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Biomethane production from co-digestion of grass silage and slurry

School of Engineering
&
Environmental Research Institute

University College Cork

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

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December 2017

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

(Himanshu)

Date: 10-December-2017

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Thesis abstract

The core aim of this thesis was to quantify the effects of co-digesting forage silages with animal slurries on methane yields and to investigate if antagonistic or synergistic outcomes occur. In order to complete this assessment, the economic impacts of changing forage silage characteristics, of changing the mixing ratios of forage silage and cattle slurry in binary mixtures (and the presence of synergy or antagonism) and of changing the costs of providing these feedstocks for anaerobic digestion (AD) on the cost of methane production in an on-farm AD facility were accessed. An initial objective, however, was to define an optimal methodology for laboratory-scale anaerobic digestion, specifically to determine the impact of altering the headspace volume within incubation bottles and the overhead pressure measurement and release (OHPMR) frequency on methane yield using a manual manometric biochemical methane potential (mBMP) batch digestion method.

Two anaerobic batch co-digestion experiments were conducted with forage silages and animal slurries. In the first experiment, oven-dried perennial ryegrass (harvested at two growth stages) or red clover (harvested at two growth stages) silages and cattle slurry were co-digested. Each binary mixture had synergistic effects which resulted in 2.8-7.5% higher methane yields than predicted from mono-digestion of individual substrates. In the second experiment, cattle slurry (two types) or pig slurry was co-digested with undried perennial ryegrass silages (harvested at two growth stages). Each silage and slurry mixture had antagonistic effects which resulted in methane yields 5.7-7.6% below those predicted from mono-digestion of individual substrates.

In the initial experiment and in order to broaden the conditions under which the assessment was made, the biogas and methane yields of cellulose, barley grain, grass silage and cattle slurry were determined in response to three incubation bottle headspace volumes and four OHPMR

frequencies. The methane yields of barley, silage and slurry were also compared with those from an automated volumetric method (i.e. AMPTS). Headspace volume and OHPMR frequency effects on biogas yield were mediated mainly through headspace pressure, with the latter having a negative effect on the biogas yield measured but relatively little effect on methane yield. Two mBMP treatments that produced methane yields equivalent to AMPTS were identified.

Economic modelling results showed significant impacts of AD feedstock characteristics and their provision cost on the cost of methane production in an AD facility. The feedstock provision cost contributed about half of the total cost of methane production when the AD facility solely operated on grass silage. The total cost of methane produced from mono-digestion of cattle slurry that was supplied free of charge was more than double the cost of methane produced from grass silage. For co-digestion of grass silage and cattle slurry, the total cost of methane production progressively increased as the proportion of slurry in the co-digested mixture increased. Antagonistic and synergistic methanogenesis resulted in a corresponding 6% higher and 5% lower total cost of methane production during co-digestion of grass silage and cattle slurry (at a silage:slurry volatile solids ratio of 0.8:0.2) compared to the binary mixture without these effects.

Thesis output

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

Chapter 3: Himanshu H, Murphy JD, Grant J, O'Kiely P. Synergies from co-digesting grass or clover silages with cattle slurry in *in vitro* batch anaerobic digestion. Renewable Energy (under review).

Chapter 4: Himanshu H, Murphy JD, Grant J, O'Kiely P. Antagonistic effects on biogas and methane output when co-digesting cattle and pig slurries with grass silages in *in vitro* batch anaerobic digestion. Biomass and Bioenergy. 109, 190-198.

Chapter 5: Himanshu, H, Voelklein, MA, Murphy, JD, Grant, J, O'Kiely, P. 2017. Factors controlling headspace pressure in a manual manometric BMP method can be used to produce a methane output comparable to AMPTS. Bioresource Technology, 238, 633-642.

Chapter 6: Himanshu, H, Lenehan, JJ, Murphy, JD and O'Kiely, P. 2017. Impact of characteristics of the feedstocks grass silage and cattle slurry on the cost of methane production. Renewable Energy (under review).

Contribution to the papers

Chapter 3: I was responsible for undertaking the laboratory work, data analysis, producing the first draft of the manuscript (first author) and updating reviewed drafts. I was also involved in the development of the hypotheses and designing the experiment.

Chapter 4: I was responsible for undertaking the laboratory work, data analysis, producing the first draft of the manuscript (first author) and updating reviewed drafts. I was also involved in the development of the hypotheses and designing the experiment.

Chapter 5: I was responsible for the development of hypothesis, designing the experiment, data analysis, producing the first draft of the manuscript (first author) and updating reviewed drafts. The laboratory trials were achieved in conjunction with my colleagues at University College Cork, Cork.

Chapter 6: I was responsible for researching and collecting the required data, data analysis, producing the first draft of the manuscript (first author) and updating reviewed drafts. I was also involved in the development of the hypotheses.

Abbreviations

AA	Acetic acid
ABAI	Anaerobic Biodegradation, Activity and Inhibition
AD	Anaerobic digestion
ADF	Acid detergent fibre
ADL	Acid detergent lignin
AFBI	Agri-Food and Biosciences Institute
AMPTS	Automated methane potential test system II
BA	Butyric acid
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
C:N	Carbon:Nitrogen
CHP	Combined heat and power
COD	Chemical oxygen demand
CP	Crude protein
CS	Cattle slurry
CS1	Cattle slurry 1
CS2	Catttle slurry 2
CSTR	Continuously stirred tank reactor
Eth	Ethanol
EU	European Union
F	Frequency with which overhead pressure was measured and released
GFCM	Grange feed costing model
GHG	Green house gas
GS	Grass silage
HRT	Hydraulic retention time
IWA	International Water Association
k	First order decay constant
LA	Lactic acid
mBMP	Manual manometric BMP
NA	Not available
NDF	Neutral detergent fibre
NH_3-N	Ammonia-nitrogen
NLB	Non-linear blending
OHPMR	Overhead pressure measurement and release
OLR	Organic loading rate
P	Level of significance
P day	Time at which the maximum pressure was recorded during the mBMP test
PA	Propionic acid
P_{Max}	Maximum pressure measured during the mBMP test
PRG1	Perennial ryegrass 1
PRG2	Perennial ryegrass 2
PS	Pig slurry
RC1	Red clover 1
RC2	Red clover 2
SEM	Standard error of the mean
SFI	Science Foundation Ireland
T_{50}	Time taken (d) to produce 50% of the gas production (Half-life)
TS	Total solids
TSD	Total solids digestibility
U	Maximum methane or biogas production rate
V	Headspace volume
VFA	Volatile fatty acid
VS	Volatile solids
WSC	Water-soluble carbohydrates
λ	Lag phase
λ_T	Exponential phase

1. Introduction

1.1. Introduction and background to thesis

Climate change and high fossil fuel prices ($157 per barrel of crude oil in July 2008) have led to a focus on alternative and renewable sources of energy and subsequently to policies such as the European Union's (EU) Renewable Energy Directive and Paris climate agreement. The Renewable Energy Directive, requires the EU to provide at least 20% of its total energy requirement with renewables and each EU country to source at least 10% transport fuel from renewables by 2020 (EC, 2009). Ireland is committed to provide at least 16% of its energy requirements from renewables (EC, 2009). During the Paris climate agreement 195 countries adopted the first-ever universal, legally binding global climate deal to keep the global temperature rise less than 2 °C above pre-industrial levels by controlling the green house gas (GHG) emissions.

About 20% of the world's total energy is supplied by renewables including biomass, geothermal, hydro, solar, tidal, wave and wind; biomass makes the most significant contribution supplying 10-15% of total energy (Murphy et al., 2011). Biomass can produce energy either by combustion or by anaerobic digestion (AD). The biogas (50-60% methane) produced during AD can either be upgraded to biomethane (> 97% methane), which has an energy content equivalent to that of natural gas, or, with minimal purification, can be used in a combined heat and power (CHP) engine for simultaneous production of heat and electricity. Germany has adopted AD as an strategy for renewable energy provision and GHG mitigation and its ca. 9000 AD facilities produce about 29.4 TWh of electricity each year (Fachverband Biogas, 2017). In Ireland, 1.1% of the grassland can supply 10% renewable energy in the transport sector via AD (Wall et al., 2013).

Grass can be a major AD feedstock in Ireland since over 90% of its agricultural land is under grassland (CSO, 2013). This grassland has the potential to produce biomass in excess of current or expected livestock requirements (McEniry et al., 2013). Furthermore, methane production from grass has been reported to produce more energy in fuel per hectare, be superior in energy balance, be economical under good farm management, and be more sustainable (i.e. more greenhouse gas savings) compared to the indigenous European first generation liquid biofuels such as wheat ethanol and rapeseed biodiesel (Korres et al., 2010; McEniry et al., 2011; Smyth et al., 2009). At present, most of grass produced in Ireland is utilized by ruminants which are accommodated indoors for at least part of the year, thereby producing slurry that can be utilized for AD.

Long term mono-digestion of grass silage at high organic loading rates carries a risk of process imbalance (Thamsiriroj et al., 2012). However, co-digestion of grass silage with animal slurry, a feedstock which usually produces a lower methane yield compared to grass silage, can complement each other and enhance the longevity of stable and productive AD (Wall et al., 2014). Furthermore, co-digestion of these contrasting substrates may result in antagonistic (i.e. the mixture produces less yield than the arithmetically calculated yield from sole substrates) or synergistic (i.e. the mixture produces more methane than the arithmetically calculated yield from sole substrates) (Wall et al., 2013) effects on methanogenesis, and in a commercial scale AD facility, these effects would likely have an effect on profitability.

The methanogenic potential of grass silage and its production cost can be effected by several factors such as biomass yield, total solids digestibility (TSD), harvest date, preservation and farm management (McEniry et al., 2014). Similarly, the methanogenic potential of cattle slurry can be effected by cattle type (Triolo et al., 2013), diet type (Amon et al., 2006; Hellwing et al., 2014),

dilution with other materials or the presence of antibiotics (Hashimoto et al., 1981; Triolo et al., 2011) and the duration and conditions of slurry storage (Browne et al., 2015). Furthermore, different biochemical methane potential (BMP) test methods, which are commonly used to determine the biogas and methane yields from organic substrates using anaerobic batch digestion processes, can also influence the methane yields (Nolan et al., 2016; Valero et al., 2016; Wang et al., 2014; Yilmaz, 2015).

A significant share to the total cost of methane production in an AD facility is contributed by the feedstock provision cost (Smyth et al., 2010). However, the feedstock characteristics and its provision cost are too often assumed to be constant during operation of the AD facility (Dennehy et al., 2017; Smyth et al., 2010).

This thesis investigates (a) the anaerobic co-digestion of silages with slurries for possible antagonistic or synergistic impacts on biogas and methane yields, (b) the impacts of incubation bottle headspace volume and overhead pressure measurement and release (OHPMR) frequency on biogas and methane yield for a manual manometric BMP (mBMP) method, and (c) the impacts of changing silage characteristics, silage and slurry provision costs and their binary mixing ratios (including any effects of synergy/antagonism) on the cost of methane production in an AD facility.

1.1.1. Thesis aims and objectives

The aims and objectives of the thesis were as follows:

- To measure the biogas and methane yields of the mixture of oven-dried grass or legume silages with cattle slurry and to assess the possible antagonistic/synergistic outcomes using *in vitro* batch digestion.

- To measure the biogas and methane yields of the mixture of cattle or pig slurry with non-dried perennial rye grass silage and to assess the possible antagonistic/synergistic outcomes using *in vitro* batch digestion.
- To study the impacts of headspace volume and OHPMR frequency on biogas and methane yields in *in vitro* anaerobic digestion using a mBMP method and compare these yields with those from an automated volumetric method i.e. Automated Methane Potential Test System II (AMPTS).
- To quantify the responses in methane production costs due to changes in (a) grass silage characteristics and its provision cost, (b) cattle slurry provision cost, (c) ratios of silage and slurry volatile solids (VS) in binary mixtures (and the presence of synergy or antagonism), and (d) operational efficiency of the AD facility.

1.1.2. Thesis outline and link between chapters

The thesis consists of seven chapters. Chapter 2 is a review that assesses the potential of co-digestion of silage and slurry as a feedstock for renewable gaseous biofuel production in Ireland. Chapters 3 to 5 present the majority of the laboratory work undertaken over the research period. Chapter 6 is an economic analysis of the feedstock characteristics, their provision cost and their binary mixing ratios on the cost of methane production in an AD facility. Chapters 3, 4 and 6 appear as per the manuscripts submitted for publication and are currently under review and/or in press. Chapter 5 is a peer-reviewed journal paper and appears as per the published manuscript. Each chapter describes a separate topic; however, a sequential theme combines the study into one unit. The thesis follows the academic paper model, that is, a succession of published or submitted journal papers that can be read independently or as a whole. A summary of Chapters 2 to 6 is given below:

Chapter 2: *Literature review*

This chapter examines the scientific literature and reviews previous work undertaken which reported the potential of grass and slurry as feedstocks for renewable sources of energy and AD studies reporting antagonistic or synergistic effects on methanogenesis during co-digestion of contrasting substrates. Furthermore, different BMP methods and the experimental factors that could influence the methane yield are reviewed. Also, the Grange feed costing model (GFCM), previously developed to identify the relative costs of feeds produced for ruminants and which has been used in Chapter 6 to calculate the cost of feedstock provision in an AD facility, is also briefly described.

Chapter 3: *Synergies from co-digesting grass or clover silages with cattle slurry in in vitro batch anaerobic digestion*

This chapter investigates the co-digestion of grass or legume silages with cattle slurry using *in vitro* batch digestion. Oven dried perennial ryegrass silage (harvested at two growth stages, PRG1 and PRG2) or red clover silage (harvested at two growth stages, RC1 and RC2) with cattle slurry were incubated as sole substrates or as part of binary mixtures (silage:slurry ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.75:0.25 and 0:1 on a volatile solid (VS) basis). The two forages, which represent the two main botanical families found in north-western European permanent grassland, and at contrasting growth stages, were selected to broaden the conditions under which the linearity of methane output in response to co-digestion in a series of ratios with cattle slurry would be assessed. For mono-digestion, the biogas and methane yields were higher for perennial ryegrass and, for both forages, these yields decreased with the advancing stage of maturity. The

methane yields of PRG1, PRG2, RC1, RC2 and cattle slurry were 318, 286, 287, 255 and 282 L CH_4 kg^{-1} VS, respectively. During co-digestion, both forages displayed non-linear blending and the maximum effect, which was always synergistic and observed at different silage:slurry ratios for each mixture, differed with the forage species and its growth stage when harvested.

Chapter 4: *Antagonistic effects on biogas and methane output when co-digesting cattle and pig slurries with grass silage in in vitro batch anaerobic digestion*

Chapter 4 investigates the biogas and methane yields of the mixtures of cattle (two types, CS1 and CS2) or pig slurry (PS) with two contrasting undried perennial ryegrass silages (GS1 and GS2) using *in vitro* anaerobic batch digestion. The three slurries and two grass silages used in this study are examples from within the diverse range of livestock slurries and conserved forages likely to be used for AD on Irish farms. Slurries and silages were incubated as sole substrates or as part of binary mixtures (slurry:silage ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.75:0.25 and 0:1 on a VS basis). The methane yields of CS1, CS2, PS, GS1 and GS2 were 270, 246, 380, 428 and 359 L CH_4 kg^{-1} VS, respectively. During co-digestion, with all slurries, the sequential replacement of slurry by silage caused a progressive change in biogas and methane yields from the values obtained with slurries to those with silages. However, methane yield for slurry and silage mixtures displayed non-linear blending and the maximum effect, which was always antagonistic, was at a 0.5:0.5 VS ratio and ranged from 5.7-7.6% below the yields predicted from mono-digestion of sole substrates.

Chapter 5: *Factors controlling headspace pressure in a manual manometric BMP method can be used to produce a methane output comparable to AMPTS*

This chapter investigates the effects of two different experimental parameters i.e. headspace volume and OHPMR frequency on the biogas and methane yields in a mBMP method and compares these yields with those from an automated volumetric BMP method. In the mBMP test method, cellulose, barley, oven dried silage and slurry were incubated in three incubation bottle headspace volumes (50, 90 and 180 ml; constant 70 ml total medium) and for each headspace volume incubation bottle the overhead pressure was measured and released at four frequencies (daily, each third day, weekly and solely at the end of experiment). The methane yields of barley, silage and slurry were compared with those from an automated volumetric BMP method i.e. AMPTS. Headspace volume and OHPMR frequency effects on biogas yield were mediated mainly through headspace pressure, with the latter having a negative effect on the biogas yield and relatively little effect on methane yield. For barley, silage and slurry, two mBMP treatments produced methane yields equivalent to AMPTS. The study highlights that judicious consideration is required when selecting a BMP technique as the decision can impact on the methane yields recorded and on the relative values attributed to different substrates.

Chapter 6: *Impacts of characteristics of the feedstocks grass silage and cattle slurry on the cost of methane production*

Chapter 6 uses economic modelling to investigate the impacts of changing grass silage characteristics, grass silage and cattle slurry provision costs and their binary mixing ratios (including any synergy/antagonism) on the cost of methane production from an on-farm AD facility. The input data for this modelling exercise e.g. methane yield of feedstocks, impact of

silage characteristics such as TSD and growth stage on its methane yield was obtained from the experimental results from Chapters 3 and 4 and previously published scientific literature. The feedstock provision cost, which was calculated using GFCM, contributed about half of the total cost of methane production when the AD facility solely operated on grass silage. The management targets, in order to reduce the cost of methane production on a grass silage farm for its AD facility, are that high yields of biomass are achieved per harvest, that the grass is of high digestibility and undergoes efficient fermentation during ensiling, and that aerobic deterioration of silage during its feedout is minimised. Even though the cattle slurry was considered to be supplied free of cost the total cost of methane production from its mono-digestion, compared to grass silage, was more than double due to its low total solids (TS), VS and methanogenic potential. The total cost of methane production progressively increased as the proportion of slurry in binary mixture of silage and slurry increased during their co-digestion. Antagonistic and synergistic methanogenesis resulted in a corresponding 6% higher and 5% lower total cost of methane production during co-digestion of grass silage and cattle slurry (at silage:slurry VS ratio of 0.8:0.2) compared to the binary mixture without these effects. During co-digestion of grass silage and cattle slurry the emphasis should be to maximize the inclusion rate of grass silage commensurate with maintaining an efficient and stable long-term digestion process.

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2. Literature review

2.1. Anaerobic digestion (AD)

AD is a multi-step biochemical process, carried out by various types of anaerobic microbes where complex organic matter is decomposed in the absence of oxygen. AD naturally occurs in environments such as marshes, ponds, swamps, paddy fields, lakes, hot springs, landfills, sewage digesters, oceans and intestinal tracts of humans and animals (Christy et al., 2014).

A. Volta in 1776 showed the formation of "combustible air" from sediments in lakes, ponds, and streams and recognized that anaerobic biological processes result in the conversion of organic matter to methane (McCarty, 2001). The first full-scale anaerobic treatment of domestic wastewater in an airtight chamber known as "Mouras Automatic Scavenger" was developed in 1881. The first anaerobic digester that can use the produced methane in a gas engine for sewage pumping, lighting and cooking was first developed in 1897 in waste disposal tanks at a leper colony in Matunga, Bombay, India (Bushwell & Hatfield, 1938; Fowler, 1934; James, 1901; Khanal, 2011; Pullen, 2015).

The four different steps of AD process are (Figure 2.1):

2.1.1. Hydrolysis

During hydrolysis, several hydrolytic microbes e.g. Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas, Streptococcus excrete hydrolytic enzymes e.g. cellulase, cellobiase, xylanase, amylase, protease, lipase which decompose polymers e.g. carbohydrates, lipids, nucleic acids and proteins into mono- and oligomers e.g. glucose, glycerol, purines and pyridines. Hydrolysis is considered as rate limiting step during AD of solid lignocellulosic material due to accessibility of hydrolytic microbes to the solid matter (Christy et al., 2014). The hydrolysis products are further decomposed and used by the microbes for their

own metabolic processes. Some of these microbes utilise the residual oxygen further strengthening anaerobic conditions (Traversi et al., 2012).

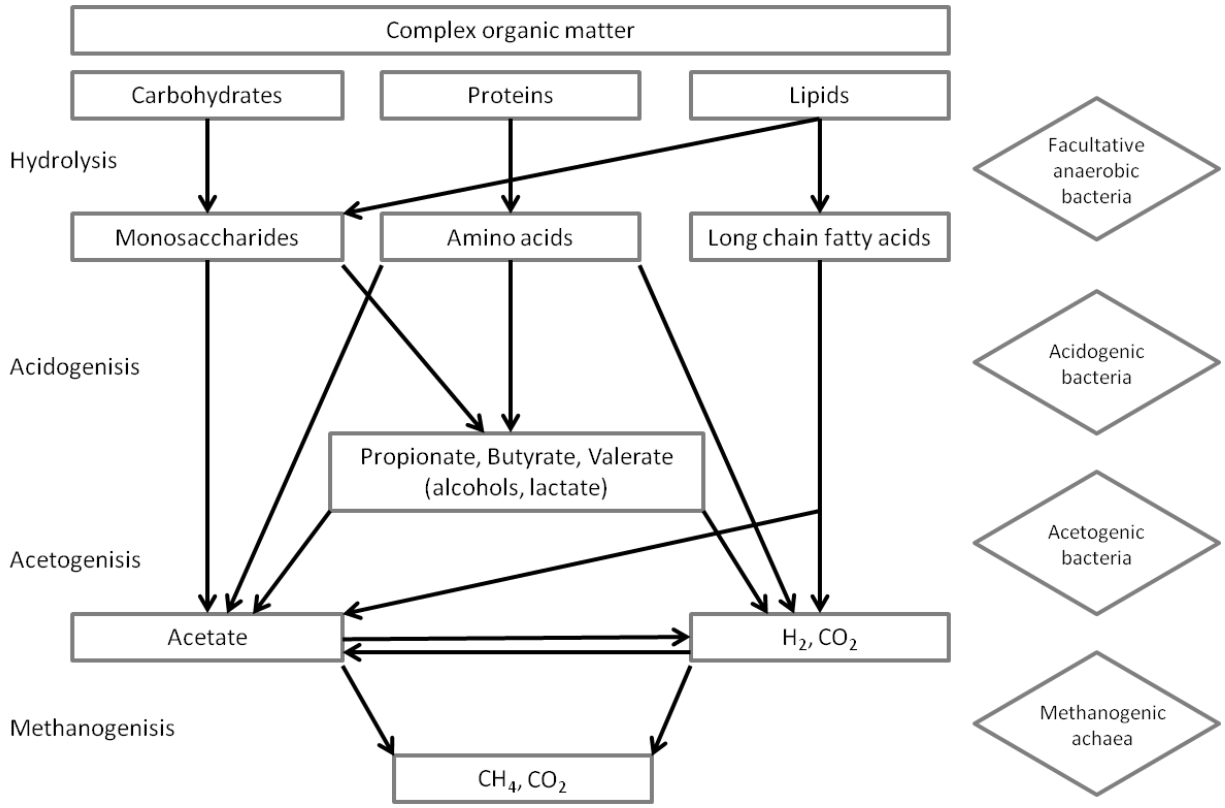


Figure 2.1. Steps of anaerobic digestion (AD) process (modified from Christy et al., 2014).

2.1.2. Acidogenisis

During acidogenesis, the products of hydrolysis are converted by acidogenic (fermentative) bacteria e.g. Streptococcus, Lactobacillus, Bacillus, Escherichia coli, Salmonella into methanogenic substrates i.e. acetate, carbon dioxide and hydrogen as well as into volatile fatty acids (VFA) and alcohols (Seadi et al., 2008). Acidogenesis is usually the fastest reaction of the AD process and acidogenic microbes grows about ten times faster than methanogens (Christy et al., 2014).

2.1.3. Acetogenisis

During acetogenisis the products of acidogensis i.e. VFAs and alcohols that are difficult to convert to methane are oxidised into methanogenic substrates like acetate, H_2 and CO_2 (Seadi et al., 2008). Acetogenic bacteria are strict anaerobes, slow growing, sensitive to fluctuations in organic loadings and environmental changes and require long acclimatisation periods to adjust to new environmental conditions. The acetogens are generally found in syntrophic associations with methanogens (Christy et al., 2014).

2.1.4. Methanogenisis

During the final phase of AD methanogenic microbes that belong to Archaea use formate, acetate and H_2 and CO_2 as energy and carbon sources for growth and produce methane (Christy et al., 2014). Typically 70% of the methane originates from acetate which involves acetoclastic methanogens, while the remaining 30% is produced from conversion of H_2 and CO_2 which involves hydrogenotrophic methanogens. Methanogens are severely influenced by operating conditions such as composition of feedstock, feeding rate, temperature, O_2 concentration and pH (Seadi et al., 2008). Acetogenesis and methanogenesis usually run parallel, as symbiosis of two groups of organisms (Seadi et al., 2008).

2.2. Current status of AD in Ireland

The AD industry in Republic of Ireland is somewhat limited with 13 operational AD plants having about 10 MW electricity capacity (Irish BioEnergy Association, 2017), even though being an agricultural country it has substantial amount of potential AD feedstocks e.g. grass, food wastes and animal slurry (McEniry et al., 2013; O'Shea et al., 2016; Wall et al., 2013).

2.3. Grass as AD feedstock in Ireland

In Ireland, approximately 61% of the total land mass is agricultural land (O'Mara, 2008) and about 80% (3.7 million hectares) of this agricultural land is grassland based farming under silage, hay and pasture while 11% (0.45 million ha) is dedicated to rough grazing (CSO, 2012). Perennial ryegrass, meadow grass and white clover dominate the grasslands (O'Mara, 2008; O'Sullivan & Murphy, 1982) with perennial ryegrass as the most widely sown grass accounting for approximately 0.95 of forage grass seed sold for commercial agricultural practice due to its high digestibility when harvested at the appropriate growth stage, high yield in response to nitrogen fertiliser application and ease of preservation as silage due to its relatively high water soluble carbohydrate content (DAFF, 2016; Whitehead, 1995). McEniry et al. (2013) estimated that Ireland has 1.7 million tonnes of grass TS per year in excess of livestock requirements and there is potential to increase this to 12.2 million tonnes per year with intensive grassland management, thereby providing a significant supply of grass as AD feedstock. If grass is to be used as a feedstock for AD it has to be harvested and stored as silage to ensure year round availability and a predictable quality. Irish farmers are already in possession of the technical know-how and expensive machinery for silage preparation as silage is currently produced on 86% of Irish farms (McEniry et al., 2007). At present, grass is not used as an AD feedstock in Ireland even though it is already an established resource in more than 50% of AD plants in Germany and Austria (Prochnow et al., 2009). Apart from agricultural functions, grasslands provide several ecosystem services such as cultural, landscape, recreation, tourism and rural development values (Prochnow et al., 2009).

A wide range of methane yields for grass silage have been reported in literature i.e. 140-650 L CH_4 kg^{-1} VS (Prochnow et al., 2009; Wall, 2015). The reported methane yield of non-dried

perennial ryegrass silage in Ireland is 258-488 L CH_4 kg^{-1} VS while the methane yield for red clover is 250-347 L CH_4 kg^{-1} VS (Lehtomäki, 2006; Seppälä et al., 2013; Weiland, 2010).

2.4. Factors affecting grass as AD feedstock

In principle an AD feedstock should be economically produced and stored and should have sufficient methanogenic potential. A maximum methane yield is especially important with AD feedstocks such as energy crops and grass as these, in contrast to animal slurries or organic wastes have production costs that have to be covered by the methane production (Walla & Schneeberger, 2008). There are multiple factors that can affect the production cost and methanogenic potential of grass silage. Some of these factors such as biomass yield and its digestibility are independent of farm management while several potential losses including field losses, effluent production, fermentation losses in the silo and aerobic deterioration during storage and at feedout can be minimized with good farm management (McDonald et al., 1991).

2.4.1. Biomass yield

A higher biomass yield can reduce the cost of feedstock production (McEniry et al., 2011) and consequently the cost of producing methane in an AD facility. However, biomass yield can differ across years even when harvested on the same date and subjected to constant management e.g. O'Kiely (2004a) and O'Kiely (2004b) reported 5-8 t TS ha^{-1} biomass yield for perennial ryegrass which was harvested at same date each year (29-May) during a six year field trial in Ireland.

2.4.2. Total solids digestibility (TSD)

TSD can directly impact the cost of methane production in an AD facility as TSD is directly proportional to methane yield (McEniry & O'Kiely, 2013). Low biomass TSD will reduce the

extent and/or rate of methane yield per unit volatile solids (VS) incubated. Similar to biomass yield, TSD can also differ across years even when harvested on the same date and subjected to constant management e.g. O'Kiely (2004a) and O'Kiely (2004b) reported 735-778 g kg^{-1} TS TSD for perennial ryegrass silage harvested at same date each year (29-May, first cut) during six year field trials in Ireland.

2.4.3. Forage growth stage at harvest

Growth stage of a crop at harvest is the single most important factor that affects both the biomass yield and TSD due to change in chemical composition of forage with the advancing stage of maturity. As the plant matures the proportion of cell walls increase while the proportion of cell contents decreases (Morrison, 1980). The increased lignin content of cell walls which increases structural strength of the plant also simultaneously leads to an overall decrease in cell wall digestibility and consequently of the whole forage (Frame & Laidlaw, 2011). Several studies have reported a decrease in methane yield of grasses and legumes at a more fibrous growth stage (Amon et al., 2007a; Kaparaju et al., 2002; Lehtomäki et al., 2008; McEniry & O'Kiely, 2013; Prochnow et al., 2005; Seppälä et al., 2009). While the TSD and consequently the methane yield of a forage is decreased with advancing maturity a higher biomass yield can be achieved due to delayed harvest thereby producing a cheaper crop (Prochnow et al., 2009). Hence, identifying the optimal stage at which to harvest to obtain high yields of high quality forage requires special attention (McEniry & O'Kiely, 2013).

2.4.4. Bad fermentation during ensiling

Bad preservation i.e. excessive fermentation reflected by high levels of clostridial activity results in high TS losses due to extensive production of carbon dioxide and hydrogen fermentation of

lactate and hexose sugars (Rooke & Hatfield, 2003). However, the resulting fermentation products e.g. ethanol, butyric acid and propionic acid may have higher methanogenic potential than the normal lactic acid dominating bacteria fermentation products e.g. lactic acid and acetic acid (Neureiter et al., 2005; Weissbach, 2009). McEniry et al. (2014) reported 16.6% higher TS loss and a 3.9% increase in methane yield (L CH_4 kg^{-1} VS) of perennial ryegrass due to bad fermentation when compared with good lactic acid bacteria dominated fermentation. However, the positive effect of enhanced specific methane yield was outweighed by the large TS losses during the ensiling process (McEniry et al., 2014).

2.4.5. Effluent loss during ensiling

Silage effluent is produced during ensiling of forage mass with low TS content (Jones & Murdoch, 1954; Purves & McDonald, 1963) by the expulsion of plant juices (Haigh, 1994). Silage effluent has high biological oxygen demand i.e. five-day BOD (12,000-90,000 g O_2 L^{-1}) due to presence of soluble nutrients i.e. carbohydrates, organic acids, proteins and minerals (Wilkinson, 1942). Abu-Dahrieh et al. (2011) reported a methane yield of 0.385 m^3 kg^{-1} COD (70-80% CH_4 content) for grass silage effluent. Silage effluent losses can either be minimized by reducing effluent production through wilting (Castle & Watson, 1973) or directly using it as a feedstock in an AD plant (McEniry et al., 2011).

2.4.6. Aerobic degradation

Exposure of ensiled forage to air (oxygen) can reactivate the aerobic microbes (McDonald et al., 1991) causing heating and chemical changes within the silage indicated by reduction in lactic acid concentration and a corresponding rise in pH. Aerobic deterioration can cause significant TS losses e.g. Honig and Woolford (1980) reported a loss of 30-50 g TS kg^{-1} within 1 d of exposure

to air while O'Kiely and Lenehan (1996) reported a loss of 269 g TS kg^{-1} during extensive exposure to air, consequently causing loss in available energy content. Baserga and Egger (1997) reported a 26% decrease in methane yield of perennial ryegrass silage after 5 d exposure to air. Furthermore, mycotoxins may be produced during aerobic deterioration (Woolford, 1990) and they have been shown to restrict microbial activity and cause foaming in the rumen (Moeller et al., 2012). Similar issues may also arise in the AD facility (Moeller et al., 2012).

2.5. Animal slurry as AD feedstock in Ireland

The dairy, beef and pig industry accounts for 30, 21 and 6% of Irish exports, respectively (Bord Bia, 2017). Ireland has about 6.6 million cattle and 1.6 million pigs (CSO, 2017) which can produce about 36 Mt and 2.5 Mt (FSAI, 2008) of slurry per year, respectively.

The wide range of methane yield has been reported for both cattle (125-240 L CH_4 kg^{-1} VS (Amon et al., 2005; Wall et al., 2013)) and pig slurry (200-417 L CH_4 kg^{-1} VS (Steffen et al., 1998; Triolo et al., 2011)). However, in direct comparison studies the methane yield per kg of VS of cattle slurry was less than pig slurry, while the TS and VS content of cattle slurry were higher (Amon et al., 2005; Kaparaju & Rintala, 2011; Triolo et al., 2013). The lower methane yield of cattle slurries compared to pig slurry was probably due to the inhibitory effects of lignin on the AD of fibre in cattle slurries (Triolo et al., 2013).

The profitability of methane produced from animal slurry is usually low due to critical quality and quantity of its organic pools. The slurry has low biodegradability due to up-concentration of indigestible fractions during animal digestion. Thus, the quantity of digestible organic pools in slurry is often too small to perform economically viable operations (Moller et al., 2007; Triolo et

al., 2011). Methane productivity per unit of feedstock volume is inevitably related to its biochemical and physical composition (Triolo et al., 2013). There are several factors that can affect the methanogenic potential of animal slurry.

2.5.1. Animal diet and animal type

Anaerobic digestibility of animal slurry is markedly influenced by the animal diet and performance (Amon et al., 2007b). Amon et al. (2007b) reported methane yields from dairy cow slurry reduced with increasing feeding intensity and milk yield. The increase in feed conversion decreases the nutrient excretion (Amon et al., 2005) resulting increase in low digestibility lignin content (Amon et al., 2007b). Hellwing et al. (2014) compared methane yields from dairy cows slurry fed with either maize silage with or without supplementation of crushed rapeseed or late cut grass silage only and reported lowest and highest methane from cows fed with grass silage and maize silage with crushed rapeseed, respectively. Triolo et al. (2013) reported slurry from dairy cows (ca. 186 L CH_4 kg^{-1} VS) had 13% less methane yield than beef cattle (ca. 213).

2.5.2. Antibiotics

Antibacterial compounds such as antibiotics are used as feed additives in cattle and pig diets to enhance production or control diseases. The fraction of antibiotics excreted with the slurry may produce toxic substances that could cause inhibition or failure of the AD process. Varel and Hashimoto (1981) reported total inhibition of methane production from slurry from cattle fed with monensin. Similarly, Fischer et al. (1981) also reported a total failure of the AD process from slurry from pig fed with lyncomycin. Lallai et al. (2002) showed amoxicillin and oxytetracycline had little effect while thiamphenicol caused significant reduction in methane

production during AD of pig slurry. Massé et al. (2000) reported presence of penicillin and tetracycline in pig slurry reduced methane production by 35% and 25%, respectively.

2.5.3. Condition and duration of slurry storage

Increase in temperature and storage duration reduces methanogenic potential of animal slurry due to increase in breakdown of its organic matter (Browne et al., 2015). Steed and Hashimoto (1994) reported little effect on methane yield of cattle slurry stored for 5 months at 10 °C while the methane yield reduced by up to 55% when stored at 20 °C. Moller et al. (2004a) and Moller et al. (2004b) reported the reduction in methane yield of slurry stored for 90 days was strongly influenced by storage temperature (15-20 °C). Browne et al. (2015) reported a 26% decrease in methane yield of cattle slurry stored at 20 °C during 26 weeks of storage.

2.6. Anaerobic co-digestion of different AD feedstocks

Anaerobic co-digestion of different feedstocks is not a new concept (Converti et al., 1997; Hills, 1979; Hills & Roberts, 1981), several studies have shown improved methane yield in laboratory trials (Callaghan et al., 1999; Hills & Roberts, 1981; Kaparaju et al., 2002; Lehtomäki et al., 2007; Macias-Corral et al., 2008; Xie et al., 2011), pilot scale studies (Comino et al., 2010; Xie et al., 2017) and full scale AD plant (Amon et al., 2002; Kaparaju et al., 2002) during co-digestion of different feedstocks.

Mono-digestion of feedstocks such as grass silage over an extended duration at significant organic loading rates is prone to process imbalance (Thamsiriroj et al., 2012) probably due to borderline concentration of trace elements while with animal slurries the AD is usually not economically viable due to low TS, VS and methane yields. However, when these feedstocks are

co-digested, complementarity between the chemical and microbiological compositions of forages (high TS and VS content) and animal slurry (rich source of trace elements and higher buffering capacity (Angelidaki & Ellegaard, 2002)) can greatly enhance the longevity of stable and productive methanogenesis (Wall et al., 2014). A successful co-digestion requires balancing several parameters in the co-substrate mixture, such as macro- and micronutrients, carbon to nitrogen (C:N) ratio, pH, inhibitors/toxic compounds, biodegradable organic matter, and soils content (Hartmann et al., 2002; Mata-Alvarez et al., 2000).

2.6.1. Antagonism and synergy during co-digestion

The anaerobic co-digestion of contrasting substrates can result in antagonistic (the mixture produces less yield than predicted from sole substrates) or synergistic (i.e. the mixture produces more methane than the arithmetically calculated yield from sole substrates). The synergistic effects are usually due to the addition of complementary elements to the co-digestion, such as additional alkalinity, trace elements, nutrients or enzymes that a substrate by itself may lack (Labatut & Scott, 2008) while the antagonistic effects can occur due to an imbalance in the C:N ratio (Kayhanian, 1999), excess, deficiency or an imbalance in the ratios of trace elements (Feng et al., 2010), ammonia toxicity, and high VFA concentration (Labatut & Scott, 2008).

2.6.2. Co-digestion of grass and animal slurries

Several researchers have studied co-digestion of grass with cattle or pig slurry and reported both antagonistic and synergistic effects on methanogenesis as shown in Table 2.1. However, in several of these studies the incomplete range of ratios employed prevented calculation of antagonistic or synergistic effects (Table 2.1). Scientific literature on co-digestion of perennial ryegrass silage with cattle or pig slurry is somewhat limited. Wall et al. (2013) reported

antagonistic effects on methane yield during co-digestion of perennial ryegrass silage and cattle slurry while Xie et al. (2011) suggested synergistic effects on methanogenesis during co-digestion of perennial ryegrass silage and pig slurry.

Table 2.1 Selected results from scientific literature reviewing co-digestion of grass and slurry.

Feedstocks	Co-digestion Ratio	Methane yield (L CH_4 kg^{-1} VS)	Reactor	Operating parameters	NLB (%)
Grass silage (GS) and cattle slurry (CS)	GS:CS		CSTR	OLR 0.7	
(Mähnert et al., 2005)	1:0	613*		kg VS m^{-3} d^{-1}	-
Perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis*	0.67:0.33	493*		at 35°C	-7
glomerata) and meadow foxtail (*Alopecurus pratensis*); dairy	0:1	361*			-
farm slurry					
Grass (G) and pig manure (PM)	G:PM		Batch	35°C	
(Dechrugsa et al., 2013)	1:0	522			-
Dried green para grass (*Branchiria mutica*); inoculum from pig	0.75:0.25	453			-1
farm digester	0.5:0.5	383			-2
	0.25:0.75	314			-3
	0:1	257			-
Grass silage (GS) and cattle slurry (CS)	GS:CS		Batch	37°C	
(Wall et al., 2013)	1:0	400			-
First cut perennial ryegrass (*Lolium perenne*) silage; dairy farm	0.8:0.2	345			-7
slurry	0.6:0.4	321			-5
	0.5:0.5	308			-4
	0.4:0.6	273			-11
	0.2:0.8	250			-8
	0:1	239			-
Grass (G) and cow dung (CD) or pig manure	G:CD		Batch	53°C	
(PM)	1:0	226			-
(Poulsen & Adelard, 2016)	0.84:0.16	125			-38
	0.67:0.33	150			-14
	0:1	68			-
	G:PM				
	0.84:0.16	125			-40

	0.73:0.27	149			-24
	0:1	117			
Grass (G) and cow manure (CM)	G:CM		CSTR	OLR 1.8	
(Alvarez & Lidén, 2008)	1:0	15		kg VS m^{-3} d^{-1}	-
Totora (*Schoenoplectus tatora*)	0.5:0.5	149		at 25°C	+173
	0:1	94			-
Grass (G) and cow feces (CF)	G:CF		Batch	35°C	
(Chen et al., 2010)	1:0	138			-
Salt water cord grass (*Spartina alterniflora*)	0.875:0.125	161			+20
	0.75:0.25	177			+35
	0.5:0.5	143			+15
	0.25:0.75	122			+4
	0.125:0.875	115			+1
	0:1	111			-
Grass (G) and cow manure (CM)	G:CM		Batch	37°C	
(Zheng et al., 2015)	1:0	131			-
Dried switch grass; dairy farm manure	0.75:0.25	143			+33
	0.5:0.5	155			+39
	0.25:0.75	134			+18
	0:1	89			-
Grass silage (GS) and cow manure (CM)	GS:CM		CSTR	OLR 2	NA
(Lehtomäki et al., 2007)	1:0	151		kg VS m^{-3} d^{-1}	
75% timothy (*Phleum pratense*), 25% meadow fescue (*Festuca pratensis*) harvested at early flowering stage; dairy farm manure	0.9:0.1	143		at 35°C	
	0.8:0.2	178			
	0.7:0.3	268			
	0.6:0.4	250			
Grass silage (GS) and cow manure (CM)	GS:CS		CSTR	OLR 2	NA
(Jagadabhi et al., 2008)	0.3:0.7	183		kg VS m^{-3} d^{-1}	

75% timothy (*Phleum pratense*), 25% meadow fescue (*Festuca pratensis*); dairy farm manure				at 35°C	
Agricultural residue (AR) and cow manure (CM)	AR:CM		CSTR	OLR 3	NA
(Alkaya et al., 2010)	0.3:0.7	181		kg VS m^{-3} d^{-1}	
30% Clover, 40% grass and 30% wheat straw; dairy farm manure	0:1	175		at 35°C	
Grass silage (GS) and pig manure (PM)	GS:PM		Batch	37°C	NA
(Xie et al., 2011)	1:0	NA*			
Dried perennial ryegrass silage; concentrated pig manure	0.75:0.25	267			
	0.5:0.5	303			
	0.25:0.75	304			
	0:1	280			
Grass (G) and cow manure (CM)	G:CM		Batch	35°C	NA
(Frigon et al., 2012)	0.4:0.6	262			
Mulched switched grass *(Panicum vergatum)*; manure from dairy farm	0:1	316			
Grass (G) and animal slurry (AS)	G:AS		Batch	35°C	NA
(Molinuevo-Salces et al., 2015)	0.85:0.15	243			
Italian ryegrass; 80% cow manure and 20% pig manure	0.15:0.85	210			
Grass (G) or grass silage (GS) and cow dung (CD)	G:CD		Batch	30°C	NA
(Prapinagsorn et al., 2017)	1:1	117			
Napier grass (*Pennisetum purpureum*) or napier grass silage	2:1	141			
	3:1	180			
	4:1	170			
	5:1	117			
	6:1	85			
	GS:CD				
	1:1	142			

	2:1	179			
	3:1	208			
	4:1	202			
	5:1	182			
	6:1	140			
Grass silage (GS) and pig manure (PM)	GS:PM		CSTR	OLR 1.1	NA
(Tsapekos et al., 2017)	0.1:0.9	367		kg VS m^{-3} d^{-1}	
Meadow grass silage	0:1	337		at 55°C	

CSTR: continuously stirred tank reactor; NLB: the deviation of the response (measured value) from the arithmetic mean of the two sole component; NA: not available; OLR: organic loading rate; VS: volatile solids; *: biogas yield and **: reactor failed

2.7. Biochemical methane potential (BMP) test

The BMP test is an anaerobic batch digestion test which is commonly used to determine the biogas and methane yields from organic substrates. Manometric (where the volume is kept constant and an increase in the overhead pressure is used to calculate the amount of gas produced) and volumetric (the pressure is kept constant and the volume of produced gas is measured by a displacement volume device) are the two most commonly used BMP test methods (Valero et al., 2016). There is no single universally accepted standard method to conduct the BMP test although several guidelines are published such as VDI 4630 guideline (2006), Anaerobic Biodegradation, Activity and Inhibition (ABAI) guideline 2009 (Angelidaki et al., 2009) and ABAI guideline 2016 (Holliger et al., 2016). These guidelines recommend both manometric and volumetric methods for the BMP test.

2.7.1. Influence of BMP method on methane yield

The methane yield of a particular feedstock can be impacted by various factors including, but not limited to, inoculum, inoculum to substrate ratio, buffering system, substrate to buffer ratio, operating temperature, duration of the assay and the specific BMP technique employed.

Raposo et al. (2011) reported a wide range of methane yields for cellulose, a relatively homogeneous and industrially synthesized substance, in an inter-laboratory study of 19 participating laboratories. Laboratories using manometric BMP methods reported lower methane yields than those using volumetric BMP methods. Furthermore, McEniry et al. (2014), Nolan et al. (2016) and Wang et al. (2014) also reported a lower methane yield from cellulose using the mBMP method compared to an automated volumetric method i.e. AMPTS (http://www.bioprocesscontrol.com/products/ampts-ii/). Logan et al. (2002) also reported a lower

biogas yield with a manometric method compared to a respirometer (a variation of the volumetric method).

Manometric method is widely used and has been recommended by several guidelines but its parameters e.g. incubation bottle size, maximum pressure limit and OHPMR frequency vary with each guideline. For example, the VDI 4630 guideline (2006) recommends an incubation bottle size of 500 - 2000 ml for homogeneous substrates and 10 l - 20 l for heterogeneous substrates whereas Holliger et al. (2016) recommend an incubation bottle size of 100 ml for homogeneous substrates and 500 - 2000 ml for heterogeneous substrates. Both these guidelines have no direct recommendation for the OHPMR frequency but identify a maximum overhead pressure 100 hPa (VDI 4630 guideline, 2006) and 3000 hPa (Holliger et al., 2016) that should not be exceeded during the BMP test. In mBMP method, change in headspace volume of the incubation bottle and/or the frequency of pressure release associated with the OHPMR frequency regime adopted can alter the overhead pressure affecting methane yields. Yilmaz (2015) reported lowering of the headspace pressure resulted in enhanced biogas yield for glucose while Valero et al. (2016) suggested that the influence of overhead pressure on methane yield varied with the substrate used. Thus, headspace volume of incubation bottle and OHPMR frequency are two important parameters of mBMP that can influence the methane yield. However, these important descriptive details are not always provided in anaerobic batch digestion tests.

2.8. Provision cost of grass as AD feedstock

The costs of grass as AD feedstock comprise of the production cost which includes costs for grassland management (fertilization, re-seeding, mechanical treatment, water table control), for

harvest (mowing, swath treatment, clearing, transport), for storage and handling (placing into silo and compaction, storing, removing from silo, feeding into biogas plant) and overhead costs (tenure, taxes, insurances, etc.) plus supply costs which mainly depend on yields, intensity of management, transport distances and field conditions for machinery use (field size, trafficability, slope, etc.) (Prochnow et al., 2009).

2.8.1. Grange feed cost model (GFCM)

The GFCM is developed by Teagasc, Ireland to identify the relative costs of feeds produced for ruminants (Finneran et al., 2010). It is a static, spreadsheet-based, agro-economic simulation model for evaluation of the physical and financial performance of alternative feed crop production and utilization options in Ireland. The GFCM employs a full bottom-up costing approach to calculate total feedstock cost and includes all fixed and variable production (e.g. sowing and crop management) and utilization (e.g. storage and labour) costs associated with the feedstock. The steps to calculate the feed costs in GFCM are shown in Figure 2.2. GFCM employs a deterministic approach for modelling feed crop cost during a single year. The default values for agricultural parameters e.g. sowing dates, field operations, and harvest and utilization options are relevant to Irish conditions (Finneran et al., 2010).

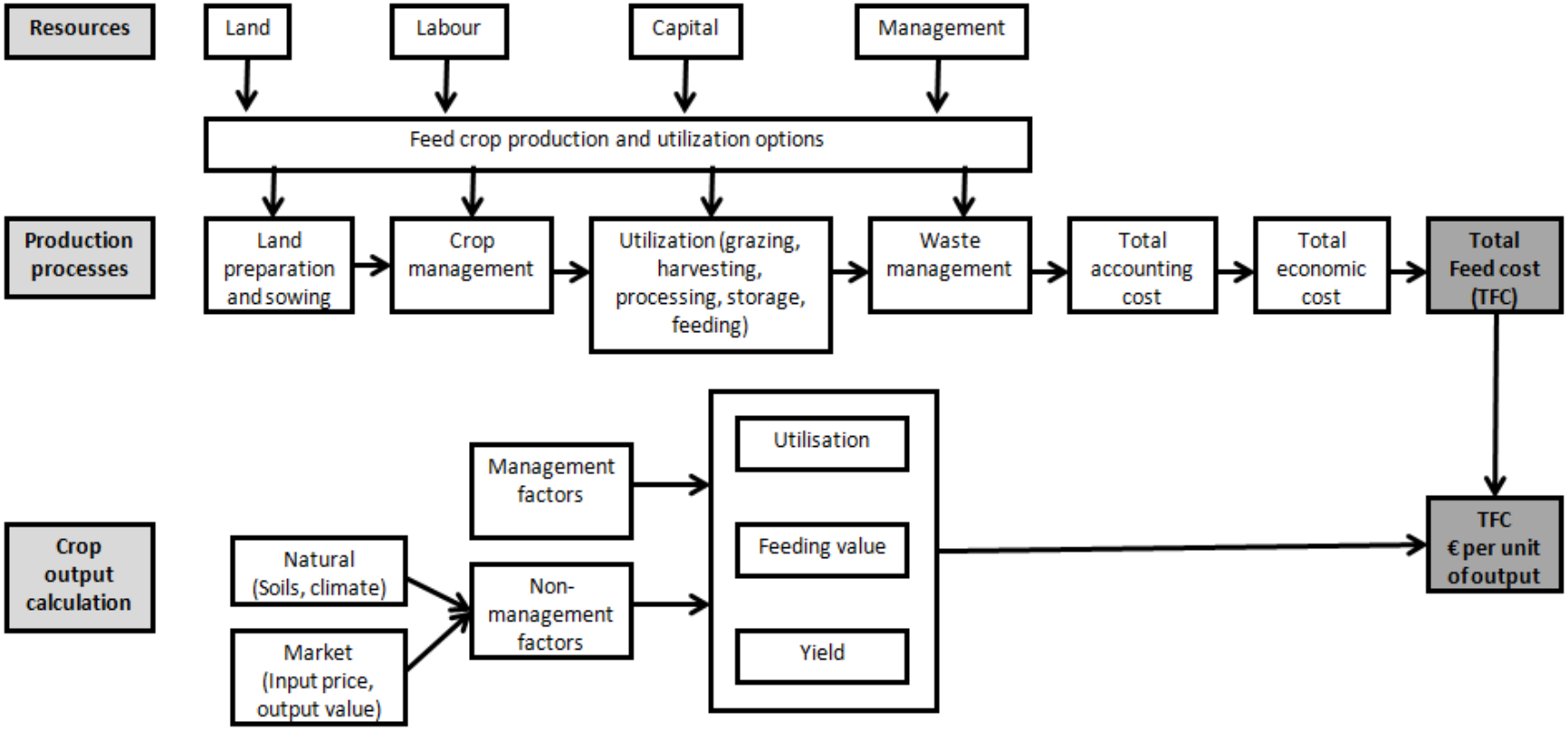


Figure 2.2. Steps involved in Grange feed costing model (GFCM) to calculate the cost of a particular feed (Finneran et al., 2010).

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3. Synergies from co-digesting grass or clover silages with cattle slurry in *in vitro* batch anaerobic digestion

Synergies from co-digesting grass or clover silages with cattle slurry in *in vitro* batch anaerobic digestion

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Abstract

Co-digestion of forage silage with cattle slurry can greatly extend the stability of methanogenesis as compared to mono-digestion of the silage. Biogas and methane yields of the mixtures of perennial ryegrass silage (at two growth stages) with cattle slurry and of red clover silage (at two growth stages) with cattle slurry were measured, and synergistic effects were investigated. Silage and slurry were incubated as sole substrates or as part of binary mixtures (forage silage:cattle slurry ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.75:0.25 and 0:1 on a volatile solid basis). The maximum measured synergistic effects for perennial ryegrass silages with cattle slurry and red clover silages with cattle slurry were observed at 0.75:0.25 and 0.5:0.5 (forage silge:cattle slurry), respectively. The forage silage:cattle slurry ratio to produce the maximum synergistic effects differed with the forage species ensiled and its growth stage when harvested.

3.1. Introduction

In Ireland over 90% of the agricultural land is under grassland (CSO, 2013). Mean yields of biomass from this grassland are relatively high, and the potential exists to greatly increase yields

so they remain in excess of current or expected livestock requirements (McEniry et al., 2013a). The 7 million cattle (CSO, 2014) currently utilising this grassland spend about one-third of each year indoors and therefore produce a substantial amount of manure primarily in the form of slurry. Although the latter is used as a fertiliser and soil conditioner, this important function would not be compromised by its use for methanogenesis prior to landspreading.

Perennial grasses and forage legumes are commonly conserved by ensiling in northern Europe, with perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.) being widely used examples of these grassland herbages. In both cases, but particularly with grass, growth stage at harvesting will significantly alter the herbages chemical composition and thus impact on the relative ease with which microbial enzymes can hydrolyse its fibre components during anaerobic digestion (Buxton, 1996; Carpita, 1996; Lewis & Davin, 1998; McEniry et al., 2013b). Ultimately this will strongly influence the rate and extent of methanogenesis that will occur (Hendriks & Zeeman, 2009; Nizami & Murphy, 2009).

Although the lower total solids (TS) and volatile solids (VS) concentrations of cattle slurry compared to grass or legume silages result in reduced methane output when expressed on a substrate fresh weight basis, forages such as grass silage are prone to process imbalance when mono-digested over an extended duration at significant organic loading rates (Thamsiriroj et al., 2012). Complementarity between the chemical and microbiological compositions of silages made from forages (e.g. high carbon to nitrogen (C:N) ratio; borderline concentrations of some minerals or trace elements; marginal buffering capacity) and cattle slurry (e.g. elevated NH_3 concentration; rich source of some minerals or trace elements; stabilising buffering capacity; source of some micro-organisms beneficial to anaerobic digestion) can greatly enhance the longevity of stable and productive methanogenesis when these substrates are co-digested.

Furthermore, some of these balancing effects have the potential to result in a synergistic outcome with reduced risk of factors such as pH instability, NH_3 inhibition and limiting C:N ratios. The synergistic effects have been reported for grass with sewage sludge (Wang et al., 2014), municipal solid waste with cow manure (Macias-Corral et al., 2008) and solid slaughterhouse wastes with agri-residue (Pagés-Díaz et al., 2014). Such synergistic effects are most often associated with co-digestion of substrates of quite contrasting C:N ratio (Allen et al., 2013). However, information on synergistic effects when forage silage is co-digested with cattle slurry is limited (Wall et al., 2013).

The innovation in this paper is that it is the first to study biogas and methane yields arising from the co-digestion of perennial ryegrass silage (harvested at two growth stages, PRG1 and PRG2) or red clover (harvested at two growth stages, RC1 and RC2) with cattle slurry and to assess synergistic effects. This involved digestion of forage silage with cattle slurry in binary mixture ratios of 0:1, 0.25:0.75, 0.5:0.5, 0.75:0.25 and 1:0 (VS basis).

3.2. Material and methods

3.2.1. Substrates

Six field plots of perennial ryegrass (PRG; *Lolium perenne* L., var. Gandalf) and of red clover (RC; *Trifolium pratense* L., var. Merviot) were grown at Teagasc Grange (53°30'N, 6°40'W, 83 m above sea level), and three plots per species were harvested at each of two dates in the primary growth (11 May and 6 July) as reported by King et al. (2012b). The growth stages of PRG were 2.4 and 3.8 (Moore et al., 1991) and of RC were 3.1 and 7.4 (Ohlsson & Wedin, 1989) (see footnotes in Table 3.1 for the explanation of growth stages). The silages from these forage

samples were prepared in laboratory silos without field-wilting or application of additives, for a period of 100 d at 15°C, as described in McEniry et al. (2013b). The silage samples were dried at 40°C for 48 h in an oven with forced air circulation and then milled (Wiley mill; 1 mm pore screen). These dried, milled samples were used for the biochemical methane potential (BMP) assay and substrate chemical analysis.

The cattle slurry sample was collected from an underground tank in a roofed slatted-floor cattle building at Teagasc Grange. It was produced by beef cows consuming grass silage *ad libitum* and consisted of faeces and urine. The collected cattle slurry was thoroughly mixed and stored at -20°C until it was assessed in the BMP assay.

The inoculum was sourced from Agri-Food and Biosciences Institute in Hillsborough, Co. Down, Northern Ireland. This on-farm anaerobic digestion (AD) facility utilize grass silage and cattle slurry as its feedstocks. The inoculum was de-gassed in anaerobic conditions in an incubator for 5 d at 38°C and then it was filtered through a 2 mm pore sieve under a continuous flow of CO_2.

3.2.2. Substrate chemical analysis

The TS of cattle slurry and inoculum, and VS of forage silage, cattle slurry and inoculum were measured according to Standard Methods 2540 G (APHA/AWWA/WEF, 2005). The chemical characteristics i.e. acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF; assayed with heat-stable amylase and sodium sulphite) of the dried milled forage samples were based on the analytical method of Van Soest (1994). In brief, ADF, ADL and NDF were determined using the filter-bag technique (Ankom, 2006a; Ankom, 2006b) with an ANKOM fibre analyser (ANKOM Technology, Fairport, NY, USA) which have been described

in detail by King et al. (2012b). The C:N ratio of forage silage and cattle slurry was determined using a LECO CN 2000 (Leco Corporation, St. Joseph, MI, USA). The TS, VS and other chemical properties of forage silage, cattle slurry and inoculum are presented in Table 3.1. Further chemical properties of forage silages are presented in Table A.1 (Appendix A).

Table 3.1. Chemical properties of perennial ryegrass (PRG) and red clover (RC) silages, cattle slurry and inoculum. Volatile solids (VS), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL). [1]From McEniry et al. (2013b).

	Growth stage[1‡]		VS (g kg^{-1} TS)	NDF[1] (g kg^{-1} TS)	ADF[1] (g kg^{-1} TS)	ADL[1] (g kg^{-1} TS)	C:N (g g^{-1})
PRG	PRG1	2.2	901	400	260	14	14.1
	PRG2	3.8	936	623	376	40	23.2
RC	RC1	1.0	892	330	270	28	12.2
	RC2	7.0	893	439	347	64	32.3
Cattle slurry	-	-	783	-	-	-	8.7
Inoculum	-	-	677	-	-	-	-

‡ Growth stage of PRG was determined according to Moore et al. (1991) where stage 2.0 to 2.9 = elongation - stem elongation and stage 3.0 to 3.9 = reproductive - floral development; growths stage of red clover was determined according to Ohlsson and Wedin (1989) where stage 1.0 = mid-vegetative stage and 7.0 = early seed-pod development.

3.2.3. Batch digestion test

The three experimental replicate samples of each of the four dried milled forage silage were individually weighed into forage silage:cattle slurry ratios (VS basis) of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1. The *in vitro* batch digestion tests were conducted in triplicate (i.e. analytical replicates) as described in McEniry and O'Kiely (2013) which follows the VDI 4630 guideline (2006). The inoculum and substrate were added to the incubation bottles (160 ml total; 70 ml working volume and 90 ml headspace) at a 2:1 VS inoculum-to-substrate gravimetric ratio to provide an organic loading of 10 g VS kg^{-1} total medium. Micro- and macro-mineral solutions were also added to prevent nutrient limitation (McEniry & O'Kiely, 2013). Six replicate bottles

of blank (i.e. without forage silage or cattle slurry) and positive control replicates (cellulose, Sigma, 22184) were also prepared. All bottles were flushed with N_2 gas for 1 minute and sealed with butyl rubber stoppers and aluminium crimp caps and were incubated at 38°C for 45 d. All bottles were mixed daily by manual swirling. A detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK) with Vaseline® lubricated needle was used to measure the headspace pressure on days 3, 6, 10, 12, 15, 19, 26, 35 and 45 of the incubation. The biogas produced was estimated using the equation:

$$\text{Gas produced (ml)} = \left(\frac{\text{vh}}{\text{Pa}}\right).\text{Pt} \qquad \text{(Equation 3.1)}$$

where, vh is the headspace volume (ml), Pa is the atmospheric pressure (hPa) and Pt is the gas headspace pressure (hPa).

0.8 ml of sample gas was used to determine the methane concentration using an automated gas chromatograph (Shimadzu GC-2014) with a flame-ionisation detector and a glass column (2.1 m × 5.0 mm × 3.2 mm packed with molecular sieve 5A 60/80 mesh). The temperatures were 120°C, 150°C and 170°C in the column, injector and detector, respectively, and hydrogen was the carrier gas (Bodas et al., 2008; Lovett et al., 2006). The methane and biogas yield data was corrected for inert gas on day 3 only, for blank samples (inoculum-induced gas); and for standard temperature and pressure (273K, 1013 hPa) conditions.

3.2.4. Kinetics

The kinetic parameters were calculated as described by Wall et al. (2013) using Matlab® R2009a software. The decay constant or k-value for both biogas and methane was determined using first-order kinetics.

First order kinetics equation

$$y(t) = y_m x(1 - e^{(-kt)}) \quad \text{(Equation 3.2)}$$

where, y(t) is the cumulative specific methane yield at time t (L CH_4 kg^{-1} VS), y(m) is the specific methane yield at the end of the 45 d batch test (L CH_4 kg^{-1} VS), t is the time (d; days) and k is the first order decay constant (d^{-1}).

The lag phase (λ), half-life (T_{50}) and maximum production rate (U) for both biogas and methane were calculated using second-order kinetics

Second order kinetics equation

$$y = y_{max}.\exp\left\{-\exp\left[U.\frac{e}{y_{max}}.(\lambda - t) + 1\right]\right\} \quad \text{(Equation 3.3)}$$

where, y is the cumulative specific methane yield (L CH_4 kg^{-1} VS), y_{max} is the predicted specific methane yield at the end of the 45 d batch test (L CH_4 kg^{-1} VS), U is the maximum specific methane production rate (L CH_4 kg^{-1} VS day^{-1}), λ is the lag phase (d) and t is the time (d). λ_T i.e. duration of exponential phase (d) was calculated by linear approximation of the fitted curve using the equation 3.4.

$$\lambda_T = \frac{y_{max} - y_\lambda}{U} \quad \text{(Equation 3.4)}$$

where, y_λ is the cumulative specific methane yield (L CH_4 kg^{-1} VS) at the end of lag phase.

3.2.5. Statistical analysis

The averaged values of methane and biogas yield data from triplicate analytical measurements were analysed using the MIXED procedure in SAS, Version 9.3. A regression equation i.e.

Equation 3.5 was used to fit the characteristic curves of the mixtures in response to the proportions of forage silage and cattle slurry(Purcell et al., 2012).

$$y = \beta_{FS}X_{FS} + \beta_{CS}X_{CS} + \beta_{FSCS}X_{CS}X_{FS} + \delta_{FSCS}X_{CS}X_{FS}(X_{FS} - X_{CS}) \quad \text{(Equation 3.5)}$$

where the X_{FS} and X_{CS} variables are the proportions of forage silage and cattle slurry, respectively, in the mixtures, while β_{FS}, β_{CS}, β_{FSCS} and δ_{FSCS} are coefficients for pure forage component, pure slurry component, nonlinear blending (NLB) component and asymmetry in the response curves component, respectively. Thus, for example, $\beta_{FSCS}X_{FS}X_{CS}$ term, at a forage silage:cattle slurry ratio of 0.5:0.5, describes the deviation of the response from the arithmetic mean of the two sole component responses (NLB; synergistic (+) or antagonistic (-) associations).The $\delta_{FSCS}X_{CS}X_{FS}(X_{FS} - X_{CS})$, at forage silage:cattle slurry ratios of 0.25:0.75 and 0.75:0.25, describes the asymmetry in the response curves to occur (i.e. it allows NLB deviations at forage silage:cattle slurry ratios of 0.75:0.25 and 0.25:0.75).

3.3. Results and discussion

The forages used in this study were selected to represent the two main botanical families found in north-western European permanent grassland (perennial ryegrass – family Poaceae; red clover – family Fabaceae) and by using samples obtained at contrasting growth stages to encompass the recalcitrant effects of lignin on their digestion. Collectively the aim was that this diverse set of forages should broaden the conditions under which the linearity of methane output in response to co-digestion in a series of ratios with cattle slurry would be assessed.

The results summarised in Table 3.1 show that the estimated concentrations of the fibre components hemicellulose (NDF-ADF), cellulose (ADF-ADL) and lignin (ADL) increased with advancing growth stage, in agreement with Bosch et al. (1992); King et al. (2012a) and King et al. (2012b). In general, as the concentration of lignin increases within a species the extent to which it impedes microbial hydrolysis of hemicellulose and cellulose increases (Carpita, 1996; Lewis & Davin, 1998; Nizami & Murphy, 2009; Prochnow et al., 2009). Finally, the increase in C:N with advancing growth stage of both species likely reflects the expected decline in crude protein concentration as these forages become more fibrous (Bosch et al., 1992; King et al., 2012a; King et al., 2012b).

The greater concentration of hemicellulose in perennial ryegrass than red clover at both the less (140 vs. 60 g kg^{-1} TS) and more (247 vs. 92 g kg^{-1} TS) mature growth stages, and the absence of or smaller differences in cellulose concentration (246 vs. 242 and 336 vs. 283 g kg^{-1} TS, respectively) were as expected (Bosch et al., 1992; King et al., 2012a; King et al., 2012b). Similar to the results shown in Table 3.1, Van Soest (1994) reported generally greater lignin concentrations in clovers than grasses, although the recalcitrant effects of lignin on fibre digestibility can be less severe with clovers (Buxton, 1996).

Table 3.2. The effect of perennial ryegrasses (PRG 1 and 2), red clovers (RC 1 and 2) or cattle slurry and the associated non-linear blending (NLB) at a forage silage:cattle slurry ratio of 0.5:0.5.

	PRG1	NLB	PRG2	NLB	RC1	NLB	RC2	NLB	Cattle slurry
Biogas									
L kg^{-1} VS	513.1	$+8.4^{NS}$	469.1	+46.8***	476.9	+23.6***	433.0	$+10.7^{NS}$	454.1
λ	1.9	$+0.3^{NS}$	2.4	-0.6*	1.9	-0.4^{NS}	2.4	-0.1^{NS}	3.2
λ_T	10.9	-0.301^{NS}	9.4	+1.933***	11.8	-0.686^{NS}	10.3	-1.354***	11.3
k	0.106	-0.003^{NS}	0.094	-0.003^{NS}	0.093	+0.009**	0.107	+0.009**	0.080
U	46.1	-0.9^{NS}	41.4	-0.9^{NS}	35.1	+4.8***	41.1	+4.8***	36.1
T_{50}	7.6	$+0.26^{NS}$	8.0	$+0.26^{NS}$	8.4	-0.7***	7.6	-0.7***	9.2
CH_4 % (vol. $vol.^{-1}$)	61.3	$+1.074^{NS}$	60.4	-1.454^{NS}	60.1	-1.461^{NS}	59.2	+2.713***	62.3
Methane									
L kg^{-1} VS	317.5	+9.4** (1.0, 13.0)	285.8	+20.4*** (9.3, 21.3)	286.7	+8.1* (7.4, 4.8)	255.0	+20.1*** (16.4, 13.8)	281.9
λ	3.5	$+0.117^{NS}$	3.6	-0.223*	4.5	-0.158^{NS}	3.9	-0.497***	4.5
λ_T	13.2	$+0.209^{NS}$	12.7	+1.360**	12.2	-0.425^{NS}	11.8	-1.028**	13.4
k	0.077	-0.003**	0.071	-0.003**	0.064	+0.005**	0.084	+0.005**	0.063
U	23.7	-0.3^{NS}	19.5	-0.3^{NS}	18.7	+2.7***	20.6	+2.7***	19.3
T_{50}	10.3	$+0.3^{NS}$ (0.03, 0.44)	10.7	+0.59* (0.23, 0.65)	11.8	-0.63* (-0.68, -0.26)	10.6	-1.38*** (-1.25, -0.26)	11.5

* - $P<0.05$,** - $P<0.01$, ***-$P<0.001$, NS - not significant. k: the first order decay constant (d^{-1}); U: the maximum specific methane or biogas production rate (L CH_4 or biogas kg^{-1} VS day^{-1}); λ: the lag phase (d); T50: half-life i.e. time taken (days) to produce 50% of the gas production; and λ_T: the duration of the exponential phase (days). The NLB mean is the deviation of the response from the arithmetic mean of the sole component responses. The sign of the NLB mean indicates whether the NLB was a synergistic (+) or antagonistic (–) effect. The values within brackets () are only mentioned where asymmetry in the NLB was observed at forage silage:cattle slurry ratios of 0.75 : 0.25 and 0.25 : 0.75, and the mean values reported are the synergistic (+) or antagonistic (-) deviations at these ratios, respectively.

The greater output of both biogas and methane from the mono-digestion of perennial ryegrass compared to red clover agrees with Lehtomäki (2006) when comparing reed canary grass and red clover. Allowing herbages advance to more mature growth stages usually reduces their subsequent methane output (McEniry & O'Kiely, 2013), reflecting the inhibitory effects of increased lignification as growth stage advances. This outcome was observed equally with both herbage species in the present study. This similar species response to advancing growth stage is interesting as it occurred despite larger increases in hemicellulose and cellulose and a smaller increase in lignin for the perennial ryegrass than the red clover. Hence, these relatively conventional indices for describing changes in herbage chemical composition may not always reliably reflect changes in methanogenesis during *in vitro* AD.

The biogas yield, methane yield and the kinetic parameters of sole components and their binary mixtures are presented in Table 3.2. The methane yield for sole component PRG1 is at the lower end of the values reported by Wall et al. (2013). This could reflect Wall et al. (2013) using undried silages compared to the oven dried silages in this study. Dried silage would have lost some fermentation products during oven drying (McEniry et al., 2014). McEniry and O'Kiely (2013) used the same grass silage sample set as this study but reported lower methane yields (263 and 229 L CH_4 kg^{-1} VS for PRG1 and PRG2, respectively, vs. corresponding values of 318 and 286 L CH_4 kg^{-1} VS in this study). The differences between these studies appears to be due to lower microbial activity (259 vs. 343 L CH_4 kg^{-1} cellulose VS, representing 69 and 80% of the theoretical yield, respectively) or a shorter duration of AD (35 vs. 45 d) by McEniry and O'Kiely (2013). However, as indicated above, the growth stage of PRG silage had a similar effect on methane yields in both studies.

Although as a generalisation it can be expected that methane production will be greater from the AD of grass or clover silages compared to slurry produced by cattle consuming that silage as their sole dietary input, methane output from the AD of cattle slurry will differ depending on a range of factors including the type of cattle (Triolo et al., 2013), diet type (Amon et al., 2006; Hellwing et al., 2014), dilution with other materials or the presence of antibiotics (Hashimoto et al., 1981; Triolo et al., 2011) and the duration and conditions of slurry storage (Browne et al., 2015). Hence, the greater output of both biogas and methane from cattle slurry than RC2 in the current study evidently reflects a relatively high methanogenic potential of the VS in this cattle slurry. The latter was obtained from a tank beneath beef cows consuming different silage than subjected to AD in this study.

The approach of using NLB was employed to objectively quantify the extent to which there was or was not a linear response in methane output to co-digestion of forage silage across a series of ratios with cattle slurry. A linear response would indicate that the actual methane output from the co-digestion of some ratio of forage silage:cattle slurry was directly predictable from their proportional contribution of methane output when incubated as sole ingredients. Where the response was not linear (i.e. where NLB was statistically significant) the positive or negative deviation from the linear prediction could be either symmetrical (necessarily greatest at a 0.5:0.5 ratio and returning at similar rates above and below this ratio towards the sole component outputs) or asymmetrical (not necessarily greatest at a 0.5:0.5 ratio and returning at different rates above and below the ratio of maximum methane output towards the sole component outputs). The four coefficients (i.e. $\beta_{FS,}$ $\beta_{CS,}$ β_{CSFS} and δ_{CSFS}) of the NLB model which can be used to calculate the value of a methane yield at a given slurry:silage ratio are presented in Table A.2 (Appendix A). A major finding of the current study is that each of the four forage silages

investigated produced significantly more methane when co-digested with cattle slurry at a VS ratio of 0.5:0.5 than would have been predicted from the sole ingredients. This synergistic effect (+ 3.1, 7.2, 2.8 and 7.5% for PRG1, PRG2, RC1 and RC2, respectively) was relatively similar for perennial ryegrass and red clover, but in both cases was markedly greater when the silage had been harvested at the more mature growth stage. Furthermore, the asymmetry that occurred in the NLB effect again reflected the trend for higher values (at forage silage:cattle slurry ratios of both 0.75:0.25 and 0.25:0.75) at the more mature growth stage for both forage species. However the nature of the asymmetry differed between the silages made from perennial ryegrass and red clover. At both growth stages of perennial ryegrass, the magnitude of the synergistic effect was lesser at 0.75:0.25 than at 0.25:0.75, and with the latter ratio in each case giving a numerically greater synergistic effect on methane output than recorded for the 0.5:0.5 ratio. In contrast, when red clover at either growth stage was co-digested with cattle slurry the synergistic effect was numerically greater at 0.75:0.25 than at 0.25:0.75, and both ratios gave numerically smaller synergistic effects on methane output than recorded for the corresponding 0.5:0.5 ratios. A larger number of ratios of forage silage: cattle slurry than were employed would have allowed a more accurate identification of the ratio at which the maximum synergy in methane output occurred. Overall, however, the outcomes discussed above suggest quite complex chemical and microbiological relationships between each of the forage silage (as influenced by species and growth stage when harvested) and those of the cattle slurry and the inoculum, under the prevailing test conditions.

Whereas the total output of methane per unit VS incubated is the index of primary interest, the proportion of biogas composed of methane also has commercial significance (Nachtmann et al., 2015). The values of 59 to 62% reported in this study were markedly greater than Dandikas et al.

(2014) reported for non-ensiled clover (52%) and grass (51%). Triolo et al. (2011) also reported a lower methane proportion of 54% to 56% for perennial grass but their methane proportion for cattle slurry was similar to this study.

Contrasting patterns of methane production occurred with each of the sole component substrates (i.e. standard cellulose, four forage silages, cattle slurry) (see second order kinetics curves fitted in Figure 3.1). PRG1 had a greater decay constant (k-value), a shorter lag phase (λ) and a longer exponential phase (λ_T) than PRG2 resulting in higher biogas and methane yields for PRG1 compared to PRG2. The greater output of methane with PRG1 than RC1 reflected its greater k, λ_T and U values and its shorter λ. The greater methane yield with RC1 compared to RC2, despite its lower k-value and greater λ_T, λ, and T_{50} values, can be explained from Figure 3.1 by RC1 continuing to produce methane after its exponential phase (after day 24) and during which time RC2 produced negligible methane. A similar pattern of differential extended methanogensis can be observed when comparing PRG2 and RC.

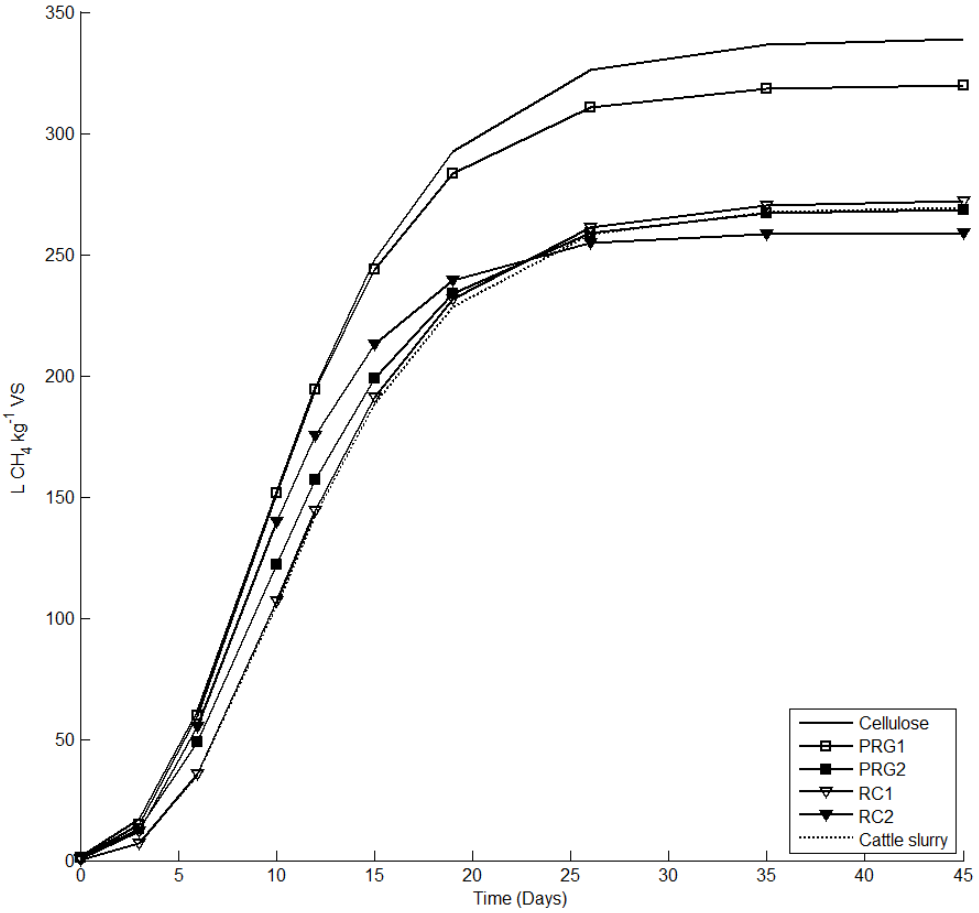


Figure 3.1. Fitted curves for cellulose, perennial ryegrass silage (PRG1 and PRG2), red clover silage (RC1 and RC2) and cattle slurry using second order kinetics.

3.4. Conclusion

Even though forage silage species and growth stage at harvest resulted in contrasting biogas and methane yields, co-digesting any of these silages with cattle slurry resulted in synergistic methanogenesis. The forage silage:cattle slurry ratio to produce the maximum synergistic effects differed with the forage species ensiled and its growth stage when harvested.

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4. Antagonistic effects on biogas and methane output when co-digesting cattle and pig slurries with grass silage in *in vitro* batch anaerobic digestion

Antagonistic effects on biogas and methane output when co-digesting cattle and pig slurries with grass silage in *in vitro* batch anaerobic digestion

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Abstract

Anaerobic co-digestion of contrasting substrates can result in synergistic or antagonistic effects on methanogenesis. Biogas and methane yields of the mixtures of cattle slurry (CS1 and CS2) or pig slurry with grass silages (GS1 and GS2) were measured using *in vitro* anaerobic batch digesters, and synergistic and antagonistic effects were investigated. Slurries and silages were incubated as individual substrates or as part of binary mixtures (slurry:silage mass ratios of volatile solids (VS) of 1:0, 0.75:0.25, 0.5:0.5, 0.75:0.25 and 0:1).The biogas yields of CS1, CS2, pig slurry, GS1 and GS2 were 405.9, 380.4, 550.8, 673.7 and 610.6 L kg^{-1} of VS, respectively while the corresponding methane yields were 269.1, 246.4, 380.1, 427.7 and 359.0 L kg^{-1} of VS. The sequential replacement of either cattle slurry by either grass silage caused a progressive increase in biogas and methane yields, but there was not as clear-cut increase when pig slurry was replaced by grass silages. The methane yield for slurry and silage mixtures displayed non-linear blending and the maximum effect, which was always antagonistic, was at a 0.5:0.5 mass ratio of VS, and ranged from 5.7-7.6% below the yields predicted from mono-digestion of individual substrates.

Abbreviations

AA: Acetic acid; AD: Anaerobic digestion; ADF: Acid detergent fibre; ADL: Acid detergent lignin; AFBI: Agri-Food and Biosciences Institute; AR: Agricultural residue; AS: Animal slurry; BA: Butyric acid; BMP: Biochemical methane potential; BS: Beef cow slurry; C:N: Carbon:Nitrogen; CD: Cow dung; CF: Cow faeces; CM: Cow manure; CP: Crude protein; CS1: Cattle slurry 1; CS2: Catttle slurry 2; CSTR: Continuously stirred tank reactor; DM: Dairy cow manure; DS: Dairy cow slurry; Eth: Ethanol; G: Grass; GS: Grass silage; GS1: Grass silage 1; GS2: Grass silage 2; k : First order decay constant; LA: Lactic acid; NA: Not available; NDF: Neutral detergent fibre; NH_3-N: Ammonia-nitrogen; NLB: Non-linear blending; OLR: Organic loading rate; P: Level of significance; PM: Pig manure; PS: Pig slurry; T_{50}: Time taken to produce 50 % of the gas production (Half-life); TS: Total solids; TSD: Total solids digestibility; U: Maximum methane or biogas production rate; VFA: Volatile fatty acids; VS: Volatile solids; WSC: Water-soluble carbohydrates; λ: Lag phase and λ_T: Exponential phase

4.1. Introduction

The on-farm anaerobic digestion (AD) plants in some European countries utilize energy crops such as maize but the Irish climate limits reliable economic production of maize. However, cattle slurry, pig slurry and grass silage are three major potential AD substrates in Ireland. For example, the 6.6 million cattle and 1.6 million pigs (CSO, 2017) produce about 36 Mt and 2.5 Mt (FSAI, 2008) of slurry per year, respectively. Over 90% of the Irish agricultural land is under grassland (CSO, 2013), and its relatively high yield results in an estimate of 1.7 Mt of grass total solids per year in excess of livestock requirements. With intensive grassland management, there is potential to increase this to 12.2 Mt y^{-1} (McEniry et al., 2013).

Cattle slurry usually supports a lower methane yield compared to energy crops or grass silage as the livestock have already utilised much of the more easily digestible organic components in the feeds (Triolo et al., 2013). When expressed on a volatile solids (VS) basis, pig slurry can produce higher methane yields than cattle slurry, but pig slurry often has a lower VS mass fraction (Amon et al., 2005; Kaparaju & Rintala, 2011; Moller et al., 2004; Triolo et al., 2013). Furthermore, a challenge with AD of grass silage relates to a risk of process imbalance when mono-digested over an extended duration at high organic loading rates (Thamsiriroj et al., 2012). Thus, the co-digestion of animal slurry (rich source of trace elements and stabilising buffering capacity) with grass silage (more easily digestible organic content, borderline trace elements concentrations and marginal buffering capacity) could complement each other and greatly enhance the longevity of stable and productive AD.

The anaerobic co-digestion of contrasting substrates can result in synergistic (i.e. the mixture produces more methane than the arithmetically calculated yield from individual substrates) or antagonistic (the mixture produces less yield than predicted from individual substrates) effects. The synergistic effects are usually due to the addition of complementary elements to the co-digestion mixture, such as additional alkalinity, trace elements, nutrients or enzymes that a substrate by itself may lack (Labatut & Scott, 2008) while the antagonistic effects can occur due to an imbalance in the C:N ratio (Kayhanian, 1999), excess, deficient or an imbalance in the ratios of trace elements (Feng et al., 2010), ammonia toxicity, and high VFA concentration (Labatut & Scott, 2008).

Although several researchers have studied co-digestion of cattle or pig slurry with different grass or grass silage and reported both antagonistic and synergistic effects (Table 4.1), in some studies the incomplete range of binary mixture ratios employed prevented calculation of synergistic or

antagonistic effects (Table 4.1). The co-digestion of cattle slurry and perennial ryegrass has contradictory results with both antagonistic (Wall et al., 2013) and synergistic (Himanshu et al., under review) effects on methane yields while there is limited information on co-digestion of pig slurry and perennial ryegrass (Xie et al., 2012).

The aim of this experiment was to quantify the antagonistic or synergistic effects of co-digesting cattle slurry (two types) or pig slurry with two contrasting perennial ryegrass silages using *in vitro* batch digestion. This involved digestion of each slurry with each silage in binary mixture mass ratios of VS of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1.

Table 4.1. Summary of methane yields from published comparisons of animal slurry with grass or grass silage.

Substrates	Co-digestion ratio	Methane yield (L CH_4 kg^{-1} of VS)	Reactor	Operating parameters	NLB (%)
Dairy cow slurry (DS) and grass silage (G)	DS:G		CSTR	OLR 0.7	
(Mähnert et al., 2005)	1:0	361*		kg of VS m^{-3} d^{-1}	-
Perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*)	0.33:0.67	493*		at 35°C	-7
and meadow foxtail (*Alopecurus pratensis*) silage	0:1	613*			-
Pig manure (PM) and grass (G)	PM:G		Batch	35°C	
(Dechrugsa et al., 2013)	1:0	257			-
Dried green para grass (*Branchiria mutica*); inoculum from pig farm	0.75:0.25	314			-3
digester	0.5:0.5	383			-2
	0.25:0.75	453			-1
	0:1	522			-
Dairy cow slurry (DS) and grass silage (GS)	DS:GS		Batch	37°C	
(Wall et al., 2013)	1:0	239			-
First cut perennial ryegrass (*Lolium perenne*) silage	0.8:0.2	250			-8
	0.6:0.4	273			-11
	0.5:0.5	308			-4
	0.4:0.6	321			-5
	0.2:0.8	345			-7
	0:1	400			-
Cow dung (CD) or pig manure (PM) and grass (G)	CD:G		Batch	53°C	
(Poulsen & Adelard, 2016)	1:0	68			-
	0.67:0.33	150			-14
	0.84:0.16	125			-38
	0:1	226			-
	PM:G				
	1:0	117			-
	0.73:0.27	149			-24
	0.84:0.16	125			-40
Cow manure (CM) and grass (G)	CM:G		CSTR	OLR 1.8	
(Alvarez & Lidén, 2008)	1:0	94		kg VS m^{-3} d^{-1} at	-

Totora (*Schoenoplectus tatora*)	0.5:0.5	149		25°C	+173
	0:1	15			-
Cow faeces (CF) and grass (G)	CF:G		Batch	35°C	
(Chen et al., 2010)	1:0	111			-
Salt water cord grass (*Spartina alterniflora*)	0.875:0.125	115			+1
	0.75:0.25	122			+4
	0.5:0.5	143			+15
	0.25:0.75	177			+35
	0.125:0.875	161			+20
	0:1	138			-
Dairy cow slurry (DS) and grass (GR)	DS:GR		Batch	37°C	
(Zheng et al., 2015)	1:0	89			-
Dried switch grass	0.75:0.25	134			+18
	0.5:0.5	155			+39
	0.25:0.75	143			+33
	0:1	131			-
Beef cow slurry (BS) and grass silage 1 (GS1) or grass silage 2	BS: GS1		Batch	37°C	
(GS2)	1:0	282			-
(Himanshu et al., under review)	0.75:0.25	304			+4
Perennial ryegrass (*Lolium perenne*) silage from grass harvested at	0.5:0.5	309			+3
two growth stages	0.25:0.75	310			+0
	0:1	318			-
	BS: GS2				
	0.75:0.25	304			+7
	0.5:0.5	304			+7
	0.25:0.75	294			+3
	0:1	286			-
Dairy cow manure (DM) and grass silage (GS)	DM:GS		CSTR	OLR 2	NA
(Lehtomäki et al., 2007)	0.4:0.6	250		kg of VS m^{-3} d^{-1}	
75% timothy (*Phleum pratense*), 25% meadow fescue (*Festuca*	0.3:0.7	268		at 35°C	
pratensis) harvested at early flowering stage	0.2:0.8	178			
	0.1:0.9	143			

	0:1	151			
Dairy cow manure (DM) and grass silage (GS)	DM:GS		CSTR	OLR 2	NA
(Jagadabhi et al., 2008)	0.7:0.3	183		kg VS m^{-3} d^{-1} at	
75% timothy (*Phleum pratense*), 25% meadow fescue (*Festuca pratensis*)				35°C	
Dairy cow manure (DM) and agricultural residue (AR)	DM:AR		CSTR	OLR 3	NA
(Alkaya et al., 2010)	0:1	175		kg of VS m^{-3} d^{-1}	
30% Clover, 40% grass and 30% wheat straw;	0.3:0.7	181		at 35°C	
Pig manure (PM) and grass silage (GS)	PM:GS		Batch	37°C	NA
(Xie et al., 2011)	1:0	280			
Concentrated pig manure; dried perennial ryegrass (*Lolium perenne*)	0.75:0.25	304			
silage	0.5:0.5	303			
	0.25:0.75	267			
	0:1	NA**			
Dairy manure (DM) and grass (G)	DM:G		Batch	35°C	NA
(Frigon et al., 2012)	1:0	316			
Switch grass *(Panicum vergatum)*	0.6:0.4	262			
Animal slurry (AS) and grass (G)	AS:G		Batch	35°C	NA
(Molinuevo-Salces et al., 2015)	0.85:0.15	210			
80% cow manure and 20% pig manure; Italian ryegrass	0.15:0.85	243			
Cow dung (CD) and grass (G) or grass silage (GS) and	CD:G		Batch	30°C	NA
(Prapinagsorn et al., 2017)	1:6	85			
Napier grass (*Pennisetum purpureum*) or napier grass silage	1:5	117			
	1:4	170			
	1:3	180			
	1:2	141			
	1:1	117			
	CD:GS				
	1:6	140			

	1:5	182			
	1:4	202			
	1:3	208			
	1:2	179			
	1:1	142			
Pig manure (PM) and grass silage (GS)	PM:GS		CSTR	OLR 1.1	NA
(Tsapekos et al., 2017)	1:0	337		kg of VS m^{-3} d^{-1}	
Meadow grass silage	0.9:0.1	367		at 55°C	

CSTR: Continuously Stirred Tank Reactor; NLB: Non-linear blending (deviation of the measured value from the arithmetic mean of the two individual components); NA: Not available; OLR: Organic loading rate; VS: Volatile solids; * Biogas yield; ** Reactor failed

4.2. Material and methods

4.2.1. Substrates

Two types of cattle slurry were collected from underground tanks beneath roofed slatted-floor cattle buildings in separate beef production systems at Teagasc Grange, Ireland. The first slurry (CS1), produced by beef cows consuming grass silage *ad libitum*, was collected during the November - March indoor feeding period and sampled after manual agitation in March. The second slurry (CS2), produced by finishing beef bulls consuming cereal grain-based concentrates *ad libitum* and supplemented with 1 kg grass silage total solids (TS) per head daily, was collected during indoor feeding between July and May and sampled after mechanised agitation in May. The pig slurry, produced during the preceding 10 weeks by housed finisher pigs consuming a cereal grain-based concentrate diet at the Agri-Food and Biosciences Institute (AFBI) in Hillsborough, Co. Down, Northern Ireland, was mechanically sampled. All three slurries consisted of faeces and urine. Each collected slurry was thoroughly mixed and stored at -20°C until used in the biochemical methane potential (BMP) assay.

Six field plots of perennial ryegrass (*Lolium perenne* L., an equal mixture of the late-heading date diploid varieties Denver, Soriento and Tyrella) were grown at Teagasc Grange (53°30'N, 6°40'W, 83 m above sea level). Immediately prior to harvesting, growth stage was determined with 20 randomly selected tillers according to methods described by Moore et al. (1991). Three plots were harvested on 14 May and the remainder on 11 June using a Haldrup forage plot harvester (J. Haldrup, Løgstor, Denmark) cutting to an average 5 cm stubble height, and precision chopped (Pottinger Mex VI; Grieskirchen, Austria). Grass samples from each plot were ensiled in laboratory silos (O'Kiely & Wilson, 1991) for approximately 120 d at 15°C. Grass

silage samples were then stored at -18°C until the BMP assay. For the BMP assay, the silage samples were thawed at room temperature (about 20°C) for 24 h and to obtain a representative sample were manually milled in a pre-cooled stainless steel mortar, using liquid nitrogen (approximately -196°C) and a stainless steel pestle, until all particles passed through a 1 mm sieve (Nolan et al., 2014).

The inoculum was obtained from an on-farm AD facility digesting cattle slurry and grass silage at AFBI. The inoculum was de-gassed in an incubator for one week at 37°C. The inoculum was then mixed with a wooden spatula and, under a continuous flow of N_2, filtered through a 2 mm pore sieve.

4.2.2. Substrate chemical analysis

The TS of all three slurries and the inoculum, and the VS of silages, all three slurries and the inoculum, were measured according to Standard Methods 2540 G (APHA/AWWA/WEF, 2005). The TS of silages were determined by drying at 85^{o}C for 16 h and these values were corrected for the loss of volatiles according to the equation of Porter and Murray (2001).

Dried milled silage samples were assayed for chemical characteristics as described by King et al. (2012). In brief, acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF; assayed with heat-stable amylase and sodium sulphite) were determined using the filter-bag technique (Ankom, 2006a; Ankom, 2006b) with an ANKOM fibre analyser (ANKOM Technology, Fairport, NY, USA) based on the analytical method of Van Soest (1994) and expressed exclusive of residual ash. Aqueous extract of post-ensilage herbage was assayed for volatile fatty acids (VFA; i.e. acetic, propionic and butyric acids) and ethanol using an automated gas chromatograph (Shimadzu GC-8A; Shimadzu Corporation, Kyoto, Japan) with a flame

ionisation detector and equipped with a Chrompack column (2.4 m × 5.0 mm × 3.4 mm glass column packed with 9% Carbowax 20 M + 1% H_3PO_4 on Chrom WHP 80-100 mesh) using iso-valeric acid (25 g L^{-1}) as an internal standard as described by Ranfft (1973). Temperatures were 150°C in the column, 150°C in the injector and 180°C in the detector; N_2 was the carrier gas. Lactic acid was assayed using a SP-Ace Clinical Chemical Analyser (Alfa Wasserman, NJ, USA) and an l-lactic acid UV method test kit (catalogue number 101309084035; Boehringer Mannheim/R-Biopharm, Darmstadt, Germany), with d-lactate determined using the enzyme d-lactate dehydrogenase (catalog number 1016941001; Boehringer Mannheim/R-Biopharm). The C:N mass ratio was determined using a LECO CN 2000 (Leco Corporation, St. Joseph, MI, USA). The TS, VS and other chemical properties of slurries, silages and inoculum are presented in Table 4.2.

Table 4.2. Chemical properties of cattle slurries (CS1 and CS2), pig slurry, grass silages (GS1 and GS2), and inoculum. All units in g kg^{-1} TS except pH and unless indicated otherwise.

	Growth stage	TS	VS	Chemical composition												
		(g kg^{-1})		TSD	NDF	ADF	ADL	C:N (g:g)	CP	WSC	LA	AA	PA	BA	Eth	NH_3-N (g kg^{-1}N)	pH
CS1	-	122	789		-	-	-	8.7		-	-	-	-	-	-	-	-
CS2	-	78	755		-	-	-	8.9		-	-	-	-	-	-	-	-
Pig slurry	-	60	766		-	-	-	7.8		-	-	-	-	-	-	-	-
GS1	2.3	138	901	642	641	399	33	19.9	130	9.6	4.3	21.3	5.9	36.6	8.6	460	5.1
GS2	2.8	183	936	596	661	391	36	24.1	116	17.5	64.4	5.1	0.8	15.0	10.3	99	4.0
Inoculum	-	41	700		-	-	-	-		-	-	-	-	-	-	-	-

Growth stage determined according to Moore et al. (1991). TS: Total solids; VS: Volatile solids; TSD: Total solids digestibility; NDF: Neutral detergent fiber; ADF: Acid detergent fibre; ADL: Acid detergent lignin; C:N: Carbon to nitrogen mass ratio; CP: Crude protein; WSC: Water-soluble carbohydrates; LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; Eth: Ethanol and NH_3-N: Ammonia-nitrogen.

4.2.3. Batch digestion test

Each of the three slurries and two silages were individually weighed into slurry:silage VS mass ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1. For each silage, a subsample from each of their three experimental replicate samples was used to produce the three slurry:silage experimental replicates. The methane yield of each of these individual or combined substrate samples was determined in triplicate (i.e. analytical replicates) as previously described in McEniry and O'Kiely (2013), with a few minor adjustments. Briefly, the inoculum and substrate were added to 160 cm^3 serum bottles at a 2:1 VS inoculum-to-substrate mass ratio to provide an organic loading of 10 g of VS kg^{-1} total medium. Micro- ($MgSO_4.7H_2O$, 5 mg L^{-1}; H_3BO_3, 0.3 mg L^{-1}; $ZnCl_2$, 0.1 mg L^{-1}; $NiCl_2.6H_2O$, 0.75 mg L^{-1}; $MnCl_2.4H_2O$, 1 mg L^{-1}; $CuCl_2.2H_2O$, 0.1 mg L^{-1}; $CoCl_2.6H_2O$, 1.5 mg L^{-1}; $Na_2SeO_3.5H_2O$, 0.02 mg L^{-1}; $Al_2(SO_4)_3.18H_2O$, 0.1 mg L^{-1}; $(NH_4)6Mo_7O_{24}.4H_2O$, 0.1 mg L^{-1}) and macro- (NH_4HCO_3, 0.4 g L^{-1}; $KHCO_3$, 0.4 g L^{-1}; $NaHCO_3$, 0.4 g L^{-1}) mineral solutions were also added (Gonzalez-Gil et al., 2001). The final substrate volume of each bottle was adjusted to 70 cm^3 using distilled water leaving a headspace of 90 cm^3 in each bottle. Six blank replicates (i.e. without slurry or silage) and six positive control replicates (238 mg cellulose, Sigma, 22184) were also prepared. All bottles were flushed with N_2 for 1 minute and sealed with butyl rubber stoppers and aluminium crimp caps to rapidly create anaerobic conditions. Bottles were incubated at 37°C for 45 d and mixed daily by manual swirling. The headspace pressure was recorded and excess gas was released on days 2, 4, 7, 10, 13, 16, 20, 24, 30, 37 and 45 of the batch digestion using a detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK) and a Vaseline® lubricated needle. The biogas produced was estimated using the equation:

$$\text{Gas produced} = \frac{\text{Vh}}{\text{Pa}}.\text{Pt} \qquad \text{(Equation 4.1)}$$

where, Vh is the headspace volume, Pa is the atmospheric pressure and Pt is the gas headspace pressure.

The methane concentration of biogas was determined using a Shimadzu GC-2014 gas chromatograph equipped with a flame-ionisation detector and a glass column (2.1 m × 5.0 mm × 3.2 mm packed with molecular sieve 5A 60/80 mesh). The temperatures in the column, injector and detector were 120°C, 150°C and 170°C, respectively, with hydrogen as the carrier gas (Bodas et al., 2008; Lovett et al., 2006). The methane yield was corrected for inert gas on day 2 only, corrected for inoculum-induced gas production and the volume normalised to standard temperature and pressure (273°K, 1013 hPa) conditions.

4.2.4. Kinetics

The decay constant or k-value for both biogas and methane was determined using first-order kinetics. Lag phase (λ), half-life (T_{50}) and maximum production rate (U) for both biogas and methane were calculated using second-order kinetics, as described by Wall et al. (2013). Matlab® R2009a software was used to run both first and second order kinetics.

First order kinetics equation

$$y(t) = y_m.(1 - e^{(-kt)}) \qquad \text{(Equation 4.2)}$$

where, y(t) is the cumulative specific methane (or biogas) yield on VS fed at time t, y(m) is the specific methane (or biogas) yield at the end of the 45 d batch test, t is the time and k is the first order decay constant.

Second order kinetics equation

$$y = y_{max}.\exp\left\{-\exp\left[U.\frac{e}{y_{max}}.(\lambda - t) + 1\right]\right\}$$ (Equation 4.3)

where, y is the cumulative specific methane (or biogas) yield on VS fed, y_{max} is the predicted specific methane (or biogas) yield at the end of the 45 d batch test, U is the maximum specific methane (or biogas) production rate , λ is the lag phase and t is the time.

4.2.5. Statistical analysis

Triplicate analytical measurements were averaged to give a single value for each experimental replicate per treatment. Biogas and methane data were analysed using the MIXED procedure in SAS, Version 9.3. The characteristic curves for the mixtures were fitted by regressing the responses on the proportions of slurry and silage, as described by Purcell et al. (2012), with a basic equation of the form:

$$y = \beta_{CS}X_{CS} + \beta_{FS}X_{FS} + \beta_{CSFS}X_{CS}X_{FS} + \delta_{CSFS}X_{CS}X_{FS}(X_{CS} - X_{FS})$$ (Equation 4.4)

where the X_{CS} and X_{FS} variables are the proportions of slurry VS and silage VS, respectively, in the mixtures. This allows a convenient interpretation of the coefficients where, for example, β_{CS} is the individual component response for slurry when slurry = 1 and silage = 0 and, similarly, β_{FS} is the individual component response for silage when slurry = 0 and silage = 1. At a slurry:silage ratio of 0.5:0.5, the $\beta_{CSFS}X_{CS}X_{FS}$ term describes the deviation of the response from the arithmetic mean of the two individual component responses (the non-linear blending (NLB); synergistic (+) or antagonistic (-) associations). For mixtures with slurry:silage ratios of 0.25:0.75 and 0.75:0.25, $\delta_{CSFS}X_{CS}X_{FS}(X_{CS} - X_{FS})$ allows asymmetry in the response curves to be assessed (i.e. it allows different NLB deviations at ratios of 0.75:0.25 and 0.25:0.75).

4.3. Results and discussion

The three slurries and two grass silages used in this study are examples from within the diverse range of livestock slurries and conserved forages likely to be used for AD on Irish farms. This diversity within each substrate broadened the conditions under which the linearity of biogas and methane outputs in response to co-digestion in a series of slurry:silage ratios were assessed.

The biogas yield, methane yield and associated kinetic parameters of individual components and their binary mixtures are presented in Table 4.3 while the corresponding levels of significance are presented in Table 4.4. The biogas and methane yields from the cellulose positive control were 623 (83% of theoretical yield (VDI 4630 guideline, 2006)) and 327 L kg^{-1} of VS (79% of theoretical yield (Wang et al., 2014a)), respectively, reflecting an active inoculum. On average 84% of the methane yield occurred by day 20 of AD (short-term methane potential).

Table 4.3. Effects of cattle slurries (CS1 and CS2), pig slurry (PS) or grass silages (GS 1 and GS2) on biogas and methane output variables, and the associated non-linear blending (NLB) at a slurry:silage ratio of 0.5:0.5 (volatile solids mass basis). The values within brackets () are for the slurry:silage ratios of 0.75:0.25 and 0.25:0.75, respectively.

	CS1	CS2	PS	GS1	GS2	NLB					
						CS1:GS1	CS1:GS2	CS2:GS1	CS2:GS2	PS:GS1	PS:GS2
Biogas											
L kg^{-1} of VS	405.9	380.4	550.8	673.7	610.6	-29.8 (-22.3, -22.3)	-29.8 (-22.3, -22.3)	-29.8 (-22.3, -22.3)	-29.8 (-22.3, -22.3)	-29.8 (-22.3, -22.3)	-29.8 (-22.3, -22.3)
λ	5.2	5.1	6.0	1.4	1.1	-0.50 (-0.47, -0.29)	-0.50 (-0.47, -0.29)	-0.35 (-0.35, -0.17)	-0.35 (-0.35, -0.17)	-0.78 (-0.68, -0.49)	-0.78 (-0.68, -0.49)
k	0.060	0.060	0.060	0.081	0.081	+0.0013 (+0.0010, +0.0010	+0.0013 (+0.0010, +0.0010	+0.0091 (+0.0068, +0.0068)	+0.0091 (+0.0068, +0.0068)	-0.0012 (-0.0009, -0.0009	-0.0012 (-0.0009, -0.0009
U	25.5	25.2	36.1	41.0	35.2	-2.79 (-2.10, -2.10)	-2.79 (-2.10, -2.10)	+2.34 (+1.76, +1.76)	+2.34 (+1.76, +1.76)	-3.28 (-2.46, -2.46)	-3.28 (-2.46, -2.46)
T_{50}	13.6	12.3	13.6	9.6	10.0	-0.25 (-0.19, -0.19)	-0.25 (-0.19, -0.19)	-1.19 (-0.89, -0.89)	-1.19 (-0.89, -0.89)	-0.21 (-0.16, -0.16)	-0.21 (-0.16, -0.16)
CH_4 %	65.9	64.0	70.1	63.5	58.5	-1.05 (-0.47, -1.11)	-1.05 (-0.47, -1.11)	-1.05 (-0.68, -0.90)	-1.05 (-0.68, -0.90)	-1.05 (-2.21, +0.64)	-1.05 (-2.21, +0.64)
Methane											
L kg^{-1} of VS	269.1	246.4	380.1	427.7	359.0	-22.9 (-17.2, -17.2)	-22.9 (-17.2, -17.2)	-22.9 (-17.2, -17.2)	-22.9 (-17.2, -17.2)	-22.9 (-17.2, -17.2)	-22.9 (-17.2, -17.2)
λ	7.2	6.8	7.5	4.0	4.0	-0.40 (-0.30, -0.30)	-0.40 (-0.30, -0.30)	-0.40 (-0.30, -0.30)	-0.40 (-0.30, -0.30)	-0.40 (-0.30, -0.30)	-0.40 (-0.30, -0.30)
k	0.052	0.053	0.056	0.066	0.066	+0.0025 (+0.0019, +0.0019)	+0.0025 (+0.0019, +0.0019)	+0.0079 (+0.0059, +0.0059)	+0.0079 (+0.0059, +0.0059)	+0.0019 (+0.0014, +0.0014)	+0.0019 (+0.0014, +0.0014)
U	18.4	16.7	29.2	27.7	22.3	-2.03 (-1.53, -1.53)	-2.03 (-1.53, -1.53)	+2.66 (+1.99, +1.99)	+2.66 (+1.99, +1.99)	-2.19 (-1.64, -1.64)	-2.19 (-1.64, -1.64)
T_{50}	14.5	13.6	14.2	11.7	12.2	-0.42 (-0.31, -0.31)	-0.42 (-0.31, -0.31)	-1.45 (-1.10, -1.10)	-1.45 (-1.10, -1.10)	-0.23 (-0.19, -0.19)	-0.23 (-0.19, -0.19)

λ: Lag phase (d); k: First order decay constant (d^{-1}); U: Maximum biogas or specific methane production rate (L biogas or CH_4 kg^{-1} of VS day^{-1}); T_{50}: Half-life i.e. time taken (days) to produce 50% of the gas production; and CH_4% (vol. $vol.^{-1}$): Methane volume fraction in biogas. The NLB mean is the deviation of the response from the arithmetic mean of the individual component responses. The sign of the NLB mean indicates whether the deviation was synergistic (+) or antagonistic (–).

Table 4.4. The levels of significance (*P*) for non-linear blending (NLB) of silage and slurry binary mixtures at a slurry:silage ratio of 0.5:0.5 (volatile solids mass basis).

	NLB					
	CS1:GS1	CS1:GS2	CS2:GS1	CS2:GS2	PS:GS1	PS:GS2
Biogas						
L kg^{-1} of VS	***	***	***	***	***	***
λ	***	***	***	***	***	***
k	NS	NS	***	***	NS	NS
U	***	***	**	**	***	***
T_{50}	NS	NS	***	***	NS	NS
CH_4 %	**	**	***	***	**	**
Methane						
L kg^{-1} of VS	***	***	***	***	***	***
λ	***	***	***	***	***	***
k	*	*	***	***	*	*
U	**	**	***	***	**	**
T_{50}	NS	NS	***	***	NS	NS

* = *P*<0.05, ** = *P*<0.01, ***= *P*<0.001, NS = not significant. λ: Lag phase (d); k: First order decay constant (d^{-1}); U: Maximum biogas or specific methane production rate (L biogas or CH_4 kg^{-1} of VS day^{-1}); T_{50}: Half-life i.e. time taken (days) to produce 50% of the gas production; and CH_4% (vol. $vol.^{-1}$): Methane volume fraction in biogas.

4.3.1. Mono-digestion of animal slurries

CS1, CS2 and pig slurry produced 79, 77 and 86% of their total methane yield during the short-term methane potential time-frame, respectively. The measured methane yields for pig slurry agree with previous reports of 200-417 L kg^{-1} of VS (Steffen et al., 1998; Triolo et al., 2011) VS while the measured methane yields from cattle slurries (246-269 L kg^{-1} of VS) were higher than those reported by Triolo et al. (2011) (197-237 L kg^{-1} of VS). With both cattle slurries, the sequential replacement of slurry by silage caused a progressive increase in biogas and methane yields from the values obtained with slurries to those with silages.

The methane yield for both cattle slurries was less than pig slurry, and this agrees with (Amon et al. (2005); Kaparaju and Rintala (2011); Moller et al. (2004); Triolo et al. (2013)). The slower and lower methane yield of cattle slurries compared to pig slurry (Figure 4.1) was probably due

to the inhibitory effects of lignin on the AD of fibre in cattle slurries (Triolo et al., 2013) and to the likely higher content of readily digestible lipid in pig slurry (Kothari et al., 2014). The difference in methane yields between the two cattle slurries was possibly due to differences in factors such as type of cattle (Triolo et al., 2013), diet type (Amon et al., 2005; Hellwing et al., 2014) and the duration and conditions of slurry storage (Browne et al., 2015).

The methane volume fraction for cattle slurries (64.0-65.9%) was higher than reported by Triolo et al. (2011) (58-62%) but the fraction for pig slurry (70%) was similar to values reported by Steffen et al. (1998) (70-80%).

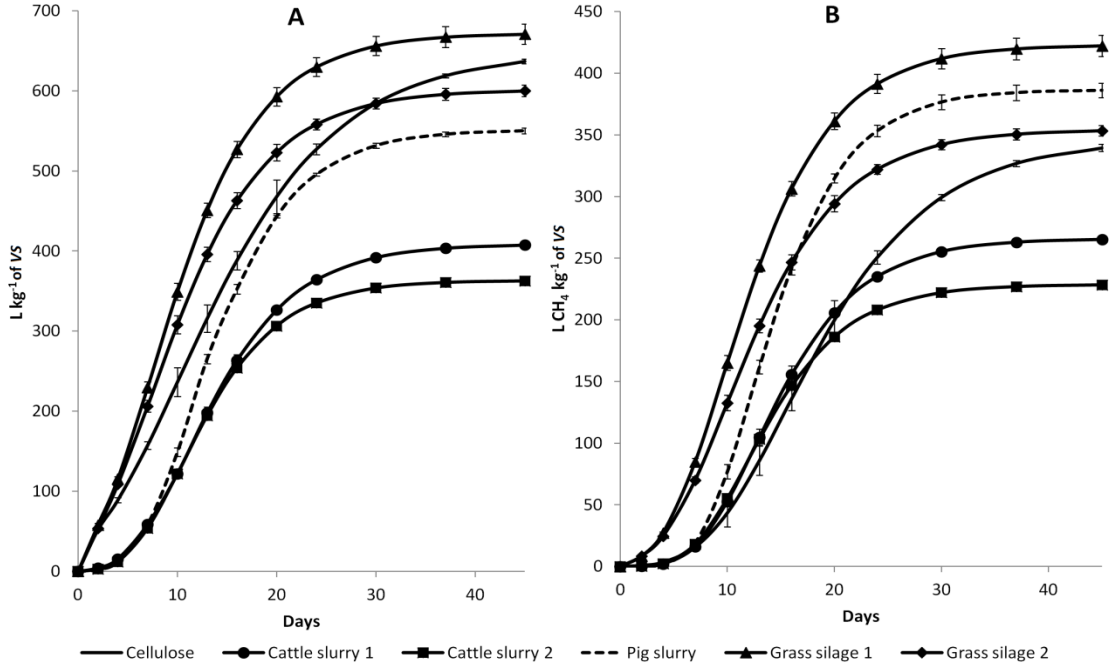


Figure 4.1. Cumulative biogas (A) and methane (B), along with the standard error of mean, fitted curves for the individual components cellulose, cattle slurries, pig slurry and grass silages using second order kinetics.

4.3.2. Mono-digestion of grass silages

Both grass silages produced more than 80% their total methane yield during the short-term methane potential time-frame. The measured methane yields for both grass silages are within the

published range of 229-650 L kg^{-1} of VS (McEniry & O'Kiely, 2013; Prochnow et al., 2009). The two grass silages clearly differed in the growth stage of the perennial ryegrass crop when harvested, reflecting the four week difference in harvest dates. The expected much greater total solids digestibility and lower fibre component proportions for GS1 were considerably smaller than anticipated (Table 4.2). This is most likely due to a considerably greater loss of digestible soluble VS via effluent for the wetter GS1, as would be predicted from the findings of Miller and Clifton (1965). Furthermore, the extensive clostridial fermentation (high butyric acid, high NH_3-N and high pH) for GS1 compared to the apparent dominance by lactic acid bacteria (high lactic acid, low NH_3-N and low pH) for GS2 (Table 4.2) would also result in a greater loss of digestible VS for GS1 (Savoie & Jofriet, 2003). However, the greater butyric acid than lactic acid concentration for GS1 would favour the latter having an elevated methane yield when expressed on a VS basis (Weissbach, 2009). Overall, the greater methane yield recorded per unit VS for GS1 likely reflects the combined effects of its less advanced growth stage at harvest and its more methanogenic silage fermentation acid profile compared to GS2.

The methane volume fraction for silages in this study (58.5-63.5%) was higher than reported by Dandikas et al. (2014) (51%) or Triolo et al. (2011) (54-56%).

4.3.3. Co-digestion of animal slurries and grass silages

4.3.3.1. Statistical approach for quantification of antagonistic and synergistic effects

The NLB approach was used to describe and objectively analyse the nature of the response of methane yield and other variables to a progressive change in the proportions of slurry and silage VS in the AD substrate. The NLB model uses four coefficients that can be used to calculate the value of a parameter at a given slurry:silage ratio using Equation 4.4. For example, the

coefficients for methane yield for all six slurry and silage binary mixtures are presented in Table 4.5. The coefficients, β_{CS} and β_{FS} are for the pure slurry and pure silage components, respectively. $\beta_{CSFS}=0$ would indicate no NLB and thus that the measured methane yield from the co-digestion of slurry and silage was directly predictable from their proportional contribution relative to when incubated as individual ingredients. $\beta_{CSFS}<0$ and $\beta_{CSFS}>0$ would indicate antagonism and synergy, respectively. In addition, the coefficient, δ_{CSFS} predicts if the NLB is either symmetrical (necessarily greatest at a 0.5:0.5 ratio and returning at similar rates above and below this ratio towards the individual component values; $\delta_{CSFS}=0$) or asymmetrical (not necessarily greatest at a 0.5:0.5 ratio and returning at different rates above and below the ratio of maximum methane output towards the individual component values; $\delta_{CSFS}\neq0$). Figure 4.2 shows the modelled response curves of methane yield with a change in the grass silage proportion for each of the six binary mixtures. Thus, the absence of non-linear blending would result in the curve from a silage proportion of 0 to 1 being a straight line whereas for antagonism or synergy the response curve would be lower or higher than the straight line, respectively.

Table 4.5. The coefficients of non-linear blending (NLB) equation (Equation 4) for methane yield for the six binary mixtures of slurry and silage.

	Binary mixtures of slurries and silages					
	CS1:GS1	CS1:GS2	CS2:GS1	CS2:GS2	PS:GS1	PS:GS2
β_{CS}	269.06	269.06	246.43	246.43	380.05	380.05
β_{GS}	427.65	359.01	427.65	359.01	427.65	359.01
β_{CSFS}	-91.74	-91.74	-91.74	-91.74	-91.74	-91.74
δ_{CSFS}	0	0	0	0	0	0

CS1: Cattle slurry 1; CS2: Cattle slurry 2; GS1: Grass silage 1; GS2: Grass silage 2; PS: Pig slurry; β_{CS} and β_{FS} are coefficient for pure slurry and pure silage; and β_{CSFS} describes deviation of the response from the arithmetic mean of the two individual components and δ_{CSFS} describes asymmetry in the response curves

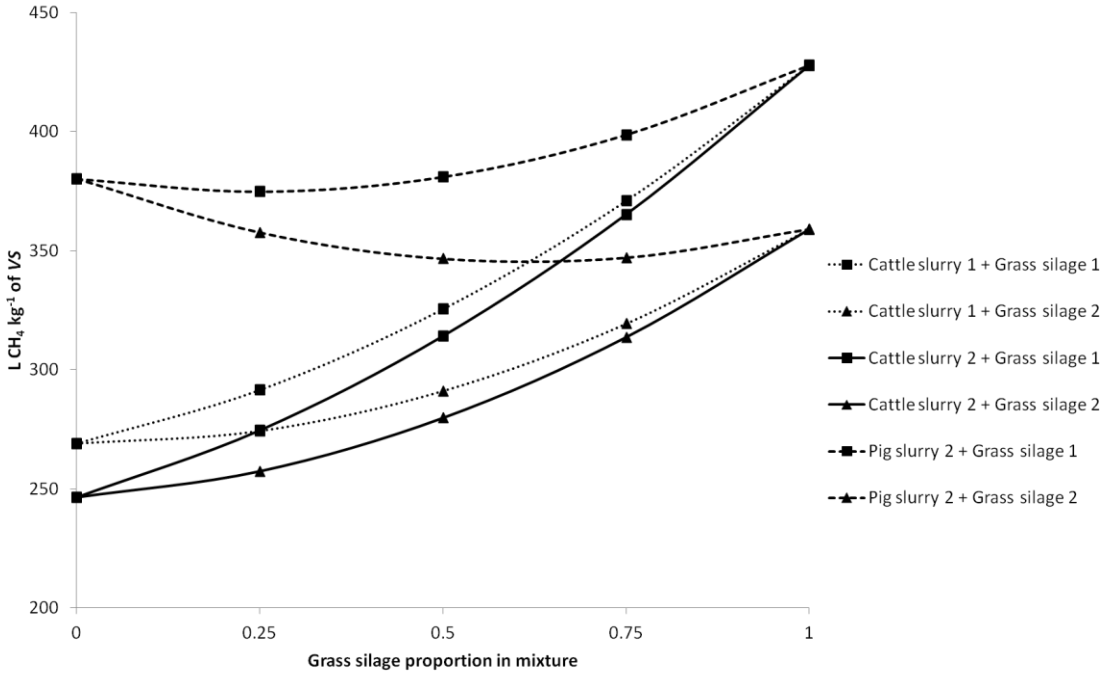


Figure 4.2. Modelled response curves of methane yield with change in grass silage proportion for each of the six binary grass silage and animal slurry mixtures.

4.3.3.2. Antagonistic effects on methanogenesis

A major finding of the current study is that each of the three slurries investigated produced significantly less methane when co-digested at a VS mass ratio of 0.5:0.5 with either of the two silages than would have been predicted from the methane yields for the individual ingredients. The antagonistic effects ranged from 5.7-7.6% below the yields predicted for a linear relationship at a slurry:silage VS mass ratio of 0.5:0.5. Furthermore, these antagonistic effects were symmetric and occurred consistently for the various combinations of the three slurries with the two silages.

The NLB statistical model considered the effects of slurry:silage ratio, slurry type, grass silage type and interactions between these factors. It is interesting to note from Table 4.3 that for biogas

and methane yields, and for methane volume fraction and lag phase, the slurry:silage ratio was the only factor to significantly influence the NLB outcome. Thus, this outcome appeared to be independent of the type of slurry or silage used in this study.

In the present study, although the C:N ratio for a slurry:silage ratio of 0.5:0.5 was *circa* 15, a value below the suggested optimum range of 20-30 (Kayhanian, 1999; Wang et al., 2017), this does not explain the observed antagonism as the three slurries also had more extreme sub-optimal C:N ratio values (7.8-8.9) but with the pig slurry in particular still supporting a relatively high methane yield. The effects of C:N ratio appear not to be consistent, with Ramos-Suárez and Carreras (2014) showing both synergy and antagonism at a C:N ratio of 10 for different co-digestion mixtures. Furthermore, Wang et al. (2014b) reported synergistic effects during the co-digestion of grass and sewage sludge for a C:N ratio of *circa* 10.

It seems unlikely that there was a deficiency, excess or imbalance of trace elements as the incubation medium was fortified with trace elements and the individual component substrates produced good methane yields.

Anaerobic digestion of nitrogen rich substrates can result in high concentrations of ammonia depending on the pH and temperature of the medium (Chen et al., 2014; Chen et al., 2008). Neither pH, VFA nor ammonia were measured during the AD process as the equipment used was not suited for repeated liquid sampling, However, as the incubation medium was fortified with buffer to prevent a change in pH and since the silages did not contain high concentrations of nitrogen, it is unlikely that the AD of the co-digested mixtures was inhibited by excessive concentrations of ammonia.

The present study agrees with the findings of Wall et al. (2013) where co-digestion of non-dried grass silage (similar to the present study) and cattle slurry also resulted in antagonistic effects on methane yield. However, Himanshu et al. (under review) reported a synergistic effect on methane yield during the co-digestion of grass silage with CS1 (the same CS1 cattle slurry was used in both studies). Although operational conditions such as incubation temperature, substrate:inoculum ratio and addition of buffer and trace elements were similar in both studies, the silages and their form differed. Thus, in the present study, non-dried grass silage was used whereas Himanshu et al. (under review) used oven dried (40°C for 48 h) grass silage. Thermal drying can result in loss of some silage fermentation products, loss of organic matter due to continued plant enzyme activity and formation of condensation products. Thus, thermal drying can change the chemical composition of a substrate which may impact on the methane yield (McEniry et al., 2014).

Overall, the biological mechanisms that mediated the antagonistic outcomes when slurry and grass silage were co-digested are not evident and require further research.

4.4. Future perspectives

Considering this and other studies, co-digestion of cattle or pig slurries with grass silage can result in either synergy or antagonism, and in a commercial scale AD facility this would likely have an effect on profitability. Hence, future research should study the economic implications of synergistic and antagonistic effects on methane yield for a commercial scale AD facility. It will also be important to develop the ability to predict the direction and scale of non-linear blending effects if they occur during co-digestion.

4.5. Conclusion

The biogas yields of cattle slurry 1, cattle slurry 2, pig slurry, grass silage 1 and grass silage 2 were 405.9, 380.4, 550.8, 673.7 and 610.6 L kg^{-1} of VS, respectively while the methane yields of these substrates were 269.1, 246.4, 380.1, 427.7 and 359.0 L kg^{-1} of VS, respectively. Biogas and methane yields were impacted by slurry type, grass silage type, and slurry:silage ratio. Each slurry and silage mixture displayed non-linear blending for methane yield and its maximum effect, which was always antagonistic, was at a 0.5:0.5 VS mass ratio and ranged from 5.7-7.6% below the yields predicted from mono-digestion of individual substrates. The biological mechanisms that mediated the antagonistic outcomes were not elucidated in this study.

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5. **Factors controlling headspace pressure in a manual manometric method can be used to produce methane output comparable to AMPTS**

Factors controlling headspace pressure in a manual manometric method can be used to produce methane output comparable to AMPTS

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Abstract

The manual manometric biochemical methane potential (mBMP) test uses the increase in pressure to calculate the gas produced. This gas production may be affected by the headspace volume in the incubation bottle and by the overhead pressure measurement and release (OHPMR) frequency. The biogas and methane yields of cellulose, barley, silage and slurry were compared with three incubation bottle headspace volumes (50, 90 and 180 ml; constant 70 ml total medium) and four OHPMR frequencies (daily, each third day, weekly and solely at the end of experiment). The methane yields of barley, silage and slurry were compared with those from an automated volumetric method (AMPTS). Headspace volume and OHPMR frequency effects on biogas yield were mediated mainly through headspace pressure, with the latter having a negative effect on the biogas yield measured and relatively little effect on methane yield. Two mBMP treatments produced methane yields equivalent to AMPTS

Keywords: Manometric biomethane potential assay; Silage; Slurry; Pressure; Headspace

Abbreviations

ABAI: Anaerobic biodegradation activity and inhibition; AD: Anaerobic digestion; ABAI: Anaerobic Biodegradation, Activity and Inhibition; AFBI: Agri-Food and Biosciences Institute; AMPTS: Automatic methane potential test system (Automated volumetric method); BMP: Biochemical methane potential; F: Frequency with which overhead pressure was measured and released; IWA: International Water Association; k : First order decay constant; mBMP: Manual manometric biochemical methane potential; OHPMR: Overhead pressure measurement and release; P: Level of significance; P day: Time at which the maximum pressure was recorded during the mBMP test; PMax: Maximum pressure measured during the mBMP test; SEM: Standard error of the mean; T50: Time taken (d) to produce 50% of the gas production (Half-life); TS: Total solids; U: Maximum methane or biogas production rate; V: Headspace volume; VS: Volatile solids; λ: Lag phase

5.1. Introduction

The biochemical methane potential (BMP) test is an anaerobic batch digestion process which is commonly used to determine the biogas and methane yields from organic substrates. The two most commonly used BMP test methods are the manometric and volumetric methods. In the manometric method the volume is kept constant and an increase in the overhead pressure is measured and used to calculate the amount of gas produced. In the volumetric method the pressure is kept constant and the volume of produced gas is measured by a displacement volume device (Valero et al., 2016). There is no single universally accepted standard method to conduct the BMP test although several guidelines are published such as VDI 4630 guideline (2006), the method by members of the ABAI of the IWA (Angelidaki et al., 2009), and the updated

guidelines from ABAI group (Holliger et al., 2016). These guidelines recommend both manometric and volumetric methods for the BMP test.

Although the manometric method is widely used, its parameters (incubation bottle size, maximum pressure limit and overhead pressure measurement and release (OHPMR) frequency) vary with different guidelines. For example, the VDI 4630 guideline (2006) recommends an incubation bottle size of 500 - 2000 ml for homogeneous substrates and 10 l - 20 l for heterogeneous substrates whereas Holliger et al. (2016) recommend an incubation bottle size of 100 ml for homogeneous substrates and 500 - 2000 ml for heterogeneous substrates. Both these guidelines have no direct recommendation for the OHPMR frequency but identify a maximum overhead pressure 100 hPa (VDI 4630 guideline, 2006) and 3000 hPa (Holliger et al., 2016) that should not be exceeded during the BMP test.

The manual manometric method (mBMP) can have a lower capital cost but a higher labour input than either the automated manometric or the volumetric methods. In the mBMP method it may be difficult to pinpoint the maximum overhead pressure achieved if readings are only taken once daily. Researchers using the mBMP have used different incubation bottle sizes and OHPMR frequencies (Ferrer et al., 2008; Hosseini Koupaie et al., 2014; McEniry et al., 2014; Nolan et al., 2016) but important descriptive details of these parameters are not always provided.

The methane yield of a particular substrate can be impacted by various factors including, but not limited to, inoculum, inoculum to substrate ratio, buffering system, substrate to buffer ratio, operating temperature, duration of the assay and the specific BMP technique employed. A wide range of methane yields have been reported, even for a relatively homogeneous and industrially synthesized feedstock such as cellulose (Raposo et al., 2011). However, in the inter-laboratory

study (19 participating laboratories) reported by Raposo et al. (2011), laboratories using manometric BMP methods reported lower methane yields from cellulose than those using volumetric BMP methods. Furthermore, when compared within controlled experiments, McEniry et al. (2014), Nolan et al. (2016) and Wang et al. (2014) reported a lower methane yield from cellulose using the mBMP method compared to an automated volumetric method i.e. AMPTS (http://www.bioprocesscontrol.com/products/ampts-ii/). Also, Logan et al. (2002) reported a lower biogas yield with a manometric method compared to a respirometer (a variation of the volumetric method).

Biogas and methane yields with the mBMP method may be affected by the overhead pressure. The latter can be altered by differences in headspace volume in the incubation bottle and/or by the frequency of pressure release associated with the OHPMR frequency regime adopted. There is limited literature that thoroughly assesses the influence of these factors on biogas and methane yield. However, Yilmaz (2015) reported enhanced biogas yield for glucose with a lowering of the headspace pressure. Furthermore, Valero et al. (2016) suggested that the influence of overhead pressure on methane yield varied with the substrate used. The innovation in this study is that other papers have not compared a manual manometric method (with varied headspace volume and OHPMR frequency) with an automated volumetric method for assessing the biomethane potential values of energy crops and slurry. By undertaking these comparisons with substrates of contrasting anaerobic digestion characteristics this study provides the opportunity to identify manual manometric methods that best replicate the methane outputs obtained with an automated volumetric method.

The objectives of the present study were to compare the effects of different headspace volumes and the frequency of pressure release associated with different OHPMR frequency regimes on

biogas and methane yields using a mBMP test, and to compare the outputs for these mBMP treatment combinations with the output for an industry standard automated volumetric method i.e. AMPTS. In order to broaden the circumstances under which these comparisons were made, contrasting substrates (cellulose, barley, silage and slurry) with different digestion profiles were used.

5.2. Material and methods

5.2.1. Substrates

Silage was prepared from the first cut of perennial ryegrass (*Lolium perenne* L.) while whole barley (*Hordeum vulgare* L.) grains were purchased from a livestock feed merchant. Both silage and barley samples were dried at 40°C for 48 h in an oven with forced air circulation and then milled (Wiley mill; 1 mm pore screen). These dried and milled samples were used for the BMP assay. Cellulose powder was obtained from Sigma-Aldrich (product id. 22184). The cattle slurry was collected from a tank under a roofed slatted-floor cattle building at Teagasc, the Irish Agricultural and Food Development Authority Research Centre in Grange, County Meath, Ireland. It was produced by cattle consuming grass silage *ad libitum* and consisted of faeces and urine. The collected cattle slurry was thoroughly mixed and stored at -20°C until required. The inoculum was obtained from an on-farm anaerobic digestion (AD) reactor digesting cattle slurry and grass silage at the Agri-Food and Biosciences Institute in Hillsborough (AFBI), Co. Down, Northern Ireland. This was de-gassed in an incubator for 5 d at 37°C. The inoculum was then mixed with a wooden spatula and, under a continuous flow of N_2, filtered through a 2 mm pore sieve. The total solids (TS) and volatile solids (VS) of the four substrate samples were measured

according to Standard Methods 2540 G (APHA, 2005). The TS of cellulose, barley, silage, slurry and inoculum was 966, 846, 901, 136 and 48 g kg^{-1}, respectively. While, the VS of cellulose, barley, silage, slurry and inoculum was 1000, 924, 978, 794 and 715 g kg^{-1}VS, respectively.

5.2.2. mBMP

The biogas and methane yields were determined in triplicate incubation bottles for each of the four substrates in each of three different volume serum bottles (i.e. 120, 160 and 250 ml) and were subjected to each of four gas sampling and gas pressure release frequencies throughout incubation, using the method described in McEniry and O'Kiely (2013) with a few minor adjustments. The relative design and shape of all the bottles were similar but they differed in the diameter of their base and in height. The outer base diameter × height of the 120, 160 and 250 ml bottles were 52 mm × 95 mm, 54 mm × 108 mm and 64 mm × 117 mm, respectively. The inoculum and substrate were added at a 2:1 VS inoculum-to-substrate gravimetric ratio to provide a total organic loading of 10 g VS kg^{-1} total medium. Micro- and macro-mineral solutions were also added to prevent mineral nutrient deficiency (McEniry & O'Kiely, 2013). The final total medium volume of each bottle was adjusted to 70 ml using distilled water. The headspace volume in 120, 160 and 250 ml bottles was 50, 90 and 180 ml, respectively. Three blank replicates (inoculum only) were also prepared for each different bottle volume set at each sampling frequency. All bottles were flushed with N_2 gas for about 1 min and sealed with butyl rubber stoppers and aluminium crimp caps. Bottles were incubated at 37°C for 35 d and mixed daily by manual swirling. The overhead pressure in the incubation bottles was measured, and gas was released to equilibrate to atmospheric pressure, at four different frequencies i.e. daily, each third day, weekly and only after 35 d incubation, using a detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK) and Vaseline® lubricated needle.

Thus 180 mBMP incubation bottles were used as follows:

[(three headspace volumes × four OHPMR) × triplicate replication] for each of four substrates and one blank, where headspace volumes were 50, 90 and 180 ml, OHPMR were daily, each third day, weekly and only after 35 d incubation, the substrates were cellulose, barley, silage and slurry and the blank was inoculum only.

For 50 ml headspace bottles designated to be sampled only after 35 d incubation, overhead pressure was not measured at day 35 because the high pressure had ruptured the butyl rubber stopper on the incubation bottles. Thus 15 incubation bottles did not survive the study, leaving the data from 165 incubation bottles for statistical analyses.

The biogas produced was estimated using the equation:

$$\text{Gas produce (ml)} = \frac{\text{vh}}{\text{Pa}}.\text{Pt} \qquad \text{(Equation 5.1)}$$

where, vh is the headspace volume (ml), Pa is the atmospheric pressure (hPa) and Pt is the gas headspace pressure (hPa).

The methane concentration of biogas was determined using a Shimadzu GC-2014 gas chromatograph with a flame-ionisation detector equipped with a glass column (2.1 m × 5.0 mm × 3.2 mm packed with molecular sieve 5A 60/80 mesh). The temperatures in the column, injector and detector were 120, 150 and 170°C, respectively, with helium as the carrier gas. Evaluation of biogas and methane yield included a correction for inert gas, a correction for inoculum-induced gas production and a normalisation of gas output (normalised litres) to standard temperature and pressure (273 K, 1013 hPa) conditions.

5.2.3. AMPTS

The methane yield of three substrates (silage, barley and slurry) was also determined using a volumetric gas production method i.e. the Automated Methane Potential Test System II (AMPTS; Bioprocess Control AB, Lund, Sweden). To avoid possible confounding due to factors such as differences in substrate or inoculum, sub-samples of the same substrate and inoculum used in the mBMP system were simultaneously used in the AMPTS. The AMPTS employed similar characteristics to the mBMP system where feasible i.e. both systems started at the same date and continued for 35 d, using triplicate samples of each substrate, and using the same inoculum-to-substrate ratio, buffer, blanks, flushing with N_2 and incubation at 37°C. However, each AMPTS bottle (500 ml total volume; 400 ml working volume and 100 ml headspace) was equipped with an individual mechanical mixer (60 revolutions per min; for 10 min after a 10 min pause; repeat) and the biogas produced in each bottle passed through a second bottle (one per incubation bottle, containing 3 M NaOH which retains CO_2 and H_2S while allowing methane to pass through). The upgraded gas was sent to a flow measurement device (one for each incubation bottle) which measures gas through water displacement. A specific volume (approximately 10 ml) of methane caused the tipping device to tip. This movement was recorded via a digital pulse and output was recorded in a software package as volume of methane produced. For each tipping the pressure and temperature were recorded to allow normalization of the methane produced (normalised litres) to standard temperature and pressure (273K, 1013 hPa) conditions. AMPTS is further described in McEniry et al. (2014) and Bioprocess Control Sweden AB (2014).

Thus there were 12 AMPTS bottles: [three substrates and one blank] × triplicate replication, where the substrates were barley, silage and slurry and blank was inoculum only.

5.2.4. Kinetics

First and second order kinetics were run in Matlab® R2009a software, as described by Wall et al. (2013). The average decay constant or k value for both biogas and methane were determined using first-order kinetics: $y(t) = y_m x(1 - e^{(-kt)})$

(Equation 5.2)

where, y(t) is the cumulative methane (or biogas) yield at time t (L kg^{-1} VS), y(m) is the methane (or biogas) yield at the end of the 35 d batch test (L kg^{-1} VS), t is the time (d) and k is the first order decay constant (d^{-1}).

Lag phase (λ), half-life (T_{50}) and maximum production rate (U) for both biogas and methane were calculated using second-order kinetics:

$$y = y_{max}.\exp\left\{-\exp\left[U.\frac{e}{y_{max}}.(\lambda - t) + 1\right]\right\} \quad \text{(Equation 5.3)}$$

where, y is the cumulative methane (or biogas) yield (L kg^{-1} VS), y_{max} is the predicted methane (or biogas) yield at the end of the 35 d batch test (L kg^{-1} VS), U is the maximum methane (or biogas) production rate (L kg^{-1} VS d^{-1}), λ is the lag phase (d) and t is the time (d).

5.2.5. Statistical analysis

The data were analysed using the MIXED procedure in SAS 9.3. Methane yield, biogas yield and kinetics data for the mBMP system were analysed as a split plot design with incubation bottle headspace volume as the main plot and OHPMR frequency as the sub plot. The methane yield and methane kinetics from mBMP and AMPTS were compared using a one-way classification where Dunnett's adjustment was used to correct for multiple comparisons effects when comparing all means to the AMPTS control.

Within each substrate, linear regression and R^2 values were derived for the relationships between the P_{Max} and each of biogas yield, methane yield and methane proportion of treatment means using the 'format trendline' for XY scatter graphs within Microsoft Excel.

5.3. Results

5.3.1. Cellulose

Mean and standard error of the mean (SEM) values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 5.1, and the corresponding levels of significance are presented in Table 5.2. Biogas yield and the associated λ, k and U values increased with an increase in headspace volume, although the scale of this response was greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume, and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a reduction in OHPMR frequency. Declining OHPMR frequency reduced λ and k when the headspace volume was 50 ml. The U value decreased but T_{50} and P_{Max} increased with a reduction in OHPMR frequency.

Increasing headspace volume did not significantly alter methane yield for daily OHPMR frequency but it resulted in an increase during weekly and solely after 35 d OHPMR frequencies. The k value decreased with an increase in headspace volume for each third day OHPMR frequency but it increased for the solely 35 d OHPMR frequency. The U value decreased with an increase in headspace volume when the OHPMR was done daily or each third day but it increased for the weekly OHPMR frequency. Reducing OHPMR frequency reduced methane yield when headspace volume was 50 ml. λ decreased with decline in OHPMR frequency when

the headspace volume was 90 ml. The U value declined as OHPMR frequency declined when the headspace volume was 50 or 90 ml.

Table 5.1. Biogas yield, methane yield and kinetic parameters when cellulose was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each third day			Weekly			After 35 days		SEM[1]
V (ml)	50	90	180	50	90	180	50	90	180	90	180	
						Biogas						
L kg^{-1} VS	611.6^{d}	626^{d}	713.3^{e}	543.9^{c}	616.9^{d}	701.9^{e}	434.9^{b}	604.9^{d}	679.5^{e}	126.2^{a}	590.5^{cd}	15.96
λ[2]	1.4^{c}	1.5^{c}	1.8^{cd}	0.4^{b}	1.6^{cd}	2.1^{d}	-1.2^{a}	0.4^{b}	1.5^{c}			0.19
k[3]	0.088^{de}	0.088^{de}	0.097^{f}	0.069^{b}	0.083^{cd}	0.091^{ef}	0.057^{a}	0.078^{c}	0.094^{ef}			0.0026
U[4]	39.3^{cd}	40^{cd}	54.4^{e}	23.3^{b}	36.5^{c}	50.5^{e}	12.8^{a}	29.4^{b}	43.9^{d}			2.13
T_{50}[5]	9.3^{ab}	9.4^{ab}	8.3^{a}	12.7^{d}	10.1^{bc}	9.1^{ab}	24.6^{e}	10.9^{c}	9.4^{ab}			0.49
CH_4%[6]	49.0^{bcd}	46.6^{ab}	42.1^{a}	49.0^{bcd}	49.0^{bcd}	42.2^{a}	52.9^{cde}	48.4^{bc}	51.5^{bcd}	57.8^{e}	54.0^{de}	1.80
P_{Max}[7]	676.6^{d}	358.3^{b}	210.1^{a}	1340.1^{f}	892.6^{e}	486.8^{c}	1889.9^{h}	1629.2^{g}	946.2^{e}	2618.4^{j}	2217.1^{i}	31.45
P day[8]	9	9	9	9	9	9	14	14	14	35	35	0
						Methane						
L kg^{-1} VS	299.7^{de}	291.4^{cd}	300.3^{de}	266.7^{c}	302.5^{de}	296.4^{de}	230.5^{b}	292.5^{cde}	349.7^{f}	74.5^{a}	319.0^{e}	9.21
λ[2]	4.2^{c}	9.2^{f}	3.8^{c}	4.3^{cd}	6.0^{e}	6.5^{e}	5.1^{d}	1.5^{a}	2.9^{b}			0.30
k[3]	0.072^{d}	0.056^{b}	0.069^{d}	0.080^{e}	0.066^{cd}	0.060^{bc}	0.048^{a}	0.068^{d}	0.080^{e}			0.0025
U[4]	34.9^{f}	29.2^{e}	20.8^{d}	21.9^{d}	18.0^{c}	17.1^{c}	8.0^{a}	13.3^{b}	21.4^{d}			0.66
T_{50}[5]	11.7^{ab}	15.2^{d}	12.6^{abc}	11.2^{a}	12.5^{abc}	13.3^{c}	38.8^{e}	13.1^{bc}	11.3^{a}			0.47

[1] SEM: Standard error of mean; [2] λ: the lag phase (d); [3] k: the first order decay constant (d^{-1}); [4] U: the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}); [5] T_{50}: half-life i.e. time taken (d) to produce 50% of the gas production; [6] CH_4%: the methane proportion in biogas (vol. $vol.^{-1}$); [7] P_{Max} (hPa): the maximum pressure measured during the mBMP test; and [8] P day: the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other.

Table 5.2. The level of significance (*P*) for biogas yield, methane yield and kinetic parameters for cellulose, barley, silage and slurry.

F[1]	Cellulose			Barley			Silage			Slurry		
V[2] (ml)	F	V	FxV	F	V	FxV	F	V	FxV	F	V	FxV
						Biogas						
L kg^{-1} VS	***	***	***	***	***	***	***	***	***	***	***	***
λ[3]	***	***	***	***	***	***	***	***	***	*	***	NS
k[4]	***	***	***	***	***	NS	***	***	*	***	***	***
U[5]	***	***	**	***	**	***	***	***	**	***	***	***
T_{50}[6]	***	***	***	***	***	***	***	***	***	***	***	***
CH_4%[7]	**	***	NS	***	***	***	***	***	***	***	***	***
P_{Max}[8] (hPa)	***	***	***	***	***	***	***	***	***	***	***	***
						Methane						
L kg^{-1} VS	***	***	***	***	*	***	***	***	***	NS	NS	***
λ[3]	***	***	***	NS	***	NS	***	NS	***	***	NS	NS
k[4]	***	*	***	***	***	***	***	***	***	***	***	***
U[5]	**	***	***	***	***	***	NS	***	***	***	***	***
T_{50}[6]	***	***	***	***	***	***	*	***	***	***	***	***

[1] F: overhead pressure measurement and release frequency; [2] V: headspace volume; [3] λ: the lag phase (d); [4] k: the first order decay constant (d^{-1}); [5] U: the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}); [6] T_{50}: half-life i.e. time taken (d) to produce 50% of the gas production; [7] CH_4%: the methane proportion in biogas (vol. $vol.^{-1}$) and [8] P_{Max} (hPa): the maximum pressure measured during the mBMP test

5.3.2. Barley

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 5.3, and the corresponding levels of significance are presented in Table 5.2. Biogas yield and the associated λ, k and U increased with an increase in headspace volume, although the scale of this response was generally greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a reduction in OHPMR frequency when the headspace volume was 50 or 90 ml. The k value decreased while P_{Max} increased with a reduction in OHPMR frequency. CH_4% declined with an increase in headspace volume during daily and each third day OHMP frequency.

Increasing headspace volume did not significantly alter methane yield for daily OHPMR frequency but it resulted in an increase during each third day, weekly and solely after 35 d OHPMR frequencies. The associated kinetic parameters k and U generally increased while λ and T_{50} generally decreased with an increase in headspace volume. No clear effect of OHPMR frequency on methane yield emerged.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 5.3. Five of the 11 mBMP treatments had methane yields that differed (P<0.05) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

Table 5.3. Biogas yield, methane yield and kinetic parameters when barley was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
							Biogas							
L kg^{-1} VS	612.4^{de}	642.2^{e}	701.1^{f}	551.5^{bc}	620.0^{de}	736.9^{f}	386.7^{a}	604.0^{de}	700.9^{f}	523.1^{b}	583.0^{cd}	-	14.43	-
λ[2]	-2.8^{a}	-2.8^{a}	-1.9^{bc}	-1.2^{cd}	-1.0^{d}	-0.6^{d}	-2.2^{ab}	0.5^{e}	0.6^{e}	-	-	-	0.29	-
k[3]	0.094^{cd}	0.109^{e}	0.136^{f}	0.074^{b}	0.094^{cd}	0.113^{e}	0.056^{a}	0.085^{bc}	0.104^{de}	-	-	-	0.004	-
U[4]	28.3^{c}	33.4^{d}	47.4^{e}	22.1^{b}	32.1^{cd}	49.3^{e}	10.6^{a}	33.7^{d}	47.9^{e}	-	-	-	1.66	-
T_{50}[5]	8.1^{ab}	6.9^{ab}	5.5^{a}	12.2^{c}	8.8^{b}	7.0^{ab}	26.4^{d}	9.4^{bc}	7.8^{ab}	-	-	-	0.96	-
CH_4%[6]	51.5^{c}	48.8^{b}	44.0^{a}	54.4^{d}	53.2^{d}	47.5^{b}	53.7^{d}	50.9^{c}	57.2^{e}	60.2^{f}	60.1^{f}	-	0.52	-
P_{Max}[7]	673.2^{d}	550.0^{c}	348.0^{a}	1248.9^{g}	821.6^{e}	462.4^{b}	1901.3^{i}	1694.3^{h}	902.1^{f}	3871.4^{k}	2206.3^{j}	-	12.13	-
P day[8]	1	1	1	12	12	12	14	14	14	35	35	-	0	-
							Methane							
L kg^{-1} VS	315.2^{bc}	313.2^{bc}	**308.4^{bc}**	**300.1^{b}**	329.8^{cd}	350.0^{d}	**207.5^{a}**	**307.6^{bc}**	**401.3^{e}**	314.8^{bc}	350.6^{d}	349.7	8.75	8.76
λ[2]	6.4^{cd}	**3.0^{ab}**	**4.5^{bc}**	7.1^{d}	**2.3^{ab}**	**2.0^{ab}**	8.1^{d}	**1.5^{a}**	**1.1^{a}**	-	-	9.20	0.90	0.87
k[3]	**0.044^{a}**	**0.070^{bc}**	**0.066^{b}**	**0.066^{b}**	**0.074^{cd}**	**0.077^{d}**	**0.045^{a}**	**0.074^{cd}**	**0.089^{e}**	-	-	0.056	0.0023	0.0023
U[4]	**9.7^{a}**	**16.7^{b}**	**16.9^{b}**	**17.2^{bc}**	**17.2^{bc}**	**19.1^{c}**	**7.9^{a}**	**16.2^{b}**	**24.2^{d}**	-	-	29.2	0.71	0.72
T_{50}[5]	20.9^{b}	12.6^{a}	13.5^{a}	12.8^{a}	12.3^{a}	11.5^{a}	**49.5^{c}**	11.0^{a}	9.5^{a}	-	-	15.2	1.73	1.64

[1] SEM: Standard error of mean; [2] λ: the lag phase (d); [3] k: the first order decay constant (d^{-1}); [4] U: the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}); [5] T_{50}: half-life i.e. time taken (d) to produce 50% of the gas production; [6] CH_4%: the methane proportion in biogas (vol. $vol.^{-1}$); [7] P_{Max} (hPa): the maximum pressure measured during the mBMP test; and [8] P day: the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett's adjustment) different from AMPTS values.

5.3.3. Silage

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 5.4, and the corresponding levels of significance are presented in Table 5.2. Biogas yield and the associated λ, k and U increased with an increase in headspace volume, although the scale of this response was generally greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume, and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a decline in OHPMR frequency when the headspace volume was 50 or 90 ml. The k and U values generally decreased while P_{Max} increased with a reduction in OHPMR frequency.

The methane yield and the associated U value increased with an increase in headspace volume except for daily OHPMR frequency. The k value increased while T_{50} decreased with an increase in headspace volume. Methane yield showed a variable response to declining OHPMR frequency across the three headspace volumes. The λ value generally decreased with a decline in OHPMR frequency. The k value increased while T_{50} decreased with a decline in OHPMR frequency when the headspace volume was 90 or 180 ml.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 5.4. Eight of the 11 mBMP treatments had methane yields that differed (P<0.05) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

Table 5.4. Biogas yield, methane yield and kinetic parameters when silage was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
						Biogas								
L kg^{-1} VS	550.4^{c}	602.2^{e}	619.8^{e}	523.5^{c}	596.3^{de}	691.3^{f}	407.0^{b}	560.1^{cd}	614.5^{e}	332.3^{a}	550.7^{c}	-	13.95	-
λ[2]	-0.5^{b}	-0.4^{b}	0.3^{c}	-0.7^{b}	-0.4^{b}	0.1^{c}	-2.7^{a}	0.4^{c}	0.3^{c}	-	-	-	0.13	-
k[3]	0.101^{cd}	0.108^{de}	0.144^{f}	0.083^{b}	0.097^{c}	0.116^{e}	0.065^{a}	0.092^{bc}	0.116^{e}	-	-	-	0.0034	-
U[4]	32.6^{c}	38.8^{d}	56.7^{g}	24.5^{b}	33.5^{c}	49.5^{f}	12.7^{a}	32.1^{c}	44.6^{e}	-	-	-	1.38	-
T_{50}[5]	8.1^{cd}	7.4^{bc}	5.8^{a}	10.4^{f}	8.7^{de}	7.2^{b}	16.6^{g}	9.2^{e}	7.3^{b}	-	-	-	0.24	-
CH_4%[6]	55.0^{cd}	52.6^{c}	46.9^{a}	56.4^{d}	55.3^{d}	49.7^{b}	59.1^{e}	54.5^{cd}	64.6^{f}	67.8^{g}	63.5^{f}	-	0.85	-
P_{Max}[7]	543.8^{d}	322.4^{b}	186.6^{a}	1196.2^{f}	765.1^{e}	453.7^{c}	1896.5^{h}	1493.9^{g}	792.9^{e}	3267.9^{j}	2155.3^{i}	-	22.38	-
P day[8]	9	9	9	9	9	9	14	14	14	35	35	-	-	-
						Methane								
L kg^{-1} VS	**302.7**^{bc}	**316.7**^{cd}	**290.5**^{b}	**295.1**^{bc}	330.0^{de}	343.4^{e}	**240.7**^{a}	**305.2**^{bc}	**397.2**^{f}	**224.9**^{a}	349.6^{e}	358.7	8.43	8.13
λ[2]	**5.4**^{g}	**2.3**^{e}	3.2^{f}	**1.7**^{d}	**2.5**^{e}	**2.7**^{ef}	**-0.5**^{a}	**1.1**^{c}	**0.5**^{b}	-	-	3.80	0.20	0.20
k[3]	0.064^{b}	0.068^{bc}	0.072^{cd}	0.071^{c}	0.077^{de}	**0.081**^{e}	**0.058**^{a}	**0.088**^{f}	**0.100**^{g}	-	-	0.069	0.0021	0.002
U[4]	18.8^{d}	**16.3**^{c}	18.6^{d}	**14.2**^{b}	18.4^{d}	21.1^{e}	**7.6**^{a}	**18.3**^{d}	**24.9**^{f}	-	-	20.8	0.61	0.59
T_{50}[5]	13.7^{d}	12.2^{bc}	11.8^{bc}	12.9^{cd}	11.9^{bc}	11.1^{b}	21.1^{e}	**9.6**^{a}	**8.7**^{a}	-	-	12.6	0.47	0.45

[1] SEM: Standard error of mean; [2] λ: the lag phase (d); [3] k: the first order decay constant (d^{-1}); [4] U: the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}); [5] T_{50}: half-life i.e. time taken (d) to produce 50% of the gas production; [6] CH_4%: the methane proportion in biogas (vol. $vol.^{-1}$); [7] P_{Max} (hPa): the maximum pressure measured during the mBMP test; and [8] P day: the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett's adjustment) different from AMPTS values.

5.3.4. Slurry

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 5.5, and the corresponding levels of significance are presented in Table 5.2. Biogas yield increased with an increase in headspace volume except for daily OHPMR frequency, and the scale of this response was highest for weekly OHPMR frequency. The associated kinetic parameters k and U generally increased while λ, T_{50}, CH_4% and P_{Max} generally decreased with an increase in headspace volume. Biogas yield generally decreased with a decline in OHPMR frequency when the headspace volume was 50 or 90 ml. The U value decreased while T_{50}, CH_4% and P_{Max} generally increased with a decline in OHPMR frequency.

There was no main effect of headspace volume or OHPMR frequency on methane yield, although individual treatment differences did occur. The significant effects on the associated kinetic parameters generally did not follow a linear progression in response to either headspace volume or OHPMR frequency.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 5.5. Two of the 11 mBMP treatments had methane yields that differed (P<0.05) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

Table 5.5. Biogas yield, methane yield and kinetic parameters when slurry was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
							Biogas							
L kg^{-1} VS	371.6^{bc}	413.4^{de}	387.7^{cd}	372.6^{bc}	387.1^{cd}	445.1^{ef}	296.3^{a}	362.4^{bc}	462.0^{f}	309.5^{a}	348.1^{b}	-	12.66	-
λ^2	4.2^{bc}	3.6^{a}	3.6^{a}	4.5^{c}	4.2^{bc}	3.6^{a}	4.5^{c}	4.2^{bc}	3.8^{ab}	-	-	-	0.16	-
k^3	0.071^{bc}	0.071^{bc}	0.092^{d}	0.067^{b}	0.070^{bc}	0.074^{c}	0.051^{a}	0.070^{bc}	0.070^{bc}	-	-	-	0.0014	-
U^4	24.7^{cde}	25.9^{de}	33.5^{g}	22.2^{bc}	23.8^{bcd}	29.2^{f}	11.4^{a}	21.8^{b}	27.5^{ef}	-	-	-	0.97	-
$T_{50}{}^5$	11.6^{bcd}	11.4^{bc}	9.4^{a}	13.2^{e}	12.5^{cde}	11.1^{b}	21.6^{f}	12.7^{de}	12.4^{cde}	-	-	-	0.37	-
$CH_4\%^6$	63.9^{c}	58.1^{b}	50.8^{a}	65.0^{c}	60.3^{b}	51.4^{a}	70.5^{d}	58.4^{b}	59.5^{b}	71.0^{d}	69.4^{d}	-	0.99	-
$P_{Max}{}^7$	547.7^{d}	328.4^{b}	184.8^{a}	1223.1^{g}	705.6^{e}	423.6^{c}	1866.9^{i}	1464.2^{h}	852.9^{f}	3195.5^{j}	1835.6^{i}	-	12.08	-
P day[8]	11	11	11	12	12	12	14	14	14	35	35	-	-	-
							Methane							
L kg^{-1} VS	237.5^{de}	239.7^{de}	**196.5^{a}**	242.3^{e}	233.4^{de}	228.9^{cde}	208.7^{ab}	211.6^{abc}	**275.0^{f}**	219.6^{bcd}	241.2^{e}	233.4	7.17	6.9
λ^2	**3.6^{bc}**	**2.8^{a}**	**3.5^{ab}**	5.6^{d}	6.3^{de}	6.4^{e}	**4.1^{bc}**	**4.2^{bc}**	**4.3^{c}**	-	-	6.40	0.24	0.24
k^3	**0.073^{de}**	**0.079^{f}**	**0.077^{ef}**	**0.065^{bc}**	**0.063^{b}**	**0.061^{b}**	**0.053^{a}**	**0.072^{de}**	**0.069^{cd}**	-	-	0.044	0.0018	0.0018
U^4	**19.1^{d}**	**18.9^{d}**	**20.7^{e}**	**15.7^{c}**	**15.8^{c}**	**15.7^{c}**	8.3^{a}	**13.3^{b}**	**16.5^{c}**	-	-	9.7	0.55	0.52
$T_{50}{}^5$	**11.8^{ab}**	**11.1^{a}**	**11.4^{a}**	**13.5^{cd}**	**13.8^{d}**	**13.6^{d}**	19.7^{e}	**12.2^{abc}**	**12.8^{bcd}**	-	-	20.9	0.42	0.45

[1] SEM: Standard error of mean; [2] λ: the lag phase (d); [3] k: the first order decay constant (d^{-1}); [4] U: the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}); [5] T_{50}: half-life i.e. time taken (d) to produce 50% of the gas production; [6] CH_4%: the methane proportion in biogas (vol. $vol.^{-1}$); [7] P_{Max} (hPa): the maximum pressure measured during the mBMP test; and [8] P day: the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett's adjustment) different from AMPTS values.

5.4. Discussion

The four substrates provided contrasting chemical compositions of their VS thereby broadening the conditions under which the objectives were assessed. The progressive decline in biogas yields in the mBMP test from similarly high values with cellulose and barley, intermediate values with silage and lowest values with slurry suggest a matching decline in AD of VS. This progression at least partially reflects the negative effects of corresponding increases in lignifications. These differences in extent of AD were accompanied by contrasting kinetics of digestion, with barley showing a particularly short lag phase and a rapid early rate of AD whereas slurry had a relatively long lag phase and slow early rate of AD.

The substrates also differed in the methanogenic nature of their digested VS (i.e. methane proportion in biogas) in the order slurry > silage > barley > cellulose (daily OHPMR frequency for 180 ml headspace bottles). Published methane proportions for slurry, silage, barley and cellulose are 56-62%, 54-56% (Triolo et al., 2011), 53% (Biteco, 2017) and 55-56% (Holliger et al., 2016; Wang et al., 2014), respectively.

5.4.1. mBMP

The VDI 4630 guideline (2006) recommends that when cellulose is digested in a BMP test it should produce a biogas yield of at least 80% of its theoretical maximum yield (i.e. 592 to 600 L kg^{-1} VS (VDI 4630 guideline, 2006)). In the present mBMP test this was achieved with eight of twelve treatments imposed, and these were mainly treatments that exhibited lower P_{max} values. However, all of the P_{max} values for the treatments imposed on cellulose and on the other three substrates exceeded the recommended maximum pressure of 100 hPa in VDI 4630 (2006) but most were below the maximum pressure of 3000 hPa recommended by the ABAI guideline group (Holliger et al., 2016)

In the present study, the effects of altering headspace volume, OHPMR frequency or both factors on biogas yield were most likely mediated through their individual or combined effects on headspace pressure. Using P_{max} as an estimate of the maximum headspace pressure that occurred, it is clear that a progressive increase in maximum headspace pressure correspondingly reduced biogas yield (Figure 5.1). Although this relationship was evident with all four substrates the apparent rate of decline in biogas yield was greatest for the substrate that also had the greatest yield at low headspace pressure (i.e. cellulose) and lowest for the substrate with the lowest biogas yield at low headspace pressure (i.e. slurry).

The negative impact of headspace pressure on biogas yield could be due to increased solubilisation of carbon dioxide in the medium as headspace pressure increased. According to Henry's Law, when the partial pressure of carbon dioxide increases in the headspace an increasing amount of this gas will dissolve in the medium and thus less of it will be released at the time of OHPMR. This agrees with the findings of a recent meta-analysis of methodological factors affecting *in vitro* rumen fermentation systems (Maccarana et al., 2016) where increasing headspace pressure also resulted in reduced gas production. Whereas an increased concentration of carbon dioxide might be expected to reduce the pH of the medium, potentially perturbing some microbial activity, the robust buffering provided to the medium in this study appeared to prevent such a change in pH.

A negative effect of presumably very high P_{max} values was evident with the treatment that combined the smallest headspace volume (50 ml) with the lowest OHPMR frequency (solely after 35 d). In this case, the butyl rubber stopper on all the incubation bottles ruptured resulting in loss of data for this treatment.

The methane yield for the 11 successfully completed treatments with cellulose ranged from 70-112% and 65-99% of the minimum yields recommended by VDI 4630 (2006) and Holliger et al. (2016), respectively. The similar methane yields recorded for cellulose, barley and silage but the lower yields for slurry (during daily OHPMR frequency for 180 ml headspace volume) relate to corresponding published values of 259 to 366 L kg^{-1} VS for cellulose (McEniry & O'Kiely, 2013; Wang et al., 2014), 304-380 L kg^{-1} VS (Biteco, 2017; Braun, 2007; Heiermann et al., 2002; Rudolf et al., 2009), 229-400 L kg^{-1} VS (McEniry & O'Kiely, 2013; Wall et al., 2013) and 125-239 (Triolo et al., 2011; Wall et al., 2013).

The weak relationship between headspace pressure and methane yield contrasts with the clear negative relationship between headspace pressure and biogas yield (Figure 5.1). Since biogas is composed mainly of carbon dioxide and methane their different responses to increasing headspace pressure likely reflect the combined effects of the much greater solubility of carbon dioxide than methane (88 ml CO_2 per 100 ml H_2O vs. 3.5 ml CH_4 per 100 ml H_2O; O'Neil (2013)) and their different Henry's Law solubility constants $3.3x10^{-2}$ mol m^{-3} hPa^{-1} for CO_2 and $1.4x10^{-3}$ mol m^{-3} hPa^{-1} for CH_4; Sander (2015)). The latter indicate that a markedly greater increase in solubility of carbon dioxide occurs in response to an increase in its partial pressure than occurs for methane. This, in turn, should result in an increase in the concentration of methane in biogas as headspace pressure increases, and Figure 5.2 shows that this occurred. These findings agree with Maccarana et al. (2016) who also reported that increasing headspace pressure had little effect on methane yield but increased the concentration of methane in the headspace gases in *in vitro* rumen digestion systems.

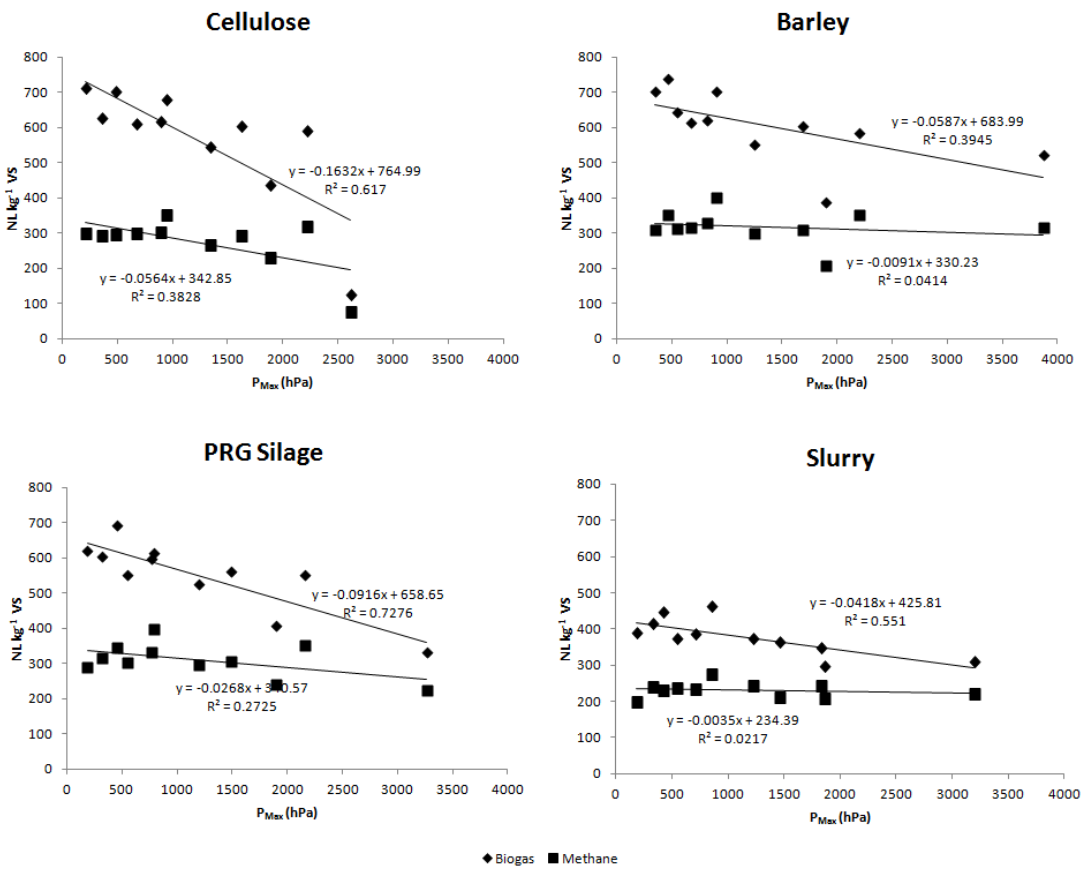


Figure 5.1. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and biogas and methane yields during 35 day anaerobic digestion.

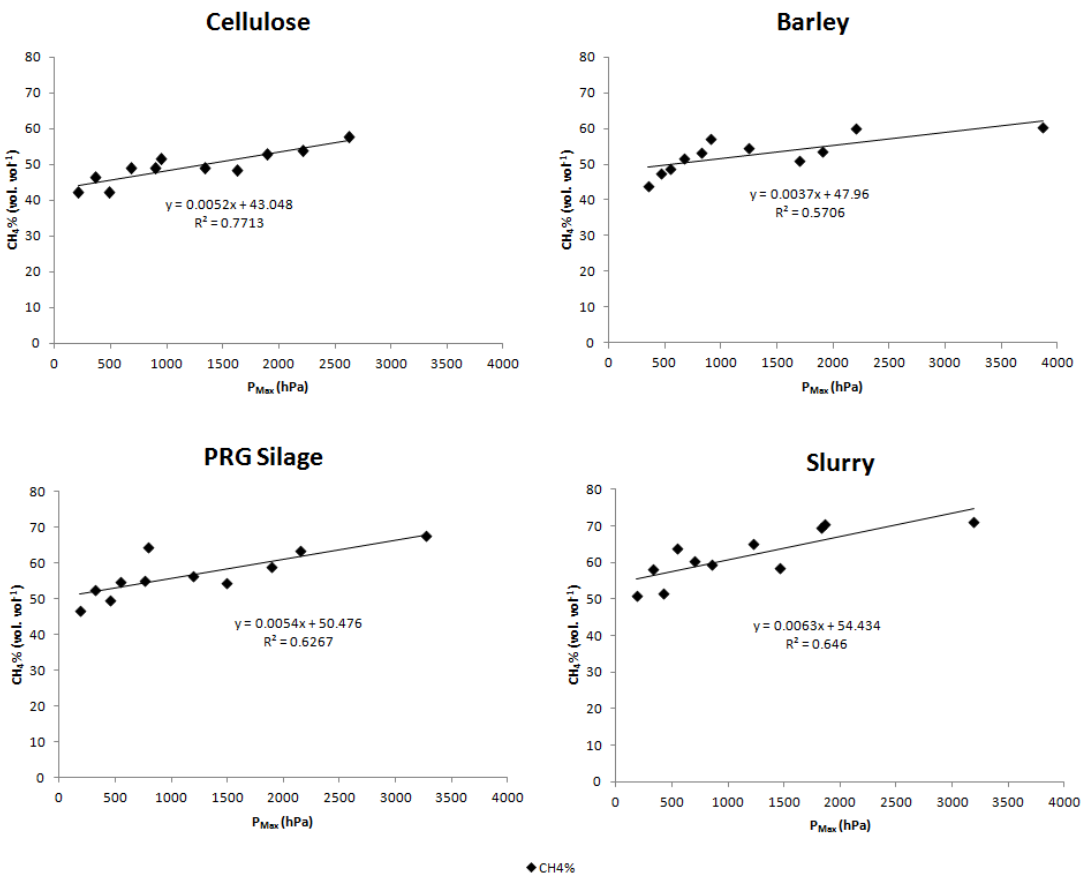


Figure 5.2. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and methane proportion in biogas during 35 day anaerobic digestion.

Headspace pressure effects alone appear not to provide a full explanation for methane yield outcomes. For example, for the three substrates that produced higher methane yields than slurry increasing headspace volume generally increased methane yield when OHPMR frequency was less than daily, but for slurry that produced a lower methane yield the headspace volume did not have a clear effect. In contrast, OHPMR frequency had little direct effect on methane yield. Thus the two factors (headspace volume and OHPMR frequency) seem to differ in the mechanisms by which they affect methane yield.

A direct comparison of the results of the present study and those of Yilmaz (2015) is difficult since different substrates, headspace volumes and OHPMR frequencies were used. However, when glucose (Yilmaz (2015) and cellulose (present study) were used as substrates, there was a general trend for methane yield to increase in response to increasing headspace volume for each third day OHPMR frequency. Also, reducing OHPMR frequency reduced methane yield only for incubation bottles with the smallest headspace volume. The results of the present study also agree with Valero et al. (2016) that headspace pressure can differentially influence the methane yield with contrasting substrates.

5.4.2. mBMP vs. AMPTS

Although the methane yields produced for many OHPMR frequency and headspace volume combinations when cellulose was digested by mBMP test were below VDI 4630 guideline (2006) and (Holliger et al. (2016) targets, the values obtained for barley, silage and slurry were 59-115%, 67-111% and 84-118% of the corresponding values recorded using AMPTS. Furthermore, the similar methane yields for barley and silage but the much lower yield for slurry when using AMPTS was repeated with eight of the 11 successfully completed mBMP treatments.

Taking the methane yields obtained using AMPTS as reference target values, two of the mBMP treatments produced comparable yields to AMPTS across the three contrasting substrates (Table 5.6). First, when the mBMP test had an each third day OHPMR frequency and a headspace volume of 180 ml it produced 100, 96 and 98% of the methane yields recorded using AMPTS for barley, silage and slurry, respectively. Furthermore, the methane yield relativities for barley, silage and slurry reflected those obtained by AMPTS (barley:silage:slurry of 1.53:1.50:1.00 and 1.50:1.54:1.00 for this mBMP treatment and AMPTS, respectively). Second, when the mBMP

test had an OHPMR solely after 35 d and a headspace volume of 180 ml it produced 100, 97 and 103% of the methane yields of AMPTS for barley, silage and slurry, respectively and had a barley:silage:slurry methane yield relativity of 1.45:1.45:1.00. For these two mBMP treatments, the option of each third day OHPMR frequency plus 180 ml headspace volume requires a greater and more frequent labour input but provides the opportunity to produce digestion kinetics results. It also poses a lower risk of septum failure due to high headspace pressure accumulation.

Table 5.6. Methane yields using mBMP for 180 ml headspace volume bottles and OHPMR frequencies of each third day and solely after 35 d and the corresponding yields with AMPTS.

	Methane yield (L kg^{-1} VS)		
	mBMP		AMPTS
	Each 3 day OHPMR	After 35 d OHPMR	
Cellulose	296.4	319.0	NA
Barley	350.0	350.6	349.7
Silage	343.4	349.6	358.7
Slurry	228.9	241.2	233.4

OHPMR: overhead pressure measurement and release and NA: not available.

5.5. Future perspectives

Judicious consideration is required when selecting a BMP technique as the decision can impact on the methane yields recorded and on the relative values attributed to different substrates. This study highlights the importance of using substrates with contrasting digestion characteristics when assessing the effects of factors of interest on biogas and methane output. Furthermore, it is important that resultant publications should report the headspace volume, OHPMR frequency and other relevant factors used in their BMP tests. Finally, where an accurate estimate of biogas yield is required, it is recommended that the duration of mBMP tests be extended sufficiently to allow dissolved CO_2 be retrieved.

5.6. Conclusion

Headspace volume and OHPMR frequency affected headspace pressure and the latter had a negative effect on biogas yield in a mBMP test. Headspace pressure had relatively little effect on methane yield but had a clear positive effect on methane concentration.

Accepting the methane yields obtained using the AMPTS system as reference target values, two mBMP treatments replicated these targets – OHPMR frequencies of each third day or solely after 35 d, in each case with a headspace volume of 180 ml (70 ml total medium).

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6. Impacts of characteristics of the feedstocks grass silage and cattle slurry on the cost of methane production

Impacts of characteristics of the feedstocks grass silage and cattle slurry on the cost of methane production

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Abstract

Feedstocks characteristics and their provision cost can have significant impact on the cost of methane production in an anaerobic digestion (AD) facility. This study investigated the impacts of changing grass silage characteristics, grass silage and cattle slurry provision costs and their binary mixing ratios on the cost of methane production from an on-farm AD facility. The feedstock provision cost contributed about half of the total cost of methane production when the AD facility solely operated on grass silage. The management targets for grass silage produced on a farm for its AD facility in order to reduce the cost of methane production are high yields of biomass per harvest,that the herbage is of high digestibility and undergoes efficient fermentation during ensiling, and that aerobic deterioration of silage during its feedout is minimised. The total cost of methane production from mono-digestion of cattle slurry, compared to grass silage, was more than double when it was supplied free of cost and was 70% higher when a gate fee of €70 t^{-1} TS was charged, due to its low total solids, volatile solids and methanogenic potential. For co-digestion of grass silage and cattle slurry, the total cost of methane production progressively increased as the proportion of slurry in the co-digested feedstocks mixture increased. Antagonistic and synergistic methanogenesis resulted in a corresponding 6% higher and 5% lower total cost of methane production during co-digestion of grass silage and cattle slurry (at

silage:slurry volatile solids ratio of 0.8:0.2) compared to the binary mixture without these effects. During co-digestion of grass silage and cattle slurry the emphasis should be to maximize the inclusion rate of grass silage commensurate with maintaining an efficient and stable long-term digestion process.

Keywords: Economic analysis**;** Co-digestion; Synergy; Antagonism; Grass silage; Cattle slurry

6.1. Introduction

Renewable biomass supplies 10-15% of the world's sustainable energy (Murphy et al., 2011). Agricultural-based anaerobic digestion (AD) is one of the ways to utilize this biomass, and it has been adopted by countries such as Germany for renewable energy production and green-house gas mitigation (Massé et al., 2011). For Ireland, Wall et al. (2013) reported 10% renewable energy in the transport sector can be supplied by utilizing 1.1% of Irish grassland through AD.

Compared to the indigenous European first generation liquid biofuels such as wheat ethanol and rapeseed biodiesel, methane production from grass has been reported to produce more energy in fuel per hectare, be superior in energy balance, be economical under good farm management, and be more sustainable (i.e. more greenhouse gas savings) (Korres et al., 2010; McEniry et al., 2011; Smyth et al., 2009). Grass can be a major AD feedstock in Ireland since over 90% of its agricultural land is under grassland (CSO, 2013). This grassland has the potential to produce biomass in excess of current or expected livestock requirements (McEniry et al., 2013). At present, most grass produced in Ireland is utilized by ruminants through grazing or as ensiled grass. Ruminant livestock that are managed within these grassland-based systems are accommodated indoors for at least part of the year, thereby producing slurry that is normally spread back on this grassland but that could also be easily utilized for AD.

Mono-digestion of grass silage carries a risk of process imbalance over an extended duration at high organic loading rates (Thamsiriroj et al., 2012). However, it can be co-digested with cattle slurry, a feedstock which usually produces a lower methane yield compared to grass silage as the livestock have already utilised much of the more easily digestible organic components in the feeds they consumed (Triolo et al., 2013). This combination of feedstocks can complement each other and enhance the longevity of stable and productive AD (Wall et al., 2014). Furthermore, co-digestion of grass silage and cattle slurry may result in synergistic (i.e. the mixture produces more methane than the arithmetically calculated yield from sole feedstocks) or antagonistic (i.e. the mixture produces less yield than predicted from sole feedstocks) methanogenesis (Himanshu et al., 2018; Himanshu et al., under review; Wall et al., 2013).

Methanogenic potential of grass silage and its production cost can be effected by several factors such as biomass yield and total solids digestibility (TSD), harvest date, preservation and farm management (McEniry et al., 2014). Similarly, the methanogenic potential of cattle slurry will differ depending on a range of factors including the cattle type (Triolo et al., 2013), diet type (Amon et al., 2007; Hellwing et al., 2014), dilution with other materials or the presence of antibiotics (Varel & Hashimoto, 1981) and the duration and conditions of slurry storage (Browne et al., 2015). In addition to slurry frequently being available to an on-farm AD facility free of a production cost it could in other circumstances be available either to purchase or with a gate fee.

The feedstock provision cost contributes a significant share to the total cost of methane production in an AD facility (Smyth et al., 2010). However, the feedstock characteristics and its provision cost are too often assumed to be constant during methane production (Dennehy et al., 2017; Smyth et al., 2010). No previous reports have been found which investigated the impacts of changing feedstock characteristics, feedstock provision costs and feedstock binary mixing

ratios on the cost of methane production in an AD facility. Thus, the aims of this study were to quantify the responses in methane production costs due to changes in (a) grass silage characteristics and its provision cost, (b) cattle slurry provision cost, (c) ratios of silage and slurry volatile solids (VS) in binary mixtures (and the presence of synergy or antagonism), and (d) operational efficiency of the AD facility.

6.2. Methodology

This study was undertaken on the assumption of an AD facility located on a grassland farm that also contained other enterprises requiring grass. The latter included a cattle production enterprise where animals were accommodated in slatted-floor housing for at least part of the year, and where slurry was collected in tanks beneath the slatted-floors. The quantities of grassland and slurry available were considered non-limiting to the operation of the AD facility, and any costs or benefits associated with digestate were omitted. The AD facility was considered to have a fixed volumetric capacity for feedstock digestion and was operated at a common constant hydraulic retention time (HRT) across all scenarios. The impacts of upgrading biogas to biomethane or of producing electricity from biogas were not considered. The study was undertaken in the context of a single year of operation of the farm and AD facility.

6.2.1. Baseline scenarios

The capital cost information used in the study was based on a farm scale AD facility located at Teagasc, Grange, Dunsany, Co. Meath, Ireland. It has a 1500 m^3 single-stage AD reactor which can co-digest grass silage and cattle slurry. Three baseline scenarios were considered on the basis of the feedstocks utilized.

6.2.1.1. Silage only baseline

The AD facility was assumed to utilize grass silage as its sole feedstock. The total solids (TS), VS and methane yield of grass silage were assumed to be 230 g kg^{-1}, 920 g kg^{-1} TS and 358 L CH_4 kg^{-1} VS, respectively. These values are within the range for perennial ryegrass silages in Ireland reported by Himanshu et al. (2018); Nizami and Murphy (2011); Nizami et al. (2012); Nolan (2017); Wall et al. (2014); Wall et al. (2013); Wall et al. (2015) and (Xie, 2012). The methane proportion (CH_4%; vol. vol^{-1}) in biogas was assumed to be 55% (Smyth et al., 2010). The AD facility can digest approximately 5000 t y^{-1} of fresh grass silage annually at an organic loading rate (OLR) of 1.94 kg VS m^{-3} d^{-1} and a HRT of 75 d. The OLR and HRT are based on the planned operating parameters of the AD facility at Teagasc, Grange.

Silage production for AD was assumed to be produced from grass harvested on the 29 May, with subsequent grass growth during the year being used by other enterprises on the farm. The mown grass received approximately 6 h of field-wilting, was precision-chop harvested with no additive applied, and was then ensiled beneath two layers of black polyethylene sheeting in a walled concrete bunker silo. The grass TS yield was considered to be 6.65 t TS ha^{-1} (O'Kiely, 2004a; O'Kiely, 2004b) and with associated field and in-silo losses of 3.0 and 17.5%, respectively. The TSD at harvest was assumed to be 756 g kg^{-1} TS (O'Kiely, 2004a; O'Kiely, 2004b), and with a 1% loss in this value occurring during harvest and ensilage. The resultant silage was assumed to be well preserved and aerobically stable. Silage effluent produced in this or other scenarios was not utilised for AD. Further details of the baseline scenario are presented in Table 6.1.

Table 6.1. Grass silage characteristics and the silage provision and methane production costs for the baseline and alternative scenarios.

Scenarios	Silage baseline	Biomass yield		Biomass TSD		Crop harvest date		Bad silage preservation	Aerobic deterioration
		Low	High	Low	High	Early	Late		
Biomass yield (kg TS ha^{-1})	6,649	5,216	8,082	6,649	6,649	4,651	8,354	6,649	6,649
Silage TS yield at feeding (kg TS ha^{-1})	5,321	4,174	6,468	5,321	5,321	3,722	6,685	4,250	3,586
Biomass TSD (g kg^{-1})	756	756	756	735	778	792	715	756	756
Silage TSD at feeding (g kg^{-1})	749	749	749	728	770	784	708	NA	NA
Silage methane yield (m^3 CH_4 t^{-1} VS)	358	358	358	354	363	366	349	372	265
Methane produced (m^3 y^{-1})	379,669	379,669	379,669	374,850	384,489	387,701	370,260	394,035	280,900
Silage provision cost (€ t^{-1} TS)	169	216	139	169	169	237	137	212	251
Total silage provision cost (€ y^{-1})	194,829	248,336	160,280	194,829	194,829	273,204	158,022	243,905	289,089
Total cost of methane production (€ m^{-3})	1.081	1.222	0.990	1.095	1.067	1.261	1.009	1.166	1.797
Feedstock component cost (€ m^{-3})	0.513	0.654	0.422	0.520	0.507	0.705	0.427	0.619	1.029
AD facility component cost (€ m^{-3})	0.568	0.568	0.568	0.575	0.561	0.556	0.582	0.547	0.767

AD - anaerobic digestion, NA - not available (since the references cited provided methane yield but not TSD values), TSD - total solids digestibility, TS - total solids and VS - volatile solids

Fixed capacity of AD reactor limits silage input to 5,009 t y^{-1} at an OLR of 1.94 kg VS m^{-3} d^{-1}. Operational and capital cost of the AD facility is €215,563 y^{-1}.

6.2.1.2. Slurry only baseline

The AD facility was assumed to utilize cattle slurry as its sole feedstock (Table 6.2). The TS, VS, methane yield and CH_4% were assumed to be 88 g kg^{-1}, 776 g kg^{-1} TS, 186 L CH_4 kg^{-1} VS and 64.5%, respectively. These values are within the range for cattle slurry collected in Ireland as reported by Browne et al. (2015); Himanshu et al. (2018); Himanshu et al. (under review); Wall et al. (2013) and Wall et al. (2014). The HRT was kept at 75 d, the same as for the silage only baseline, and this resulted in a maximum OLR of 0.91 kg VS m^{-3} d^{-1}. At these operating parameters the AD facility can digest approximately 7,300 t slurry y^{-1}. It was assumed that slurry, as a by-product of a cattle enterprise on the farm, was available free of cost at the AD facility.

Table 6.2. Cattle slurry provision and methane production costs for the baseline and alternative scenarios.

Scenarios	Slurry baseline	Slurry gate fee	Slurry purchased
Slurry provision cost (€ t^{-1} TS)	0	-70	70
Slurry added (t y^{-1})	7,307	7,307	7,307
Slurry methane yield (m^3 CH_4 t^{-1} VS)	186	186	186
Methane produced (m^3 y^{-1})	92,814	92,814	92,814
Total slurry provision cost (€ y^{-1})	0	-45,013	45,013
Total cost of methane production (€ m^{-3})	2.323	1.838	2.808
Feedstock component cost (€ m^{-3})	0.000	-0.485	0.485
AD facility component cost (€ m^{-3})	2.323	2.323	2.323

AD - anaerobic digestion and TS - total solids

Fixed capacity of AD reactor limits slurry input to 7,307 t y^{-1}. Operational and capital cost of the AD facility is €215,563 y^{-1}.

6.2.1.3. Silage and slurry co-digestion baseline

The AD facility was assumed to co-digest grass silage and cattle slurry at silage:slurry ratios of 0.8:0.2, 0.6:0.4,0.5:0.5,0.4:0.6 and 0.2:0.8 on a VS gravimetric basis, and with no synergistic or antagonistic co-digestion effects on methanogenesis. The TS, VS, methane yield and CH_4% of silage and slurry were as already mentioned for mono-digested feedstocks. The HRT of the binary mixtures remained constant at 75 d while the OLR was progressively reduced as the proportion of slurry in the co-digested feedstocks mixture increased in order to cope with the increased volume of feedstock due to slurry inclusion (Table 6.3).

Table 6.3. Silage and slurry characteristics, and feedstock provision and methane production costs during their co-digestion for the baseline and alternative scenarios.

Scenarios	No antagonism or synergy							Antagonism		Synergy	
Silage:Slurry on VS basis	1.0:0	0.8:0.2	0.6:0.4	0.5:0.5	0.4:0.6	0.2:0.8	0:1.0	0.8:0.2	0.5:0.5	0.8:0.2	0.5:0.5
Silage added (t y^{-1})	5,009	3,270	2,071	1,602	1,196	527	0	3,270	1,602	3,270	1,602
Slurry added (t y^{-1})	0	2,533	4,279	4,965	5,557	6,535	7,307	2,533	4,965	2,533	4,965
OLR (kg VS m^{-3} d^{-1})	1.94	1.58	1.33	1.24	1.16	1.02	0.91	1.58	1.24	1.58	1.24
Methane produced (m^3 y^{-1})	379,669	280,038	211,339	184,504	161,206	122,970	92,814	264,635	164,208	295,440	204,799
Total silage provision cost (€ y^{-1})	194,829	127,190	80,560	62,318	46,502	20,507	0	127,190	62,318	127,190	62,318
Total slurry provision cost (€ y^{-1})	0	0	0	0	0	0	0	0	0	0	0
Total cost of methane production (€ m^{-3})	1.081	1.224	1.401	1.506	1.626	1.920	2.323	1.295	1.692	1.160	1.357
Feedstock component cost (€ m^{-3})	0.513	0.454	0.381	0.338	0.288	0.167	0.000	0.481	0.380	0.431	0.304
AD facility component cost (€ m^{-3})	0.568	0.770	1.020	1.168	1.337	1.753	2.323	0.815	1.313	0.730	1.053

AD - anaerobic digestion, OLR - organic loading rate and VS - volatile solids

OLR was progressively reduced as the proportion of slurry in the co-digested feedstocks mixtures increased in order to cope with the increased volume of feedstock due to slurry inclusion and the fixed capacity of the AD reactor. Operational and capital cost of the AD facility is €215,563 y^{-1}.

6.2.2. Operational and capital costs

6.2.2.1. Grange feed costing model (GFCM) and feedstock cost

The Grange feed costing model (GFCM) was used to calculate the cost of providing grass silage to the AD facility. The GFCM was developed to identify the relative costs of feeds produced for ruminants (Finneran et al., 2010). It is a static, spreadsheet-based, agro-economic simulation model for evaluation of the physical and financial performance of alternative feed crop production and utilization options in Ireland and has been described in detail by McEniry et al. (2011). The GFCM employs a full bottom-up costing approach to calculate total feedstock cost and includes all fixed and variable production (e.g. sowing and crop management) and utilization (e.g. storage and labour) costs associated with the feedstock. The input costs in the model were updated for yearly inflation according to the CSO price index (CSO, 2017) using 2010 as the base year. Key commodities such as fertiliser price were corrected for inflation using category specific inflation indices while all other input costs were corrected using the general agricultural input index (CSO, 2017). The land charges were updated according to the Society of Chartered Surveyors Ireland/Teagasc (2017) while the contractor prices for harvesting and ensiling were updated according the Irish Farmers Journal (IFJ (2017)).

6.2.2.2. AD facility

The annual capital cost of the AD facility was calculated using equation 1 (Smyth et al., 2010).

$$Annual\ capital\ cost\ (€\ y^{-1}) = [P(1+r)^{N}r]/[(1+r)^{n}-1] \quad \text{(Equation 6.1)}$$

Where, P is the principal (€), r is the rate of return (5%) and N is the lifetime of the project (assumed to be 15 years for this facility).

The operational cost of the AD facility includes labour, electricity (for macerating, mixing and pumping), heating fuel (either biogas or imported fuel to meet the parasitic heat demand), feeding (from the nearby silo or slurry tanks to the AD facility) and maintenance. It does not include a combined heat and power engine or other equipment for using methane. The operational cost was assumed to be 10% of the capital cost and an additional 6.67% of the capital cost was included for depreciation of the AD facility (Smyth et al., 2010). The annual capital and operational (including depreciation) costs of the AD facility were € 78,953 y^{-1} and € 136,610 y^{-1}, respectively, giving an annualised total of €215,563.

6.2.3. Alternative scenarios investigated

Several factors related to the provision of grass silage such as biomass yield and TSD, harvest date, preservation and aerobic deterioration impact on the cost of silage utilised and its methanogenic potential. Thus, relative to the baseline scenario, alternative scenarios were investigated to quantify their impacts on the costs of the feedstock and thus on the cost of methane production:

a. Biomass yield alone. Minimum and maximum primary growth grass biomass yields on 29 May of 5.22 and 8.08 t TS ha^{-1}, respectively, were taken from Teagasc six year field trials (O'Kiely, 2004a; O'Kiely, 2004b), with the TSD and thus the methane yield being held constant (Table 6.1).
b. Biomass TSD alone. Minimum and maximum primary growth grass biomass TSDs on 29 May of 735 and 778 g kg^{-1} TS, respectively, were taken from Teagasc six year field trials (O'Kiely, 2004a; O'Kiely, 2004b), with the TS yield being held constant. The methane yield, to reflect these alternative TSD values, was back-calculated using the relationship between TSD and methane yield reported by McEniry and O'Kiely (2013) and then

adjusted for the baseline methane yield of silage (i.e. 358 L CH_4 kg^{-1} VS) on a pro rata basis according to Himanshu et al. (2018) (Table 6.1).

c. Harvest date (biomass yield and TSD change simultaneously). Harvest dates of 15 May and 12 June, with both biomass TS yield and TSD changing as predicted by the GFCM (Finneran et al., 2010). The methane yield's were adjusted as described above for changes in biomass TSD (Table 6.1).
d. Bad standard of silage preservation. Silage alternatively undergoes bad preservation resulting in an additional 16.6% TS in-silo loss relative to the baseline (i.e. total in-silo loss of 34.1%) but with a 3.9% increase in methane yield due to an altered profile of silage fermentation products (McEniry et al., 2014).
e. Aerobic deterioration. Silage alternatively undergoes aerobic deterioration for 5 d at the silage feed–face during its feedout resulting in an additional 26.9% TS in-silo loss relative to the baseline (i.e. total in-silo loss of 44.4%) (O'Kiely & Lenehan, 1996) plus a 26% decrease in methane yield (Baserga & Egger, 1997) (Table 6.1).

Relative to the baseline scenario for cattle slurry, alternative scenarios considered were a gate fee of €70 t^{-1} TS and the slurry being purchased at €70 t^{-1} TS (Table 6.2).

Relative to the silage and slurry co-digestion baseline scenario, antagonistic and synergistic effects on methanogenesis at silage:slurry ratios of 0.8:0.2 and 0.5:0.5 were considered. Both antagonism and synergy were assumed to be symmetrical (maximum deviation from linear response at a 0.5:0.5 ratio and returning at similar rates above and below this ratio towards the sole component outputs) as reported by Himanshu et al. (2018) with a deviation of ±11% (Wall et al., 2013) at silage:slurry of 0.5:0.5 and ±5.5% at silage:slurry of 0.8:0.2.

In addition to the above-mentioned scenarios consideration was also given to the impact of the AD facility operating at a lower or higher efficiency than in the baseline scenarios, resulting in corresponding lower or higher methanogenic potential values for silage, slurry and their binary mixture at a silage:slurry ratio of 0.5:0.5 (VS gravimetric basis). The low and high methane yield for silage were assumed to be 300 and 400 L CH_4 kg^{-1} VS, respectively while for slurry these values were assumed to be 120 and 240 L CH_4 kg^{-1} VS, respectively (Table 6.4). These methane yield were approximately the highest and lowest values reported in Ireland by Browne et al. (2015); Himanshu et al. (2018); Himanshu et al. (under review); Nizami & Murphy (2011); Nizami et al. (2012); Nolan (2017); Wall et al. (2013); Wall et al. (2014); Wall et al. (2015) and Xie (2012).

Table 6.4. Silage and slurry characteristics, and methane production costs, during mono- or co-digestion of grass silage and cattle slurry when there was a lower or higher efficiency of methanogenesis than in the baseline scenarios.

	Grass silage alone		Cattle slurry alone		Silage and slurry co-digestion*	
	Low methane yield	High methane yield	Low methane yield	High methane yield	Low methane yield	High methane yield
Silage added (t y^{-1})	5,009	5,009	0	0	1,602	1,602
Slurry added (t y^{-1})	0	0	7,307	7,307	4,965	4,965
OLR (kg VS m^{-3} d^{-1})	1.94	1.94	0.91	0.91	1.24	1.24
Silage methane yield (m^3 CH_4 t^{-1} VS)	300	400	-	-	300	400
Slurry methane yield (m^3 CH_4 t^{-1} VS)	-	-	120	240	120	240
Methane produced (m^3 y^{-1})	318,000	424,000	59,880	119,760	142,401	216,992
Silage provision cost (€ y^{-1})	194,829	194,829	0	0	62,318	62,318
Slurry provision cost (€ y^{-1})	0	0	0	0	0	0
Total cost of methane production (€ m^{-3})	1.291	0.968	3.600	1.800	1.951	1.281
Feedstock component cost (€ m^{-3})	0.613	0.460	0.000	0.000	0.438	0.287
AD facility component cost (€ m^{-3})	0.678	0.508	3.600	1.800	1.514	0.993

AD - anaerobic digestion and VS - volatile solids

*Co-digestion at a VS ratio for silage and slurry of 0.5:0.5 and without antagonistic or synergistic effects on methanogenesis.

6.3. Results and discussion

No costing study such as this will encompass all combinations of factors that occur on farms producing methane in an AD facility, and thus the assumptions governing this study were selected to optimally address the stated objectives within a context familiar on Irish farms. The assumed grassland farm had other enterprises requiring grass which meant that only the first cut of grass silage rather than the full annual production of grass was available for AD in the fixed volumetric capacity AD facility. However, the accompanying cattle enterprise provided sufficient slurry to meet any needs of the AD facility. Even though it has previously been shown that collection and digestion of silage effluent and recycling of digestate from the AD facility to the grassland from which the silage was produced are economically appropriate (McEniry et al., 2011) these practices were omitted in order to allow the full cost of grass silage provision emerge. Finally, grass silage was costed using a full bottom-up approach which included all fixed and variable components of feedstock provision, including a commercial land charge. It therefore produced silage provision costs that were greater than grass silage can sometimes be purchased from farms, an outcome previously demonstrated by McEniry et al. (2011).

6.3.1. AD facility mono-digesting silage

Silage characteristics and the silage provision and methane production costs for baseline and associated alternative scenarios are presented in Table 6.1. Among the six types of silage-related scenario presented, silage baseline, biomass yield, biomass TSD and crop harvest date were considered to operate under 'good management' while bad silage preservation and aerobic deterioration would reflect compromised farm management. The silage provision cost for the silage baseline scenario was €169 t^{-1} TS where fertilizers (including spreading), harvesting (including ensiling), land charges, farm facilities (such as silos), reseeding and grassland

management (e.g. weed control) contributed 38, 34, 17, 6, 3 and 2% to the total cost, respectively. The feedstock component of the total cost of methane production accounted for 47% for the silage baseline scenario.

Biomass yield and biomass TSD can differ across years even when harvested on the same date and subjected to constant management (O'Kiely, 2004a; O'Kiely, 2004b) and this variation clearly impacts on the cost of methane production. Thus, for example, a higher biomass yield can reduce the feedstock provision cost and consequently the cost of producing methane while a higher biomass TSD can directly increase the methane yield (McEniry & O'Kiely, 2013) and consequently reduce the cost of producing methane. In comparison to the silage baseline scenario, the higher biomass yield and higher biomass TSD scenarios had 8% and 1% lower total costs of methane production, respectively. Thus, normal year-to-year variation in biomass yield *per se* is likely to have a greater effect on the cost of methane production than corresponding variation in TSD and, in the present study, the high biomass yield scenario had the lowest total cost of methane production among all silage mono-digestion scenarios considered.

Crop harvest date can simultaneously impact on both biomass yield and TSD. An early harvest can result in a lower biomass yield with a higher TSD while a late harvest will usually result in a higher biomass yield with a lower TSD (McEniry et al., 2013). Although, the late harvest scenario considered in this study resulted in a 3% decrease in methane yield relative to the baseline scenario, the 26% increase in silage TS at feeding reduced the cost of methane production by 7%. Thus, were the commercial objective solely to reduce the cost of methane production then delaying harvest date in order to increase biomass yield would be justified. However, the profitability of an on-farm AD facility will depend on a number of factors including the monetary value of the methane produced each year (m^3 methane produced y^{-1} * €

earned m^{-3} methane), and in the current study later harvesting reduced the quantity of methane produced per year because of its lower methane yield, the fixed capacity of the AD facility and the constant HRT. Thus, the optimum harvest date for the AD facility or for the whole farm business may not correspond with the harvest date that results in the lowest cost of methane production.

Although 'good farm management' is highly desirable it can sometimes be inadvertently compromised (McElhinney et al., 2016). Two cases of compromised farm management considered were where silage was badly preserved and where silage aerobically deteriorated during silage feedout. Bad preservation (e.g. clostridial fermentation) will increase the loss of fully digestible VS during ensilage (McEniry et al., 2014) but will also produce some fermentation products of elevated methanogenic potential (Weissbach, 2009). In the bad silage preservation scenario, the silage TS yield at feeding was reduced by 20% compared to baseline scenario (causing a 21% increase in the feedstock component of the total cost of methane production) while the methane yield was increased by 4%. Overall, this caused a 8% increase in the total cost of methane production. It is noteworthy that badly preserved silage is highly undesirable for cattle farmers as it is unpalatable for livestock. Therefore, because livestock reduce their consumption of silage that has preserved badly (Flynn, 1981) and what silage they do consume has a reduced nutritive value, the commercial value when such silage is traded among livestock farmers is relatively low. The purchase of this silage, however, could be commercially attractive as a feedstock for an AD facility since the OLR is at the discretion of the facility manager and its methane yield should be greater than normal for its harvest date. Overall, therefore, whereas bad preservation of silage is an unwanted outcome when grass silage is being

produced for AD on the farm with the AD facility, it may in contrast provide an economically advantageous opportunity if it is purchased at low cost from another farm.

Aerobic deterioration of silage is highly undesirable as it will cause the loss of easily digestible and energy rich VS resulting in reduced TS recovery and methane yield (Baserga & Egger, 1997; Woolford, 1990). Furthermore, mycotoxins may be produced during aerobic deterioration (Woolford, 1990) and they have been shown to restrict microbial activity and cause foaming in the rumen (Moeller et al., 2012). Similar issues may also arise in the AD facility (Moeller et al., 2012). In the present study, the five day aerobic deterioration of silage resulted in a 33% reduction in silage TS yield and a 26% reduction in methane yield, causing a 66% increase in the total cost of methane production. The imperative of greatly restricting the aerobic deterioration of grass silage to be used for AD is highlighted by this scenario having the highest cost for the feedstock component, for the AD facility component and thus for the total cost of methane production among all the silage scenarios considered.

6.3.2. AD facility mono-digesting slurry

The cattle slurry provision costs and methane production costs when running the AD facility solely on cattle slurry are presented in Table 6.2. The total cost of methane production was more than double the silage baseline scenario cost even though the slurry was assumed to be supplied free of charge to the AD facility. This reflects the reduced total input of VS to the AD facility due to the low TS and VS contents of the slurry and its low methane yield.

If the AD facility is located where the demand for slurry greatly exceeds its availability (e.g. grazing livestock and tillage farming predominate) or where there is a surplus of slurry available (e.g. intensive indoor feeding of livestock using purchased feeds predominates) then the

prevailing opportunities or the requirement to conform to pricing or farming system regulations could stimulate the provider of slurry to be paid or to pay for its use in the AD facility. Thus, for example, Dutch farmers have paid about €70 t^{-1} TS for slurry to be transported from their farms and utilised elsewhere (Hari & Riiko, 2016). Hence, two alternate scenarios assumed for this study were a gate fee of €70 t^{-1} TS and slurry purchased at €70 t^{-1} TS. They resulted in a 21% reduction or increase, respectively, in the total cost of methane production. Thus, even with a gate fee €70 t^{-1} TS the total cost of methane production was 70% higher than for the silage baseline scenario.

6.3.3. AD facility co-digesting silage and slurry

Silage and slurry characteristics, and feedstock provision and methane production costs during their co-digestion for the baseline and alternative scenarios are presented in Table 6.3. The feedstock component cost progressively decreased while the AD facility component cost and total cost of methane production progressively increased as the proportion of slurry in the co-digested feedstocks mixture increased.

Although operating an AD facility solely on good quality silage should produce more methane and more profit per year than if it were co-digested with cattle slurry, inclusion of slurry can result in a more stable long-term AD process due to optimization of trace element supply and buffering conditions (Wall et al., 2013). Wall et al. (2014) reported a higher methane yield and a more stable AD operation when grass silage and dairy cow slurry were co-digested for 15 months at a silage:slurry VS ratio of 0.8:0.2 compared to the mono-digestion of grass silage. In the current study, the total cost of methane production for the binary mixture at a silage:slurry VS ratio of 0.8:0.2 was 13% higher than for silage mono-digestion i.e. silage baseline scenario. Thus, this co-digestion option supports a lower yield of methane each year and incurs a higher

cost m^{-3}, but with the potential payback of a more stable long-term AD process. An alternative approach to achieve the latter advantages in such circumstances is the addition of trace elements and buffers as a blend of salts (Wall et al., 2013). However, this scenario was not investigated but is recommended for future research.

The co-digestion of contrasting feedstocks such as grass silage and cattle slurry can result in either synergy or antagonism in methanogenesis (Himanshu et al.,2018; Himanshu et al., under review; Wall et al., 2013). The synergistic effects are usually due to the simultaneous presence of complementary elements of AD, such as improved carbon to nitrogen (C:N) ratio, additional alkalinity, trace elements, nutrients or enzymes, that one feedstock alone may lack (Labatut & Scott, 2008). Antagonistic effects of co-digestion can occur due to a resultant imbalance in the C:N ratio (Kayhanian, 1999), an excess, deficient or imbalance in the ratios of trace elements (Feng et al., 2010), ammonia toxicity and high VFA concentration (Labatut & Scott, 2008). The total cost of methane production for synergistic methanogenesis was 5 and 10% lower than the binary mixture without synergy at silage:slurry VS ratios of 0.8:0.2 and 0.5:0.5, respectively, while it was correspondingly 6 and 12% higher than the binary mixture for antagonistic methanogenesis. Since, these synergistic and antagonistic effects on methanogenesis have significant impacts on the annual yield of methane produced in the AD facility and its cost of production, it is recommended that a methodology be developed to predict the occurrence and magnitude of such effects.

6.3.4. AD facility with altered operational efficiency

The preceding parts of this study assumed generally good standards of management of an AD facility. However, the cost of methane production can also be impacted upon by the standard of technical management of the AD facility as this could result in lower or higher methanogenic

efficiency (i.e. would change the annual yield of methane produced compared to a baseline scenario). For example, Mulat et al. (2016) reported higher methane yields by optimizing the feeding regime (i.e. pulse feeding produced a higher methane yield than continuous feeding). The effects of lower and higher efficiencies of the AD facility were considered by inputting the minimum and maximum methane yield of silage and slurry from Irish literature (Browne et al., 2015; Himanshu et al., 2018; Nizami and Murphy, 2011; Nizami et al., 2012; Nolan, 2017; Wall et al., 2013; Wall et al., 2014; Wall et al., 2015; Xie, 2012). Silage and slurry mono- or co-digestion characteristics, and associated methane production costs, with a lower or higher efficiency of methanogenesis than in the respective baseline scenarios are presented in Table 6.4.

During silage mono-digestion, the low and high operational efficiency alternatives supported 16% lower and 11% higher methane yield values, respectively, and thus correspondingly 16% lower and 12% higher annual methane yields. The low efficiency therefore resulted in 19% increase while the high efficiency resulted in 10% decrease in total cost of methane production. Similarly, for slurry mono-digestion, the low and high operational efficiency of the AD facility resulted in 35% lower and 29% higher annual methane production, respectively. The low operational efficiency thus resulted in a 55% increase in the total cost of methane production while the high efficiency resulted in a 22% decrease in cost. Furthermore, during co-digestion of silage and slurry at a VS ratio of 0.5:0.5 the 23% reduction and 18% increase in the efficiency of methane production correspondingly resulted in a 30% higher and 15% lower total cost of methane production.

These findings outlined in Table 6.4 indicate that, in addition to the effects of grass silage and cattle slurry characteristics presented in Tables 6.1-6.3, the operational efficiency of the AD facility and thus the actual methane yield achieved for each feedstock can have a sizeable impact

on the annual yield of methane produced and the cost of its production. These in turn will markedly alter the profitability of the on-farm AD facility.

This study did not consider all possible circumstances that might impact significantly on the feedstock-related costs of methane production. Thus, for example, this study considered silage being utilised from a single harvest taken from only the primary growth during early summer, and did not consider the economically important savings possible (McEniry et al., 2011) when silage effluent is collected and digested or when the costs associated with fertiliser provision are reduced by incorporating the recycling of digestate from the AD facility onto the land used for silage production.

6.4. Conclusions

The feedstock component contributed about half of the total cost of methane production when the AD facility was operated solely on grass silage. The management targets for grass silage produced on-farm for AD are a high yield of biomass to harvest, and that the herbage is of high digestibility and undergoes efficient fermentation during ensilage (i.e. dominated by lactic acid production). In addition, aerobic deterioration of silage during feedout must be minimised. The cost of grass silage provision would be further reduced by collecting and digesting any effluent released from grass during its ensilage and by reducing the purchase of inorganic fertilisers by an amount that matches the nutrient contributions provided by spreading digestate from the AD facility onto the land from which the grass silage was produced (McEniry et al., 2011).

Despite the normally zero cost of cattle slurry provision, the cost of methane production from mono-digestion of cattle slurry is relatively high compared to grass silage due to its low TS, VS

and methane yield values. In addition, the annual yield of methane produced in the AD facility is reduced. Any increase in the cost of slurry provision markedly diminishes an economic rationale for its use.

Overall, based on the assumptions employed in the baseline scenarios in this study, the emphasis when co-digesting grass silage and cattle slurry should be to maximize the inclusion rate of grass silage commensurate with achieving long-term stable AD. Since, the synergistic and antagonistic effects on methanogenesis during co-digestion of grass silage and cattle slurry have significant impacts on the cost of methane production, it is recommended that a methodology be developed to predict the occurrence and magnitude of such antagonistic or synergistic effects.

The operational efficiency of an AD facility, if it alters the methane yield of grass silage, cattle slurry or their combination and thus alters the annual yield of methane produced, can have an important impact on the cost methane production.

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7. Conclusions and recommendations

7.1. Conclusions

The methane yields of oven-dried forage silages made from early growth stage perennial ryegrass (PRG1), late growth stage perennial ryegrass (PRG2), early growth stage red clover (RC1) and late growth stage red clover (RC2), and of cattle slurry, were 318, 286, 287, 255 and 282 L CH_4 kg^{-1} VS, respectively. During co-digestion, each forage silage and slurry mixture displayed non-linear blending which was always synergistic, and the maximum effects ranged from 2.8 to 7.5% above the yields predicted from mono-digestion of sole substrates. The forage silage:cattle slurry ratio to produce the maximum synergistic effects differed with the forage species ensiled and its growth stage when harvested.

The methane yields of cattle slurry 1 (CS1), cattle slurry 2 (CS2), pig slurry (PS) and two contrasting non-dried forage silages (GS1 and GS2) were 269, 246, 380, 428 and 359 L CH_4 kg^{-1} VS, respectively. Both, biogas and methane yields were impacted by slurry type, grass silage type, and slurry:silage ratio. Each slurry and silage mixture displayed non-linear blending for methane yield and its maximum effect, which was always antagonistic, was at a 0.5:0.5 VS ratio and ranged from 5.7 to 7.6% below the yields predicted from mono-digestion of sole substrates. Furthermore, these antagonistic effects were symmetric and occurred consistently for the various combinations of the three slurries with the two silages.

Thus, the co-digestion of contrasting substrates i.e. forage silage and animal slurry, can either produce antagonistic or synergistic effects on methanogenesis which can either be asymmetric or symmetric. Thermal drying of forage silage, applied to obtain a representative sample, can result in loss of some fermentation products and loss of organic matter due to continued plant enzyme activity and formation of condensation products. Thus, thermal drying can change the chemical composition of a substrate which may impact on the methane yield.

In the manual manometric biochemical methane potential (mBMP) test method, headspace volume and overhead pressure measurement and release (OHPMR) frequency affected headspace pressure and the latter had a negative effect on biogas yield. Headspace pressure had relatively little effect on methane yield but had a clear positive effect on methane concentration. The range of methane yields for cellulose, barley, silage and slurry in mBMP method were 75-350, 208-401, 225-397 and 197-275 L CH_4 kg^{-1} VS while the methane yields of barley, silage and slurry from automated methane potential test system II (AMPTS) method were 350, 359 and 233 L CH_4 kg^{-1} VS. Accepting the methane yields obtained using the AMPTS system as reference target values, two mBMP treatments replicated these targets – OHPMR frequencies of each third day or solely after 35 d, in each case with a headspace volume of 180 ml (70 ml total medium).

In an AD facility the feedstock provision cost contributes significantly to the total cost of methane production. The feedstock component contributed about half of the total cost of methane production when the AD facility was operated solely on grass silage. The management targets for grass silage produced for an on-farm AD facility was a high yield of biomass to harvest, and that the herbage was of high digestibility and underwent efficient fermentation during ensilage (i.e. dominated by lactic acid production). In addition, aerobic deterioration of silage during feedout must be minimised.

Despite the normally zero cost of cattle slurry provision, the cost of methane production from mono-digestion of cattle slurry is relatively high compared to grass silage due to its low TS, VS and methane yield values. In addition, the annual yield of methane produced in the AD facility is reduced. Any increase in the cost of slurry provision markedly diminishes an economic rationale for its use.

Overall, based on the assumptions employed in the baseline scenarios in this study, the emphasis when co-digesting grass silage and cattle slurry should be to maximize the inclusion rate of grass silage commensurate with achieving long-term stable AD.

The operational efficiency of an AD facility, if it alters the methane yield of grass silage, cattle slurry or their combination and thus alters the annual yield of methane produced, can have an important impact on the cost methane production.

7.2. Recommendations

Since, the synergistic and antagonistic effects on methanogenesis during co-digestion of grass silage and cattle slurry can have significant impacts on the cost of methane production, and since they will vary depending on the particular feedstocks co-digested, it is recommended that a methodology should be developed to predict the occurrence and magnitude of such antagonistic or synergistic effects. Furthermore, more research is required to understand the biological mechanisms that mediated the antagonistic or synergistic outcomes during co-digestion.

Variation in feedstocks characteristics can have significant impact on their provision cost and methanogenic potential thus influencing the cost of methane production in an AD facility. It is recommended that the impact of change in feedstock characteristics should be taken into account when designing an AD facility since the feedstocks characteristics will probably change during the entire operation of the AD facility.

The BMP technique can influence the methane yields, hence, judicious consideration is required comparing the methane yields of a particular feedstock measured using different BMP methods. In a mBMP test when assessing the effects of factors of interest e.g. headspace volume of

incubation bottle on biogas and methane output during mBMP test, it is important to use substrates with contrasting digestion characteristics since the headspace pressure effects the gas yield of contrasting substrates differently. It is also important that resultant publications should report the headspace volume, OHPMR frequency and other relevant factors used in their BMP tests. Furthermore, more research is required to standardize the mBMP method with respect to the headspace volume of the incubation bottle and OHPMR. Finally, where an accurate estimate of biogas yield is required, it is recommended that the duration of mBMP tests should be extended sufficiently to allow dissolved CO_2 be retrieved.

8. Appendix A

Table A.1. Chemical properties of grass silages (GS1 and GS2). All units in g kg^{-1} TS unless indicated otherwise.

	TSD	CP	WSC	LA	AA	PA	BA	Eth	NH_3-N (g kg^{-1}N)
PRG1	820	208	12.6	138.1	21.9	2.3	1.7	33.2	105.3
PRG2	628	88	47.5	47.2	2.2	0.9	4.1	4.6	62.2
RC1	717	255	9.4	29.4	41.6	5.1	3.7	40.7	131.7
RC2	617	137	11.0	89.5	4.9	1.1	3.9	5.5	45.4

TSD: Total solids digestibility; CP: Crude protein; WSC: Water-soluble carbohydrates; LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; Eth: Ethanol and NH_3-N: Ammonia-nitrogen.

Table A.2. The coefficients of non-linear blending (NLB) equation (Equation 3.5) for methane yield for the six binary mixtures of slurry and silage.

	Binary mixtures of forage and cattle slurry			
	PRG1:CS	PRG2:CS	RC1:CS	RC2:CS
β_{FS}	317.51	285.81	286.69	254.99
β_{CS}	281.91	281.91	281.91	281.91
β_{FSCS}	37.43	81.74	32.30	80.34
δ_{FSCS}	-64.07	-64.07	13.87	13.87

PRG1: Perennial ryegrass 1; PRG2: Perennial ryegrass 2; RC1: Red clover 1; RC2: Red clover 2; CS1: Cattle slurry 1; β_{FS} and β_{CS} are coefficient for pure forage and pure slurry; and β_{CSFS} describes deviation of the response from the arithmetic mean of the two individual components and δ_{CSFS} describes asymmetry in the response curves.