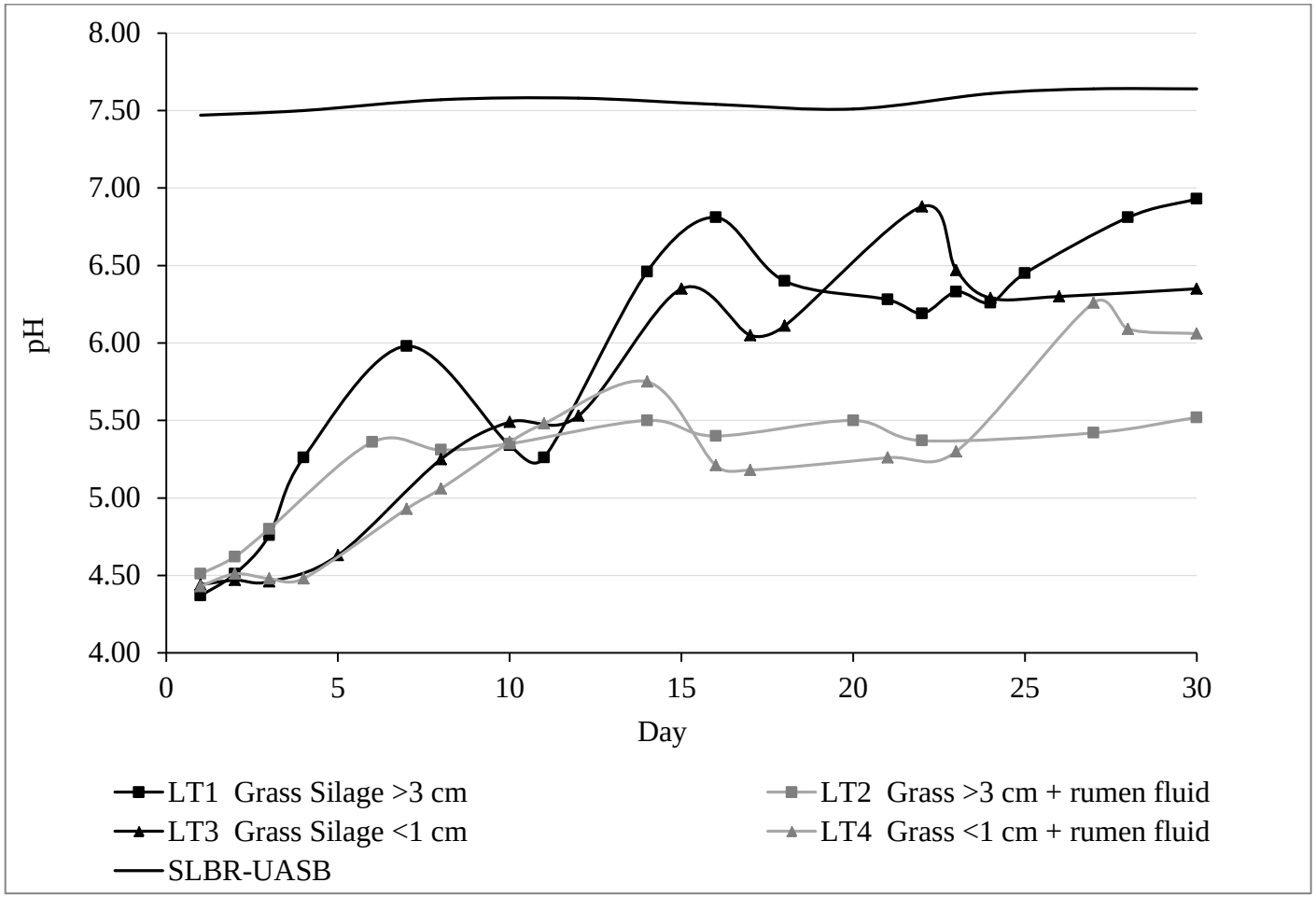


Figure 7.4 pH trend in leaching trials (LT1−LT4) and SLBR-UASB

7.3.1.2 COD, SCOD and TAN concentrations

Table 7.1 shows the average weekly values for the operational parameters monitored during the leaching trials (LT1−LT4). The hydrolytic conversion to COD was generally more effective in the trials with rumen fluid addition. LT2 saw a continual increase in COD from the second week (Day 8−14) until the end of the trial (approximately 14 to 19 g L^{-1}). This implied that hydrolytic conversion to COD was continuously active over the entire 30 days, although the rate of COD production seemed to decline significantly after week 1 (Day 1−7). Likewise LT4 began with very high COD production and remained high until the end of the trial (17 to 19 g L^{-1}). Without rumen fluid addition, the COD concentrations tended to decrease or remain largely stagnated as the trial progressed. Decreases in COD can indicate higher COD degradation rates than hydrolysis rates (Nizami *et al.*, 2010). LT1 and LT3 reported lower total COD concentrations than the corresponding leaching trials with rumen fluid addition (LT2 and LT4) by day 30. Using the conversion rate of 1.28 kg COD kg^{-1} VS (section 7.2.2), the maximum theoretical COD production (based on VS destruction rates in Table 7.2) was calculated at approximately 43.4 g L^{-1} for LT1, 29.5 g L^{-1} for LT2, 45.1 g L^{-1} for LT3 and 34.7 g L^{-1} for LT4. Taking the highest concentrations for COD produced in each individual leaching trial, LT1, LT2, LT3 and LT4 achieved 34 %, 64 %, 39 % and 55 % of its maximum theoretical COD production, respectively. This highlighted significant losses of COD from the LBRs (especially those without rumen fluid addition) potentially through respiration and oxidation processes (Browne *et al.*, 2013) and/or degradation of COD by bacteria naturally occurring in the silage (Nizami *et al.*, 2010). From the results, it is interpreted that hydrolysis to COD occurred in a very short time for all trials (approximately 1−2 days) as values of COD were initially high and did not increase

at the same rate over time. It may be inferred that continuing hydrolysis was inhibited (inefficient degradation) in the LBRs with rumen fluid addition (LT2 and LT4) due to the drop in pH.

For both LT1 and LT2 the proportion of COD that was solubilised remained high throughout the trial irrespective of COD fluctuations (Table 7.1). The best case scenario was for LT2 with rumen fluid addition with SCOD averaging approximately 93 % of the total COD over the 30 days. For LT1, an average of 84 % of the total COD was soluble over the first 28 days; however the final SCOD concentration of LT1 was approximately only half that of LT2 on day 30 (8.3 g L^{-1} v. 15.5 g L^{-1}).

There was a noticeable trend for LT3 and LT4 (with particle size <1 cm) in terms of the relationship between total COD and SCOD. The SCOD concentrations for both LT3 and LT4 represented a substantially lower fraction of the total COD from the day 22 to the end of the trial, averaging approximately 51 % for LT3 and 65 % for LT4. This meant that there was a considerable amount of particulate organics in the COD for LT3 and LT4, that is, undigested grass silage particles washing through the system. Final concentrations of SCOD were again higher when implementing rumen fluid addition, at the <1 cm particle size (11.2 g L^{-1} v. 7.4 g L^{-1}).

TAN values showed similar properties for all leaching trials, starting low, reaching a peak and then decreasing back to low values (Table 7.1). Inhibitory concentrations of TAN are reported at 5 g L^{-1} (Drosg, 2013). At no time were TAN concentrations a concern in terms of inhibition.

Table 7.1 COD, SCOD and TAN concentrations in leaching trials (LT1–LT4)

Leaching Trial 1 – Grass silage >3 cm			
Period	COD[A] (g L^{-1})	SCOD[B] (g L^{-1})	TAN[C] (g L^{-1})
Day 1-7	14.3 ± 1.7	13.0 ± 2.07	0.3 ± 0.16
Day 8-14	14.0 ± 2.04	11.5 ± 1.84	0.4 ± 0.05
Day 15-21	10.8 ± 0.28	8.9 ± 0.11	0.4 ± 0.01
Day 22-28	10.7 ± 0.21	8.6 ± 0.13	0.3 ± 0.01
Day 30	14.9	8.3	0.3
Leaching Trial 2: Grass silage >3 cm with rumen fluid addition			
Period	COD (g L^{-1})	SCOD (g L^{-1})	TAN (g L^{-1})
Day 1-7	14.9 ± 2.81	14.8 ± 2.55	0.3 ± 0.24
Day 8-14	14.2 ± 0.39	13.4 ± 0.00	0.5 ± 0.00
Day 15-21	15.6 ± 0.28	14.6 ± 0.00	0.6 ± 0.00
Day 22-28	16.5 ± 0.66	15.6 ± 0.42	0.6 ± 0.00
Day 30	19.0	15.5	0.4
Leaching Trial 3: Grass silage <1 cm			
Period	COD (g L^{-1})	SCOD (g L^{-1})	TAN (g L^{-1})
Day 1-7	17.7 ± 1.35	14.8 ± 0.00	0.2 ± 0.00
Day 8-14	16.9 ± 0.74	15.5 ± 0.28	0.5 ± 0.18
Day 15-21	14.5 ± 1.93	11.3 ± 1.41	0.5 ± 0.00
Day 22-28	13.7 ± 0.75	7.9 ± 0.35	0.3 ± 0.04
Day 30	16.8	7.4	0.1
Leaching Trial 4: Grass silage <1 cm with rumen fluid addition			
Period	COD (g L^{-1})	SCOD (g L^{-1})	TAN (g L^{-1})
Day 1-7	18.3 ± 0.85	17.4 ± 0.64	0.1 ± 0.06
Day 8-14	18.3 ± 2.52	16.5 ± 0.00	0.7 ± 0.00
Day 15-21	16.8 ± 1.01	14.4 ± 0.00	0.4 ± 0.18
Day 22-28	17.1 ± 2.80	12.3 ± 0.85	0.3 ± 0.00
Day 30	19.2	11.2	0.3

[A] COD – chemical oxygen demand
[B] SCOD – soluble chemical oxygen demand
[C] TAN – total ammonical nitrogen

7.3.1.3 Destruction of VS

The four leaching trials performed poorly with VS destruction rates in the range of 30−47 %. This is illustrated in Table 7.2. Less VS destruction was achieved in LT2 and LT4, which had rumen fluid addition as compared to LT1 and LT3, respectively, which had no rumen fluid. Longer particle sizes of >3 cm also seemed to have a

negative effect on VS destruction when contrasted to corresponding <1 cm particle size trials. The VS content (g kg^{-1}) of the grass silage output was similar in all leaching trials. It is proposed that the degradation of cellulose structures was inhibited by low pH as suggested in previous literature (Hu *et al.*, 2005).

Table 7.2 Destruction of volatile solids in leaching trials (LT1 – LT4)

Leaching Trial	Grass silage input (kg)	Average grass silage output (kg)	Average VS of grass out (g kg^{-1})	Average VS destruction (%)
LT1	3.50	2.20	185.6	42
LT2	3.50	2.58	187.2	30
LT3	3.50	1.74	192.8	49 (47*)
LT4	3.50	2.14	196.9	37 (35*)

* Including for sludge found at bottom of leachate tank when emptying system

7.3.1.4 VFA profiles

Figures 7.5–7.8 show the VFA profiles for leaching trials, LT1 – LT4, respectively. The acidification process was assessed primarily by the total production of VFAs in each leaching trial. Acidogenesis, in the form of VFA production, commenced rapidly upon start-up for all leaching trials. Total VFA concentrations, by day 1, ranged from 3.1−5.3 g L^{-1} with acetic acid the predominant constituent, comprising 81−91 % of the total VFA composition. This reinforced the suggestion of acetic acid build-ups being predominant at lower pH range as indicated in literature (Hu *et al.*, 2004). The high production of acetate in a short period could be attributed to the metabolism of readily fermentable sugars (Viéitez & Ghosh, 1999). By day 4, the concentration of acetic acid from trials with grass silage of <1 cm particle size (LT3, LT4) was approximately double that of the trials with >3 cm particle size (LT1, LT2). This implied that the reduction in particle size allowed for an increased

hydrolytic conversion to acetate. The total VFA concentrations over the 30 days varied depending on the particular leaching trial. For LT1, with grass silage of >3 cm particle size, total VFA concentrations remained largely unchanged throughout (4−5 g L^{-1} approximately). In comparison, LT2 (with grass silage of >3 cm particle size and rumen fluid addition) had over twice the total VFA concentration on day 30 as compared to day 1 (rising from approximately 3.5 to 7.5 g L^{-1}). LT3, with grass silage of <1 cm particle size, exhibited an initial increase in total VFA production and by day 12 (7.5 g L^{-1}) was over twice that on day 1 (3 g L^{-1}), but subsequently declined back to its original concentration by day 30 (3 g L^{-1}). LT4, with grass silage of <1 cm particle size and rumen fluid addition, doubled its total VFA production on day 1 by day 8 (8.7 g L^{-1}) but this subsided marginally by the end of the trial (6 g L^{-1}). The initial peak of acetic acid as a proportion of total VFAs declined over time for all trials, with a reported range of just 36−46 % by day 30. The trend of total VFA production seemed to hinge on the addition of rumen fluid, which in general allowed for the accumulation of higher concentrations (7.5−9 g L^{-1} approximately), that is, a higher rate of acidogenesis. The use of shorter particle size grass silage was inclined to produce high total VFA quantities quickly, only to decline somewhat over time. The two other acids produced in significant quantities for LT1−LT4 were propionic and butyric. Propionic acid production began at an early stage. Concentrations ranged from 0.3−0.4 g L^{-1} in trials with rumen fluid addition by day 1 (LT2 and LT4). These concentrations were higher for trials with no rumen fluid addition (approximately 0.9 g L^{-1} for LT1 and 0.5 g L^{-1} for LT3). Propionic acid concentrations increased from day 1 for LT1−LT4 but typically remained within the range of 10−20 % of the total VFA production over the 30 day period. The peak concentrations of propionic acid reported were in the region of 1.0−1.2 g L^{-1} for LT1−

LT4 but were dwarfed by the accumulation of acetic acid in each case. Butyric acid, a longer chain fatty acid, also showed significant accumulations. Each trial reported butyric acid concentrations of greater than 1 g L^{-1} after the first 7 days. LT1−LT4 also exhibited build-up of valeric acid and caproic acid representing between 5−20 % of the total VFAs present at later stages of the trials. Furthermore, concentrations (<10%) of iso-butyric, iso-caproic and enanthic acid were evident by the end of all trials. The presence of such long chain fatty acids in one-stage digestion would signify reactor instability and inhibition (Drosg, 2013), however in two-phase digestion such acids act as intermediate products and pre-cursors to methane production in the UASB.

The efficiency of acidification between leaching trials can be compared on a g VFA produced per g VS added basis (Table 7.3). Grass silage of particle size <1 cm with rumen fluid addition gave the highest productivity (113 mg VFA g^{-1} VS). The addition of rumen fluid in leaching (as in LT2 and LT4) increased the VFA production rate in comparison to the trials with the same particle size but with no rumen fluid addition (LT1 and LT3, respectively). Reduction in particle size to <1 cm also outperformed corresponding trials at >3 cm, that is, VFA production rate in LT3 was higher than in LT1 and likewise, that of LT4 was greater than that of LT2.

Table 7.3 Calculating the degree of acidification in leaching trials (LT1−LT4)

Leaching Trial	LT1	LT2	LT3	LT4
VS Input[A] (kg)	2.11	2.06	1.95	2.02
VFA production[B] (g L^{-1})	5.26	7.58	7.45	8.72
Leachate[C] (L)	26.13	26.86	26.00	26.10
Acidification (g VFA g^{-1} VS)	0.065	0.099	0.099	0.113

[A] Based on grass silage input of 10.5 kg (3 LBRs x 3.5 kg) and individual DS/VS
[B] The highest concentration of VFAs achieved at any stage in the leaching trial
[C] The total leachate collected at the end of the 30 day leaching period

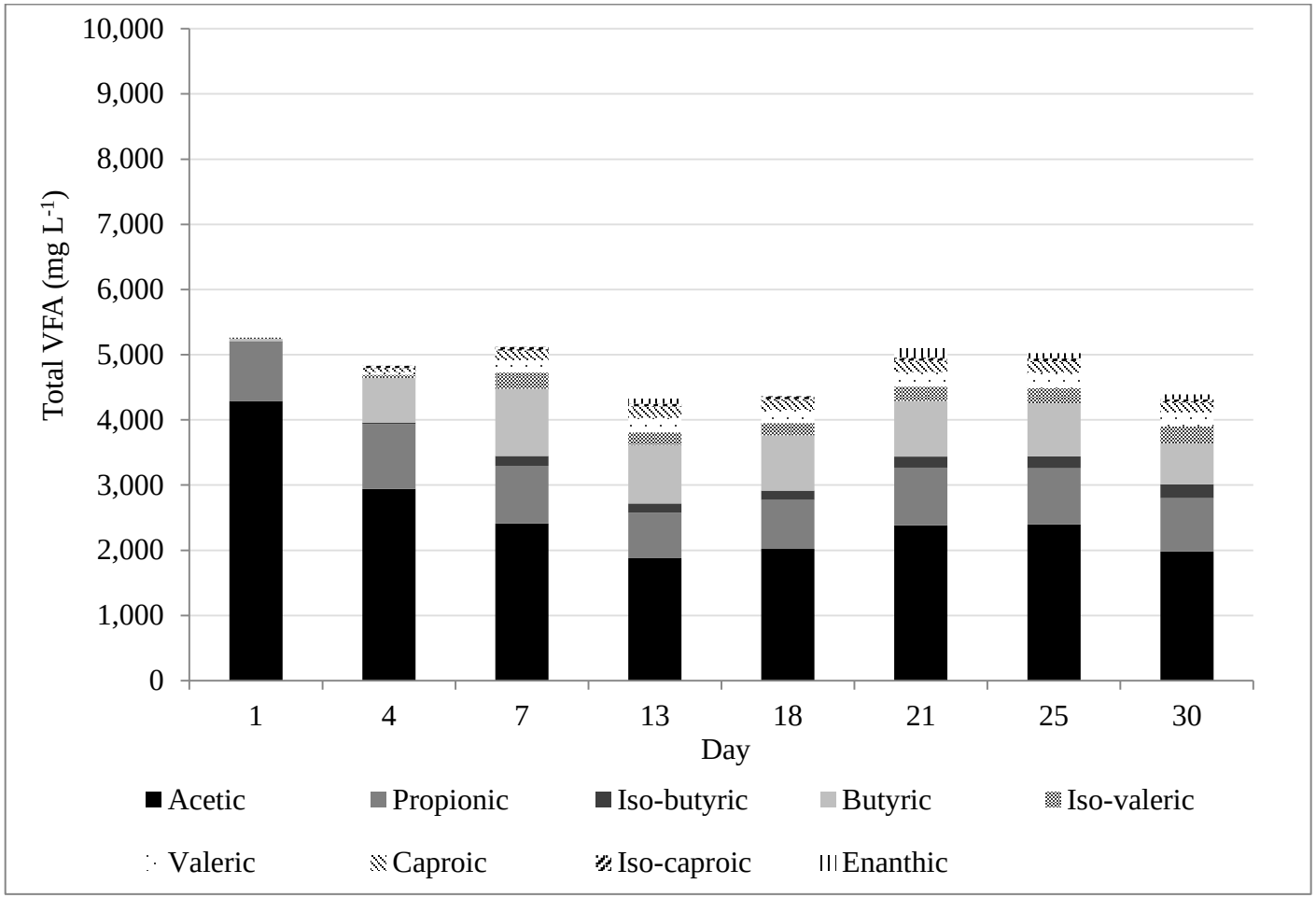


Figure 7.5 VFA profile for LT1: >3 cm particle size

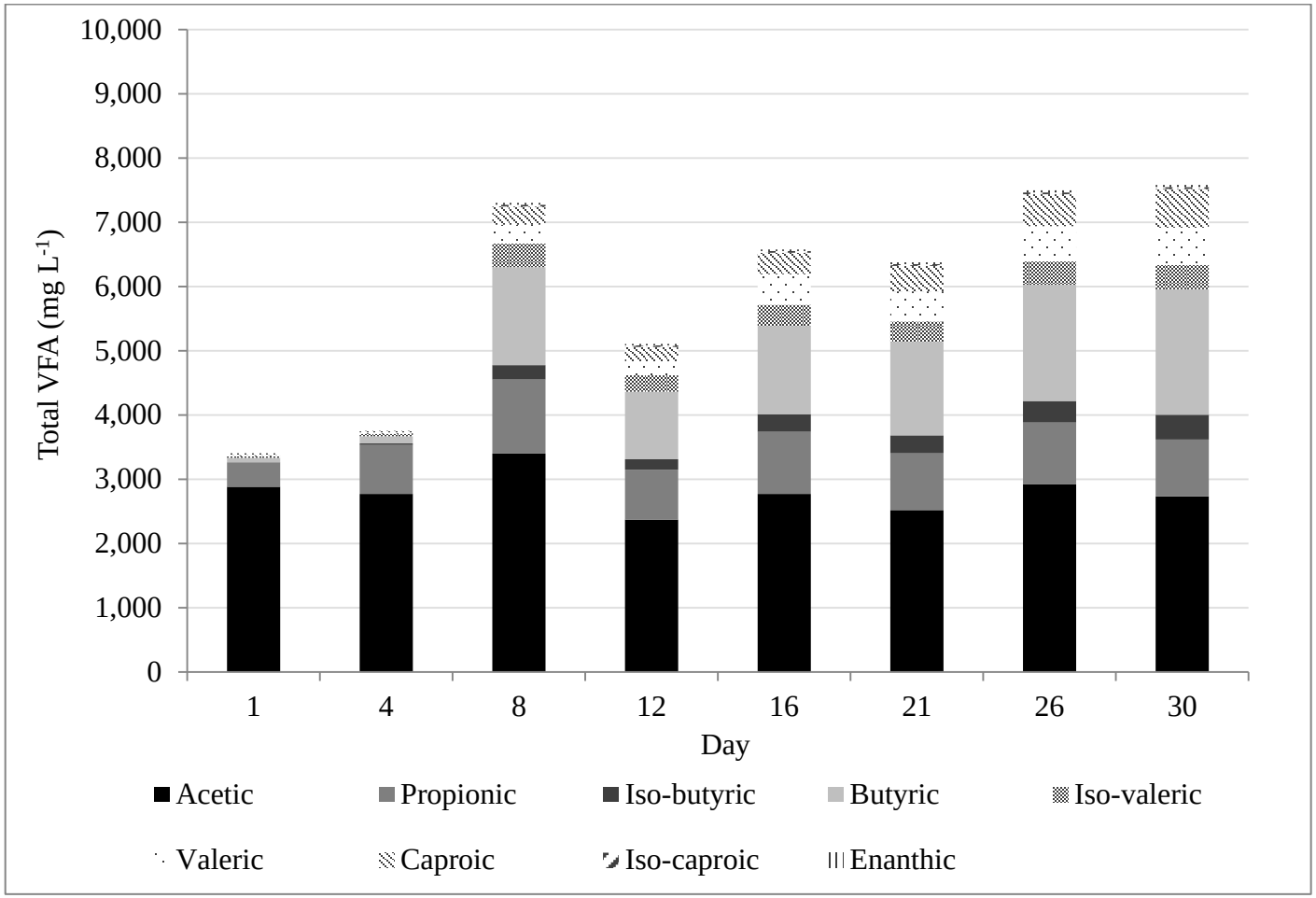


Figure 7.6 VFA profile for LT2: >3 cm particle size with rumen fluid addition

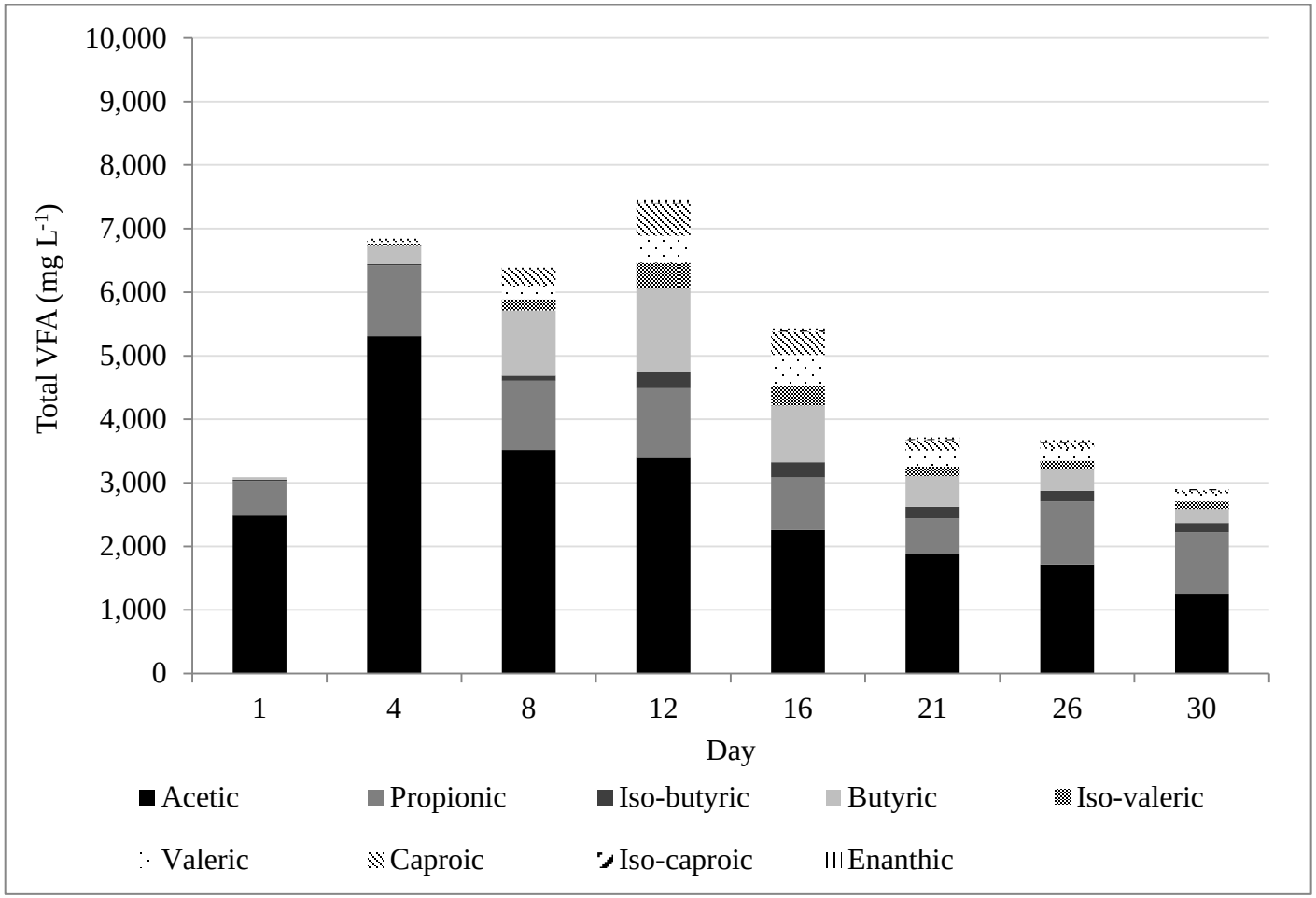


Figure 7.7 VFA profile for LT3: <1 cm particle size

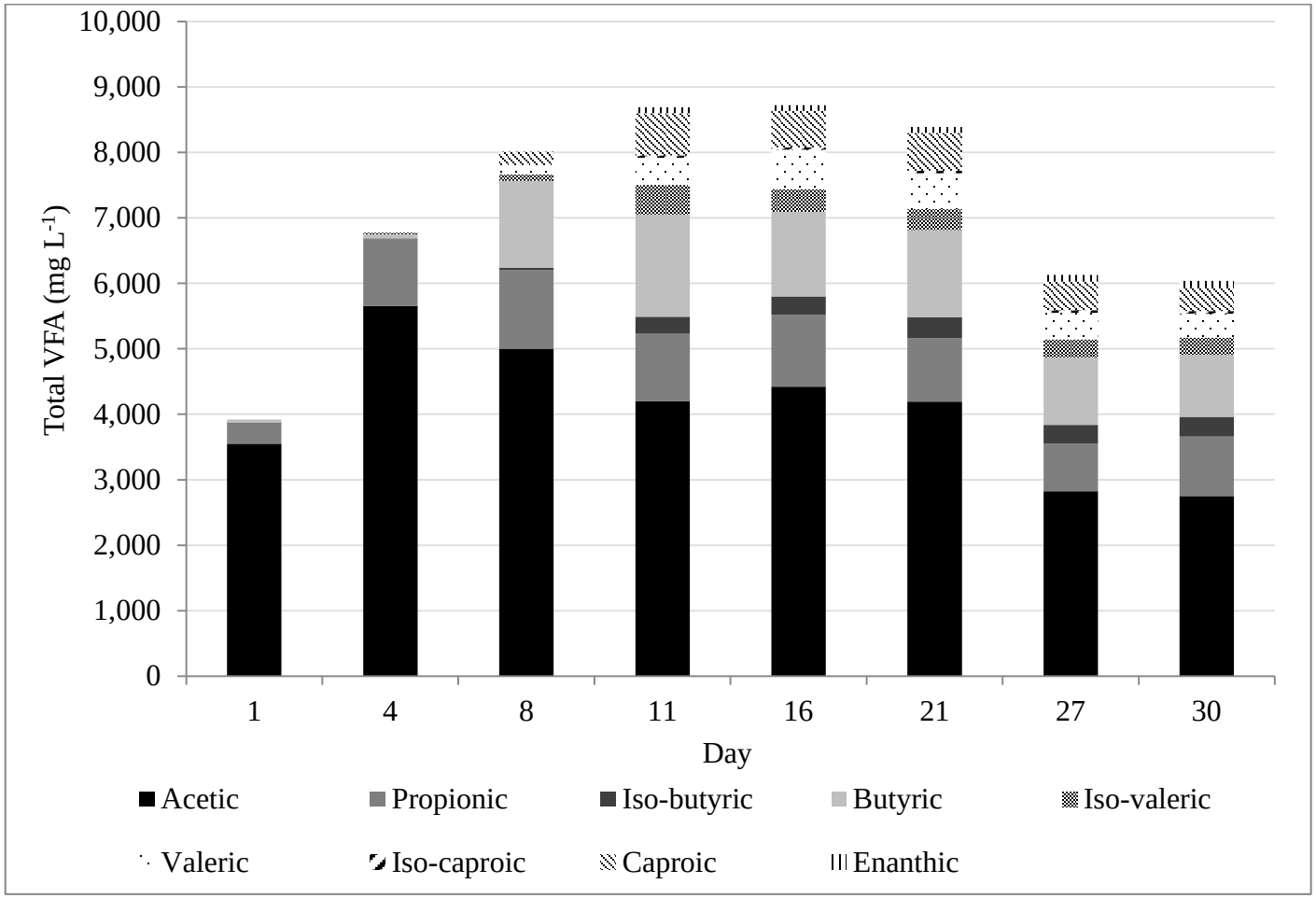


Figure 7.8 VFA profile for LT4: <1 cm particle size with rumen fluid addition

7.3.1.5 Difficulties with <1 cm particle size in LBRs

For the <1 cm particle size leaching trials (LT3 and LT4), it was evident that a significant quantity of grass silage was washed-out from the LBRs, passing through the 1 mm steel mesh and into the leachate holding tank. This caused the initial value reported for destruction of VS from the LBRs to be high. A sludge was collected from the leachate holding tank on day 30 (when emptying the system) for both LT3 and LT4. The sludge was defined as material that could not pass through a 150 µm sieve. For LT3, 0.70 kg of sludge with a DS of 71.5 g kg^{-1} and VS of 62.0 g kg^{-1} was obtained while LT4 had an accumulation of 1.08 kg with a DS of 64.5 g kg^{-1} and VS of 55.6 g kg^{-1}. Factoring the sludge as undigested material altered the VS destruction of both trials to 47 % and 35 % for LT3 and LT4, respectively, as shown in Table 7.2. The wash-out of grass silage from the leach beds had an obvious effect on the colour of the recirculating leachate, changing it from a light orange colour to a dark green (Figure 7.9). Another significant difficulty with the macerated <1 cm particle size was that the grass silage tended to compact in the LBRs. This caused the outlet for the leachate to block and thus the batch of grass silage became submerged in the recirculated leachate with no percolation. As a result the LBRs were opened once per week to unblock the outlet and release the leachate. If left for over one week, the recirculation pump was found to run dry. The process of releasing the leachate from its submerged state had the temporary effect of raising the pH and COD of the liquor. However, the soluble COD remained consistent.

Considerable foaming was also an issue with the shorter particle size of <1 cm. The foaming occurred in the first leachate holding cup (recirculation pump entry point) and was evident from the midway point of the LT3 and LT4 (approximately day 15 onwards). The foaming was removed on alternate days until the trials were

completed. A build-up of precipitate was also apparent in the second and third leaching cups as a result of the foaming. This was also removed on alternate days.

7.3.2 SLBR-UASB system

7.3.2.1 Choice of feedstock

The SLBR-UASB system was run with grass silage of >3 cm particle size. Macerating the grass silage to <1 cm was not implemented due to the issues identified in the leaching trials such as material wash-out, compaction of grass silage, foaming and low SCOD fractions in the percolated leachate. The highest VS destruction rate in the leaching trials, at >3 cm particle size, was achieved without the addition of rumen fluid. Although rumen fluid added to VFA production, this ultimately inhibited the VS destruction rate.

7.3.2.2 Destruction of VS and trend of pH

Table 7.4 illustrates the destruction rates of VS in the SLBR-UASB system. Connection to a UASB gave much higher VS destruction rates for grass silage compared to any of the leaching trials undertaken previously, averaging 61 %. However the average destruction rate still remained lower (as expected due to the high-fibre content of the grass silage) than reported by Nizami and Murphy (2011) who achieved 73−75 % VS destruction of an early growth stage grass silage. The VS content of the grass silage output was lower in all cases than that removed from the leaching trials. The higher VS destruction is suggested to have occurred due to leachate recirculation from the UASB, which increased the pH range. Figure 7.4 shows the operative pH range for the system in comparison to the leaching trials.

a) Day 1−7



b) Day 8−14



c) Day 15−30



d) Grass wash-out



Figure 7.9 Representative variation in liquor colour over 30 day leaching trials

With pH values averaging 7.59 over the 30 days of HRT 3, the hydrolysis phase was more effective and thus a higher breakdown of the grass silage substrate was achieved. These results were in line with that suggested by Veeken *et al.* (2000) who reported pH as the principal variable in controlling the hydrolysis rate in the digestion of organic solid waste.

Table 7.4 Destruction of volatile solids in SLBR-UASB

SLBR-UASB	Grass silage input (kg)	Average grass silage output (kg)	Average VS of grass out (g kg^{-1})	Average VS destruction (%)
Batch 1	3.50	1.54	142	63
Batch 2	3.50	1.43	156	66
Batch 3	3.50	1.70	141	61
Batch 4	3.50	2.14	147	53
Batch 5	3.50	1.79	160	57
Batch 6	3.50	1.56	133	67
Average	3.50	1.69	146	61

7.3.2.3 SCOD, VFA profiles and TAN concentrations

Table 7.5 shows the average weekly values for the operational parameters monitored in the SLBR-UASB. SCOD concentrations out of the UASB for HRT 3 averaged 3.4 g L^{-1}. This illustrated an average COD destruction rate of ca. 84 % (over the 30 day period) in the UASB when compared to the values produced in LT1 analysing the same feedstock input. VFA concentrations out of the UASB were also of a significantly reduced capacity to the leaching trials due to addition of the UASB. The average total VFA concentration in HRT 3 was 0.3 g L^{-1}. Again contrasting with LT1 (>3 cm grass silage), an average destruction of VFAs in the UASB was ca. 97 %. Acetic and propionic acid represented 73−93 % of the acid composition from the UASB with only traces of butyric acid evident in the VFA profile. The results of

SCOD and VFA showed the effectiveness and high performance of the UASB as a methanogenic reactor.

The concentration of TAN in the SLBR-UASB trial was very similar to that which occurred in the leaching trials (0.2−0.3 g L^{-1}). Concentrations were low and hence no inhibition was suspected.

Table 7.5 SCOD and TAN concentrations in SLBR-UASB (HRT 3)

SLBR-UASB: Grass silage >3 cm		
Period	SCOD (g L^{-1})	TAN (g L^{-1})
Day 1-7	3.9 ± 0.53	0.3 ± 0.01
Day 8-14	2.9 ± 0.32	0.2 ± 0.00
Day 15-21	3.1 ± 0.05	*
Day 22-28	3.0 ± 0.05	*
Day 30	2.9 ± 0.06	0.2 ± 0.00

* TAN values not recorded in these periods as concentrations were low and stable

7.3.3 Demand-driven biogas

7.3.3.1 Operation of SLBR-UASB

For a demand-driven biogas process, two-phase digestion systems such as the SLBR-UASB could prove beneficial. The leaching trials undertaken in this study indicate that high VFA concentrations and sustainable quantities of SCOD can be produced in the first-phase, given the right conditions. However, this must be carefully balanced with the destruction of VS.

The two treatments investigated to stimulate hydrolysis in two-phase digestion offered both advantages and disadvantages. Rumen fluid addition to grass silage, at a rate of 50 mL per kg silage, was shown to have a positive response in terms of VFA accumulation and higher SCOD production. Yet the destruction rates of VS were

low in comparison to leaching trials with no rumen fluid addition. This may be explained by the lower pH associated with the increase in VFAs reducing the hydrolysis of the grass silage. Conversely, the maceration of grass silage to particle sizes of <1 cm had a positive effect on the rate of VS destruction. It also increased the total production of VFAs. However such reduction in particle size caused substantial difficulties in terms of system operation particularly by restricting percolation of leachate. Particle sizes of <1 cm are thus not recommended for the SLBR-UASB. The highest VS destruction rate (42 %) in the leaching trials, that permitted a stable operation, was obtained in the run of >3 cm grass silage (LT1). With addition of the methanogenic reactor (UASB), the destruction of VS (hydrolysis) was increased up to 61 % for the digestion of >3 cm grass silage.

It is proposed that for optimal demand driven biogas performance, a combination of methods be used. At times when electricity demand is low, rumen fluid addition could be employed to boost VFA yields and SCOD production whilst keeping the pH low and thus minimising actual VS destruction. When demand for electricity is high, the resultant high-strength leachate could be directed to the UASB for biogas production. The destruction of volatiles would increase in the more optimal pH environment.

7.3.3.2 Potential methane production

In a demand driven biogas process when electricity is not required leachate would be produced as per LT2 (grass silage of >3 cm particle size with rumen fluid addition). This minimises VS destruction when the system is “offline”. When electricity is required the leachate is connected to the UASB and the SCOD from the leach beds would be immediately available for conversion to methane. The leachate from LT2

can generate a SMY of 87 L CH_4 kg^{-1} VS (based on the VS input and the mass of COD produced multiplied by the conversion factor 350 L CH_4 kg^{-1} COD (Sperling & Chernicharo, 2005)). If the UASB is "on-line", the specific methane yield (SMY) may be evaluated as follows. The leach beds effected 61 % destruction of VS (from Table 7.4). In section 7.2.2 it is shown that each kg of VS destroyed produces 1.28 kg COD, and each kg of COD destroyed generates 350 L CH_4. If the COD destruction rate is 84 % (section 7.3.2.3), then the SMY may be evaluated at 230 L CH_4 kg^{-1} VS. This can be compared with a SMY of 340 L CH_4 kg^{-1} VS for the same grass silage in a single-phase system (Wall *et al.*, 2015). It is of interest that in single phase digestion the <1 cm grass silage with rumen fluid gave the best result (Wall *et al.*, 2015) whilst in two-phase digestion the >3 cm grass silage was optimal.
Real-time biogas production was not measured due to operational issues with the flow meter. However the methane concentration of the biogas was measured at 70 %, which highlights a healthy process and is similar to that measured by Nizami and Murphy (2011) for grass silage run in the SLBR-UASB.

7.4 Conclusions

Two-phase digestion is beneficial to a demand-driven biogas process. When demand for electricity is low it is recommended to operate the system in leaching only mode with grass silage cut to >3 cm with rumen fluid addition. This reduces pH, limits destruction of volatile solids (30 %) but facilitates a high strength leachate rich in VFAs and SCOD. When demand for electricity is high the UASB should be connected, increasing the pH, improving VS destruction (61 %). VFAs and SCOD from the leaching only phase are available for methane production. A SMY of 230 L CH_4 kg^{-1} VS is available.

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References

Allen, E., Wall, D.M., Herrmann, C., Murphy, J.D. 2014. Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel. *Bioresour. Technol.*, 170(0), 436-444.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater 21st ed. *American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA.*, 2540 B, E. Solids (Editorial revisions, 2011).

Aslanzadeh, S., Rajendran, K., Taherzadeh, M.J. 2014. A comparative study between single- and two-stage anaerobic digestion processes: Effects of organic loading rate and hydraulic retention time. *International Biodeterioration & Biodegradation*, 95, Part A(0), 181-188.

Babel, S., Fukushi, K., Sitanrassamee, B. 2004. Effect of acid speciation on solid waste liquefaction in an anaerobic digester. *Water Res.*, 38((9)), 2417–2423.

Browne, J.D., Allen, E., Murphy, J.D. 2013. Improving hydrolysis of food waste in a leach bed reactor. *Waste Management*, 33(11), 2470-2477.

Dogan, E., Dunaev, T., Erguder, T.H., Demirer, G.N. 2009. Performance of leaching bed reactor converting the organic fraction of municipal solid waste to organic acids and alcohols. *Chemosphere*, 74(6), 797-803.

Drosg, B. 2013. IEA Task 37 - Process monitoring in biogas plants.

Ganesh, G., Karthikeyan, O.P., Joseph, K. 2010. Enhancing the hydrolysis step in anaerobic digestion of municipal solid waste using rumen fluid. *Int. J. Environmental Technology and Management*, Vol. 13(Nos. 3/4).

Guerrero, L., Omil, F., Méndez, R., Lema, J.M. 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Research*, 33(15), 3281-3290.

Hu, Z.-H., Wang, G., Yu, H.-Q. 2004. Anaerobic degradation of cellulose by rumen microorganisms at various pH values. *Biochemical Engineering Journal*, 21(1), 59-62.

Hu, Z.-H., Yu, H.-Q. 2006. Anaerobic digestion of cattail by rumen cultures. *Waste Management*, 26(11), 1222-1228.

Hu, Z.-H., Yu, H.-Q. 2005. Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. *Process Biochemistry*, 40(7), 2371-2377.

Hu, Z.-H., Yu, H.-Q., Zhu, R.-F. 2005. Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes. *International Biodeterioration & Biodegradation*, 55(3), 233-238.

Lehtomäki, A., Huttunen, S., Lehtinen, T.M., Rintala, J.A. 2008. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresource Technology*, 99(8), 3267-3278.

Nizami, A.-S., Korres, N.E., Murphy, J.D. 2009. Review of the Integrated Process for the Production of Grass Biomethane. *Environmental Science & Technology*, 43(22), 8496-8508.

Nizami, A.-S., Murphy, J.D. 2011. Optimizing the Operation of a Two-Phase Anaerobic Digestion System Digesting Grass Silage. *Environmental Science & Technology*, 45(17), 7561-7569.

Nizami, A.S., Thamsiriroj, T., Singh, A., Murphy, J.D. 2010. Role of Leaching and Hydrolysis in a Two-Phase Grass Digestion System. *Energy & Fuels*, 24(8), 4549-4559.

Persson, T., Murphy, J.D., Jannasch, A.K., Ahern, E., Liebetrau, J., Trommler, M., Toyama, J. 2014. A perspective on the potential role of biogas in smart energy grids. *IEA Bioenergy*, Task 37.

Sanders, W.T.M., Geerink, M., Zeeman, G., Lettinga, G. 2000. Anaerobic hydrolysis kinetics of particulate substrates. in: *Water Science and Technology*, Vol. 41, pp. 17-24.

Speece, R.E. 1996. Anaerobic Biotechnology for Industrial Wastewaters. *Archae Press, Nashville, USA*

Sperling, M., Chernicharo, C.A.L. 2005. Biological Wastewater Treatment in Warm Climate Regions. *London: IWA and Publishing. 1452 p.*

Tchobanoglous, G., Theisen, H., Vigil, S.A. 1993. Integrated Solid Waste Management: Engineering Principles and Management Issues. *Singapore: McGraw–Hill.*

Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M.J., Horváth, I.S. 2010. Pretreatment of paper tube residuals for improved biogas production. *Bioresource Technology*, 101(4), 1206-1212.

Veeken, A., Kalyuzhnyi, S., Scharff, H., Hamelers, B. 2000. Effect of pH and VFA on Hydrolysis of Organic Solid Waste. *Journal of Environmental Engineering*, 126(12), 1076-1081.

Viéitez, E.R., Ghosh, S. 1999. Biogasification of solid wastes by two-phase anaerobic fermentation. *Biomass and Bioenergy*, 16(5), 299-309.

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P., Murphy, J.D. 2014. 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.M., Straccialini, B., Allen, E., Nolan, P., Herrmann, C., O'Kiely, P., Murphy, J.D. 2015. Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage *Bioresour. Technol.*, (under review)

Xie, S., Lawlor, P.G., Frost, J.P., Wu, G., Zhan, X. 2012. Hydrolysis and acidification of grass silage in leaching bed reactors. *Bioresource Technology*, 114(0), 406-413.

Yu, H.W., Samani, Z., Hanson, A., Smith, G. 2002. Energy recovery from grass using two-phase anaerobic digestion. *Waste Management*, 22 (1), 1–5.

8. Conclusions and recommendations

8.1 Conclusions

- Mono-digestion of grass silage generated a specific methane yield (SMY) of 400 L CH_4 kg^{-1} VS in a batch biomethane potential (BMP) assay. Mono-digestion of slurry, collected from an Irish dairy farm, generated a SMY of 239 L CH_4 kg^{-1} VS in a batch BMP assay.
- On a fresh weight basis, the methane generated from grass silage was approximately 7 times that produced from dairy slurry.
- Co-digestion of grass silage and dairy slurry in batch BMP assays, at grass-to-slurry (G:S) ratios of 80:20, 60:40 50:50 40:60 and 20:80 (% on a VS basis), reported decreased SMYs of between 4−11 % when contrasted to pro-rata yields based on mono-digestion of the same substrates.
- In assessing the annual resource of grass available in Ireland in excess of livestock requirements, it was noted that co-digestion of the equivalent of 1.1 % of grassland in the country on a 1:1 VS basis with slurry would allow for compliance with EU RES-T 2020 targets.
- The industry proposed is 170 digesters each treating 10,000 t a^{-1} (3,000 t DS a^{-1} or 275 ha) of grass and 40,000 t a^{-1} of slurry. The digesters would each be 0.75 MWe facilities.
- An average Irish car travels approximately 15,000 km a^{-1} at a fuel efficiency of 5 L of diesel per 100 km. This equates to 750 L diesel per year. For the 170 digesters proposed, each digester would generate ca. 1,664,177 m^3 CH_4 a^{-1} (equivalent to 1,664,177 L of diesel a^{-1}). Thus, each facility would be sufficient to fuel over 2,200 cars travelling 15,000 km a^{-1}. Over 370,000 cars could be fuelled through the operation of 170 digesters.

- To maximise the potential biomethane output from a continuously stirred tank reactor (CSTR), a higher grass silage input is recommended. Slurry dilutes the SMYs achievable but can act as an important nutrient source.
- Mono-digestion of grass silage in a CSTR generated a SMY of 398 L CH_4 kg^{-1} VS at an organic loading rate (OLR) of 3.5 kg VS m^{-3} d^{-1} equating to a biomethane efficiency (B Eff.) of 1.0. Increasing the OLR to 4.0 kg VS m^{-3} d^{-1} instigated a drop in SMY of ca. 12 % and hence decreased the B Eff. to 0.90.
- Co-digestion of grass silage with 20 % dairy slurry in a CSTR maintained maximum B Eff. (1.01) at an OLR of 4.0 kg VS m^{-3} d^{-1} generating a SMY of 349 L CH_4 kg^{-1} VS.
- Hydaulic retention times (HRT) of less than 20 days was found to be process limiting for the continuous mono-digestion of grass silage. Such HRTs did not allow enough time for the microorganisms to degrade the feedstock.
- Comprehensive trace element (TE) profiles were developed for mono-digestion of grass silage in a CSTR from an OLR of 2.0−4.0 kg VS m^{-3} d^{-1}. A similar TE profile was developed for a CSTR co-digesting grass silage with 20 % dairy slurry. The TE profiles were cross-examined with FOS/TAC values and the volatile fatty acid (VFA) profiles to assess both reactors performance.
- Three TEs were found to be undersupplied at high OLRs for mono-digestion of grass silage when compared to a digester co-digesting grass silage and slurry. The TEs were cobalt, iron and nickel.
- Supplementation of the three identified TEs (cobalt, nickel and iron) increased the SMY for mono-digestion of grass silage to 404 L CH_4 kg^{-1} VS, thereby increasing the B Eff. to 1.01. The VFA profile also improved with TE addition by eliminating the build-up of propionic acid.

- Physical reduction in particle size and rumen fluid addition treatments to advanced growth stage grass silage, to stimulate hydrolysis, indicated little impact on obtainable SMYs in batch BMP assays (340-350 L CH_4 kg^{-1} VS).
- In CSTRs, shorter particles sizes of less than 1 cm were more suitable for digestion than particle sizes of greater than 3 cm, which caused significant operational issues.
- Mono-digestion of advanced growth stage grass silage in a CSTR at <1 cm particle size with daily rumen fluid addition generated a SMY of 371 L CH_4 kg^{-1} VS at an OLR of 2.5 kg VS m^{-3} d^{-1}.
- Two-phase digestion systems such as a leaching combined with an upflow anaerobic sludge blanket (SLBR-UASB system) can be adapted for demand-driven biogas application.
- At low electricity demand two-phase systems can be operated in leaching only mode for grass silage of particle size >3 cm with rumen fluid addition. This reduces pH and limits the destruction of volatile solids but produces a high strength leachate rich in VFAs and soluble chemical oxygen demand (SCOD).
- At high electricity demand, the UASB can be connected for the production of biogas to produce electricity. This increases the pH in the system, thereby improving the destruction of volatile solids.

8.2 Recommendations

This thesis demonstrates that high SMYs can be generated from grass silage of different chemical composition at a mesophilic temperature range. It is recommended that mono-digestion of early growth stage grass silage (with high dry solids digestibility) in a CSTR should be maintained at an OLR of less than 4.0 kg

VS m^{-3} d^{-1} to ensure a healthy process. If the OLR is set at 4.0 kg VS m^{-3} d^{-1} or above, TE addition is recommended. Addition of slurry as a co-substrate is suggested as a useful alternative to TE addition. Slurry is rich in micronutrients and valuable bacteria, although the obtainable SMYs from a digester will be diluted with slurry addition. Biomethane efficiencies remain high for digesters with TE addition or slurry co-substrates. Mono-digestion of advanced growth stage grass silage (low dry solids digestibility) should be undertaken at lower OLRs (2.5 kg VS m^{-3} d^{-1}) due to the highly fibrous nature of the feedstock. It is recommended that extra care is taken when digesting lower quality grass silages as the process is more prone to inhibition through VFA accumulation. Close attention should be taken to the effective HRT in a CSTR, particularly if liquor (separated from the digestate) is recirculated back into the reactor. Low HRTs lead to ineffective digestion of grass silage. Maceration of grass silage to short particle sizes of 1 cm or less is recommended for single-stage digestion processes such as digestion in a CSTR. Longer particle sizes of 3 cm or greater are recommended for two-phase digestion systems. This aligns with the low cost approach of two-phase digestion. Use of rumen fluid as a biological treatment to stimulate hydrolysis can be utilised in both single and two-phase systems. However a strategy to implement rumen fluid would need to include costs for transport, storage and heating.

Appendix A

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

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Abstract

Ulva lactuca, a green seaweed, accumulates on beaches and shallow estuaries subject to eutrophication. As a residue, and a 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 5 L reactors for 9 months. The reactors digesting a mix with 75% *U. lactuca* struggled to reach stable conditions. Volatile fatty acid levels of 14,000 mg L^{-1} were experienced. 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: Ulva lactuca, biomethane, biofuel

Highlights

- The optimum mix of fresh *U. lactuca* is 25 % by VS content with dairy slurry.
- The optimum loading rate is suggested as 2.5 kg VS m^{-3} d^{-1}.
- For stable operation it is suggested that management of trace elements is required.
- Critical parameters include high levels of chloride, calcium and VFA.
- Levels in excess of 75 % *U. lactuca* are not recommended.

Appendix B

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?

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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 BMP (biochemical methane potential), 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 VS^{-1}). *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.

Keywords: Biogas, macro-algae, 3rd generation biofuels

Highlights

- Ten species of seaweed were assessed for biomethane potential.
- Methane yields from seaweeds ranged from 13.5 to 34.5 m^3 CH_4 t^{-1} wet weight.
- Harvests of cultivated seaweed may generate 5 to 90 t dry solids ha^{-1} yr^{-1}.
- Seaweeds may generate a gross energy yield of up to 700 GJ ha^{-1} yr^{-1}.
- For Ireland *Saccharina latissima* is recommended, which may produce 365 GJ ha^{-1} yr^{-1}.