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EVALUATION OF BIOGAS PRODUCTION FROM ANAEROBIC DIGESTION OF PIG MANURE AND GRASS SILAGE

by

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Henry, Zaki Asam, Mingchuan Zhang, Kathy Carney and William Burchill.

Finally, I would like to sincerely thank my parents and my wife, Xiaoyu, for their encouragement and the sacrifices they have made in getting me to this stage! I appreciate the meaning of this more and more as time goes on.
ABSTRACT

The research project evaluated biogas production from anaerobic co-digestion of pig manure (PM) and grass silage (GS) in laboratory- and pilot- scale studies. In addition, improvement of the biogas yield from GS via thermo-chemical pretreatment and leaching bed reactors was studied.

Using the laboratory-scale batch experiment, anaerobic co-digestion of concentrated PM with GS at five PM to GS volatile solid (VS) ratios was evaluated. The highest specific CH\textsubscript{4} yields obtained were 304.2 ml CH\textsubscript{4}/g VS at a PM to GS ratio of 3:1. The results show that it is feasible to co-digestate PM and GS when PM/GS VS ratios are not less than 1:1. Three laboratory-scale 3 litre continuously stirred tank reactors were set up for further examination of biogas production with different organic loading rates (OLRs, 1.0, 1.5, 2.0 and 3.0 kg VS/m\textsuperscript{3}/d) at various separated PM to dried GS VS ratios. It was found that the OLR affected the digester performance more than the dried GS proportion in the feedstock. Tripling the OLR increased the volumetric methane yields by 88% but decreased the specific methane yields by 38%.

Thermo-chemical pretreatment of dried GS was examined at different NaOH loading rates and temperatures to determine effects of pretreatment on its bio-degradability in terms of the hydrolysis yield and degradation of ligno-cellulosic materials for biogas production. 100 °C and the NaOH loading rate of 5% is recommended as a proper GS pre-treatment condition, under which biological methane production potentials of GS pretreated were improved by 38% in comparison with untreated GS. Six identical column-type stainless steel leaching bed reactors (LBRs) with a working volume of 2-litre were set up to examine the hydrolysis and acidification of GS under three OLRs. The highest GS hydrolysis yields of 51.5-58.2%, acidification yields of 57.2-60.3% and VS removals of 62.1-66.3% were obtained in the conditions of
addition of inoculum, leachate dilution with tap water (1:1 – v/v) every 6 days and pH adjustment to 6.5. The cellulase activity can be used as an indicator to the hydrolysis process.

A pilot-scale CSTR (480 litres) with a working volume of 360 l was fabricated to further examine biogas production from PM and GS in the Teagasc pig research unit in Fermoy, Co. Cork. The experiments consisted of two phases: Phase I at an OLR of 0.87 kg VS /m$^3$/d with the feedstock of PM only and Phase II at an OLR of 1.74 kg VS / m$^3$/d with the mixture of PM and GS at 1:1 of VS basis as the feedstock. The specific methane yields were 154 ml CH$_4$/g VS added when mono-digestion of PM and 251 ml CH$_4$/g VS added when co-digestion of PM and GS.

This PhD study shows that anaerobic co-digestion of pig manure and grass silage should be adopted for sustainable animal manure management. It can produce methane-rich biogas and nutrient-rich fertilizer, and can reduce the greenhouse gas emissions in the pig industry. This study also provides experience and operation data for this co-digestion technology.

**Keywords:** Anaerobic digestion; co-digestion; energy yield; grass silage; greenhouse gases; hydrolysis yield; leaching bed reactor; pig manure; thermal-alkali pre-treatment; specific methane yield
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CHAPTER ONE

Introduction

1.1 BACKGROUND

In the European Union (EU), pig farming is a major agricultural industry and large pig farms are now the norm (Molinuevo et al., 2009). The pig production sector in Ireland contributes to 6% of the gross agricultural output and is the third most important agricultural sector (Martin, 2007). It is estimated that 3.2 million m$^3$ of pig manure (PM) is produced in Ireland annually, containing 13,050 tonnes nitrogen (N), 2,550 tonnes phosphorus (P) and 6,830 tonnes potassium (K). PM is therefore an excellent fertiliser for crops and grass and has traditionally been land spread for this purpose. However, environmental legislation, such as the EU Nitrates Directive, has placed constraints on the land application of PM. Anaerobic digestion (AD) of PM has a number of advantages over traditional PM management, such as: (i) methane production, which is a renewable fuel that can be used to displace fossil fuels; (ii) improvement of the fertiliser value due to enhanced nutrient availability and improved flow characteristics (Ward et al., 2008); and (iii) reduction of pathogens and unpleasant odour. In early 2010, about 5900 agricultural biogas plants with an installed electrical capacity of 2300 MWel are in operation in the EU, which mono-digest or co-digest energy crops with manures; Germany remains the leader in this sector with about 5800 units (Zuber, 2010). One of the advantages of digestion of energy crops is the high volumetric methane yield per unit of fresh weight (Demirel and Scherer, 2008). Ireland has a suitable climate for grass production and has 4.3
2 million hectares (ha) of grassland in comparison with only 280,000 ha of arable land. Grass growth produces high yields of gross energy, e.g. in Ireland 122.4 GJ/ha (Smyth et al., 2009). Grass is often conserved as winter forage for ruminant livestock as grass silage (GS). Grass silage has a high digestible organic matter and volatile solids (VS) content and is an excellent feedstock for AD, either as a single feedstock or co-digested with PM. Co-digestion of PM with energy crops/crop residues can increase the biogas yield by: (i) maintaining an optimal pH for methanogens; (ii) decreasing ammonia/ammonium inhibition, which may occur in AD of manure; and (iii) providing a better carbon/nitrogen ratio (C/N) in the feedstock. However, there is limited information on the effects of the PM to GS ratio on the methane production potential and the operation stability. Additional information would be beneficial to determine the maximum amount of GS to co-digest with PM.

The organic loading rate (OLR) is an important operational parameter for digesters. Whilst increasing the OLR could possibly result in an increase in the volumetric methane production it could also increase the risk of system failure due to accumulation of volatile fatty acids (VFAs), ammonium nitrogen ($\text{NH}_4^+$-N) and free ammonia ($\text{NH}_3$) in the system (Sánchez et al., 2005). However, there is limited information available on the concurrent effects of PM to GS ratio and OLR on methane production and the operation stability of AD systems.

The conversion of GS to biogas via AD consists of three steps: hydrolysis, acidogenesis and methanogenesis, in which hydrolysis is the rate-limiting step. Enhancement of hydrolysis will lead to faster anaerobic digestion. Cellulose, hemi-cellulose and lignin comprise up to 75% of dry matter of grass silage (GS). Treating ligno-cellulosic biomass prior to anaerobic digestion can accelerate hydrolysis and improve biogas yields. Chemical pre-treatment with alkali at ambient temperature is readily achieved and may have commercial potential (Rodgers et al.,
2009). Sodium hydroxide (NaOH) is typically used in alkaline pre-treatment. Thermal pre-treatment with steam or hot water is also effective in hydrolysis of crops and crop by-products. There have been few studies conducted on thermal alkali pre-treatment of GS in order to improve subsequent methane production.

Another approach to enhance hydrolysis of GS is to separate fermentative and methanogenic stages in separate reactors or reactor zones, forming a two-stage AD process. Fermentative micro-organisms, dominating in the first fermentation stage, hydrolyze and acidify biomass to hydrogen, carbon dioxide and volatile fatty acids, while methanogens, dominating in the second methanogenic stage, convert the gaseous products and volatile fatty acids produced in the first stage into methane and carbon dioxide. The separation of the two types of microorganisms in two individual reactors or reactor zones where different conditions are applied optimises their activities and thereby increases the stability of the AD process and biogas yields. Leaching bed reactors (LBRs) have been recently developed as the first-stage reactor to treat solid wastes and energy crops (Demirer and Chen, 2008; Jagadabhi et al., 2010; Myint et al., 2009; Nizami et al., 2010). LBRs are operated in a batch mode instead of a continuous feeding/withdrawing mode. The solid feedstock is added into LBRs at the start of a batch operation and liquid leachate is continuously circulated along the reactors upwards or downwards to enhance solubilisation and hydrolysis of solid biomass. The leachate in LBRs contains high concentrations of soluble organic matter mainly in the forms of volatile fatty acids (VFAs) which can be further treated in the successive reactors where acetogenesis and methanogenesis occur (Demirer and Chen, 2008).

1.2 OBJECTIVES

The overall research aim is to optimise and evaluate biogas production from anaerobic
co-digestion of pig manure and grass silage. The objectives of this study were:

1. to investigate anaerobic co-digestion of GS and PM in batch experiments at various PM to GS ratios in order to examine (i) the process stability; (ii) the system performance in terms of the specific methane yield (SMY) and VS reduction; and (iii) kinetics of hydrolysis.

2. to investigate anaerobic co-digestion of PM and GS in laboratory-scale and pilot-scale continuously stirred tank reactors (CSTRs) in order to examine: (i) process stability in terms of pH, VFAs, NH$_4^+$-N and NH$_3$-N; (ii) system performance in terms of volumetric methane yields, SMY, post methane production potential and VS reduction; (iii) the energy yield; and (iv) mitigation of GHG emissions.

3. to examine the effects of combined thermal and alkali pre-treatment of GS on the hydrolysis yield, hydrolysis kinetics, degradation of ligno-cellulosic materials, and the methane production potential of GS.

4. to investigate factors affecting hydrolysis and acidification of GS using LBRs.

1.3 PROCEDURES

The PhD research consisted of laboratory-scale and pilot-scale studies.

In the laboratory-scale research, the biological methane production potentials (BMPs) of the PM–GS mixtures at various PM:GS ratios were examined in 1-litre digesters made from glass bottles, which were placed in an incubator shaker at 35 °C. The co-digestion of separated solid fraction of PM and GS was undertaken in 3-litre CSTRs at 35±1°C. Six identical column-type stainless steel LBRs, each having an effective volume of 2 litres, were used to enhance the hydrolysis of GS. The major parameters measured included the biogas volume, the biogas composition, and digestate characteristics (like VFAs, ammonium, and solids contents). The description
of these experimental systems is detailed in individual chapters.

The pilot-scale research consisted of measurement of biogas production in a pilot-scale digester and the GHG emissions from storage of pig manure and digestate. The pilot-scale anaerobic digester had a working volume of 360 litre. Three 1 m³ intermediate bulk containers (IBCs) were set up for the measurement of GHG emissions from PM storage. The description of the experiment is detailed in Chapter 7.

1.4 STRUCTURE OF DISSERTATION

This dissertation comprises 8 chapters:

Chapter 1 is the introduction. The main objectives and research procedures are presented.

Chapter 2 reviews generation, characteristics and management of PM, policies relating to the generation and treatment of PM and energy crop, previous research on AD of PM and energy crops and mitigation of GHG emissions.

Chapter 3 details the effect of PM to GS ratio on methane production in batch anaerobic co-digestion of concentrated PM and GS.

Chapter 4 studies anaerobic co-digestion of the solid fraction of separated PM with dried GS in three identical laboratory-scale 3-l CSTRs at different feedstock mixtures and organic loading rates.

Chapter 5 details the effect of thermo-chemical pre-treatment of GS on methane production by anaerobic digestion.

Chapter 6 presents the hydrolysis of GS in LBRs under three different OLRs.
Chapter 7 studies biogas production from a pilot-scale anaerobic digester and GHG mitigation by adoption of AD technology.

Finally, Chapter 8 presents the conclusions drawn from both laboratory and pilot-scale studies with recommendations for further research.
CHAPTER TWO

Literature review

2.1 INTRODUCTION

The pig production sector is an important agricultural sector in Ireland. Environmental legislation, such as EU Nitrate Directive, has placed constraints on the land application of pig manure. Therefore, proper management of pig manure is required to implement these relevant policies. Not only can anaerobic digestion of pig manure produce renewable energy in terms of methane rich biogas, but it can reduce the emissions of greenhouse gases by replacing fossil fuel and reducing fertiliser use and production. On mainland Europe, anaerobic digestion systems are very common. In Germany, there are more than 4000 on-farm anaerobic digestion systems; while in Denmark, there were more than 20 large centralised anaerobic digestion systems in 2009.

Initially, this chapter briefly introduces the status in the pig industry with the focus on the generation and characteristics of pig manure, and it reviews the legislation on the management of pig manure and energy crops. Typical mechanisms of anaerobic digestion are presented along with a review of the anaerobic digestion process, operation parameters and co-digestion technology. Finally, a review on greenhouse gas emissions in the agricultural sector and greenhouse gas mitigation during manure management is carried out.
2.2 PIG INDUSTRY

2.2.1 Pig industry in Ireland

The pig production sector in Ireland contributes 6% of the gross agricultural output and is the third most important agricultural sector (Martin, 2007). The number of pigs increased year by year until December 2002, with the peak number of 1,796,900 in 2002 (Table 2.1). The number has decreased slightly in recent years.

Table 2.1. Selected livestock numbers in December in Ireland (×1000)

<table>
<thead>
<tr>
<th>Type of Livestock</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>6,330.2</td>
<td>6,408.1</td>
<td>6,332.8</td>
<td>6,223.4</td>
<td>6,211.5</td>
<td>6,191.7</td>
<td>6,001.6</td>
<td>5,902.2</td>
<td>5,934.7</td>
<td>5,848.1</td>
</tr>
<tr>
<td>Sheep</td>
<td>5,056.0</td>
<td>4,807.0</td>
<td>4,828.5</td>
<td>4,850.1</td>
<td>4,556.7</td>
<td>4,257.0</td>
<td>3,826.3</td>
<td>3,530.5</td>
<td>3,422.9</td>
<td>3,182.6</td>
</tr>
<tr>
<td>Pigs</td>
<td>1,731.5</td>
<td>1,777.8</td>
<td>1,796.9</td>
<td>1,731.0</td>
<td>1,754.3</td>
<td>1,670.8</td>
<td>1,620.0</td>
<td>1,574.6</td>
<td>1,604.6</td>
<td>1,602.1</td>
</tr>
</tbody>
</table>

Data from CSO (2011)

The number of commercial pig units declined from 912 to 441 from 1987 to 2007. There has been a gradual decline in sow numbers since 1999, with only a slight decrease from 154,000 in 2005 to 152,000 in 2010 (Table 2.2).

Cork, Cavan and Tipperary are the three major pig production counties in Ireland (Table 2.3). The total of 76,420 sows in these three counties represents 49% of the number of sows in commercial herds in the whole country.
Table 2.2. Trends in the number of commercial pig units and sow numbers in Ireland (Martin, 2007)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Commercial Units</th>
<th>Number of Sows (×1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>912</td>
<td>107</td>
</tr>
<tr>
<td>1989</td>
<td>785</td>
<td>107</td>
</tr>
<tr>
<td>1991</td>
<td>746</td>
<td>126</td>
</tr>
<tr>
<td>1993</td>
<td>726</td>
<td>136.5</td>
</tr>
<tr>
<td>1995</td>
<td>667</td>
<td>169.5</td>
</tr>
<tr>
<td>1997</td>
<td>729</td>
<td>168</td>
</tr>
<tr>
<td>1999</td>
<td>657</td>
<td>175</td>
</tr>
<tr>
<td>2001</td>
<td>554</td>
<td>166.6</td>
</tr>
<tr>
<td>2003</td>
<td>511</td>
<td>160</td>
</tr>
<tr>
<td>2005</td>
<td>464</td>
<td>154</td>
</tr>
<tr>
<td>2007</td>
<td>441</td>
<td>153</td>
</tr>
<tr>
<td>2010*</td>
<td>-</td>
<td>152</td>
</tr>
</tbody>
</table>

*Data from Teagasc (2010)

Table 2.3 Pig production in the three major pig producing counties in 2007 (Martin, 2007)

<table>
<thead>
<tr>
<th>County</th>
<th>Number of Sows</th>
<th>Number of Finishing Places</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork</td>
<td>29,100</td>
<td>150,600</td>
</tr>
<tr>
<td>Cavan</td>
<td>28,890</td>
<td>156,800</td>
</tr>
<tr>
<td>Tipperary</td>
<td>16,630</td>
<td>77,000</td>
</tr>
</tbody>
</table>

2.2.2 Pig industry worldwide

The worldwide population of swine by regions in the period of 2000-2009 is presented in Table 2.4.
<table>
<thead>
<tr>
<th>Regions</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>895253381</td>
<td>911863651</td>
<td>927755792</td>
<td>940745482</td>
<td>944381894</td>
<td>964973015</td>
<td>992527473</td>
<td>989744170</td>
<td>936355887</td>
<td>941776122</td>
</tr>
<tr>
<td>Africa</td>
<td>19475954</td>
<td>20589080</td>
<td>21281678</td>
<td>22014177</td>
<td>22904576</td>
<td>23832547</td>
<td>24188256</td>
<td>24640030</td>
<td>26650296</td>
<td>27491092</td>
</tr>
<tr>
<td>Americas</td>
<td>142578314</td>
<td>145251670</td>
<td>143965403</td>
<td>144166442</td>
<td>147043118</td>
<td>149697257</td>
<td>151781188</td>
<td>150594102</td>
<td>159011236</td>
<td>160106099</td>
</tr>
<tr>
<td>Asia</td>
<td>527755286</td>
<td>548084052</td>
<td>562002566</td>
<td>571136764</td>
<td>576647015</td>
<td>595290745</td>
<td>618455169</td>
<td>611515920</td>
<td>554421593</td>
<td>561228369</td>
</tr>
<tr>
<td>Europe</td>
<td>200345273</td>
<td>192435360</td>
<td>194723883</td>
<td>197906550</td>
<td>192352257</td>
<td>190771342</td>
<td>192780459</td>
<td>197697818</td>
<td>190958318</td>
<td>187748664</td>
</tr>
<tr>
<td>Oceania</td>
<td>5098554</td>
<td>5503489</td>
<td>5782262</td>
<td>5521549</td>
<td>5434928</td>
<td>5381124</td>
<td>5322401</td>
<td>5296300</td>
<td>5314444</td>
<td>5201898</td>
</tr>
</tbody>
</table>
The number of pigs in the world is around one billion. The largest number by region is in Asia, followed by Europe, Americas, Africa and Oceania. The most significant increase has occurred in Asia while the number has decreased slightly in Europe since 2000.

2.3 PIG MANURE GENERATION AND CHARACTERISTICS

A sow and her progeny produce 87 kg N/year and 1 m$^3$ of pig manure contains 4.2 kg of total nitrogen (N), 0.8 kg of total phosphorus (TP) and 2.2 kg of total potassium (K) (Table 2.5) (EC, 2006). Hence it is estimated that 20.7 m$^3$ of manure is generated per sow and progeny per year; thus 3.2 million m$^3$ of pig manure is produced in Ireland annually, containing 13,050 tonnes of N, 2,550 tonnes of P and 6,830 tonnes of K. If pig manure is not properly managed, the environment will be seriously polluted.

Table 2.5. Amount of nutrient contained in 1 m$^3$ of slurry

<table>
<thead>
<tr>
<th>Livestock type</th>
<th>Total Nitrogen (kg)</th>
<th>Total Phosphorus (kg)</th>
<th>Total potassium (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>5.0</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Pig</td>
<td>4.2</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Sheep</td>
<td>10.2</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Poultry-layers 30% DM</td>
<td>13.7</td>
<td>2.9</td>
<td>-</td>
</tr>
</tbody>
</table>

For the purposes of calculation, assume that 1 m$^3$ of slurry has a mass of 1 tonne.
The properties of pig manure/slurry are summarised in Table 2.6. Its total solids (TS) content ranges from 1% to 10%, with high contents of total volatile solids (TVS) and high concentrations of total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH\textsubscript{4}\textsuperscript{+}-N), P, K and chemical oxygen demand (COD). The pig manure/slurry has a pH around 7.5.

**Table 2.6. Properties of pig manure/slurry**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range or value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS), %</td>
<td>0.78~9.95</td>
</tr>
<tr>
<td>Total volatile solids (TVS), %</td>
<td>0.30~8.16</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (TKN), mg/l</td>
<td>1217~6698</td>
</tr>
<tr>
<td>Ammonium nitrogen (NH\textsubscript{4}\textsuperscript{+}-N), mg/l</td>
<td>540~3875</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD), mg/l</td>
<td>7138~174300</td>
</tr>
<tr>
<td>Soluble COD, mg/l</td>
<td>1112~74700</td>
</tr>
<tr>
<td>5-day biochemical oxygen demand (BOD\textsubscript{5}), mg/l</td>
<td>702~23600</td>
</tr>
<tr>
<td>Total phosphorus (P), mg/l</td>
<td>352~2720</td>
</tr>
<tr>
<td>Total potassium (K), mg/l</td>
<td>790~3530</td>
</tr>
<tr>
<td>pH</td>
<td>7.01~7.91</td>
</tr>
</tbody>
</table>

*Note: the data are summarised from a number of literatures (Scotford et al., 1998; Vanotti et al., 2007; Zhu et al., 2006; Masse et al., 2007; Moral et al., 2005; Martinez-Suller et al., 2008).*

### 2.4 LEGISLATION ON PIG MANURE MANAGEMENT

The EU Nitrates Directive and the Water Framework Directive 2000/60/EC (WFD) place significant pressure on member states to focus on the quality of their water bodies. The WFD requires that all surface and ground water bodies achieve ‘good status’ by 2015 (Environ, 2011). Legislation on pig manure management is dependent on the state policies on nitrate management.
Nitrate vulnerable zones (NVZ) are areas of land draining into ground or surface waters that are currently high in nitrate, or can become so. Different countries have taken different approaches regarding the designation of NVZ with the purpose of meeting the objective of reducing and preventing nitrate pollution (Fig. 2.1). Ireland is not in the list of states being designated as a NVZ for the whole country. The whole country has been designated as NVZ in Austria, Denmark, Finland, Germany, Luxenburg and Netherland. In countries with a federal structure (Austria and Germany), regional legislative bodies can formulate more stringent requirements if necessary. In other countries regions with significant nutrient surpluses, e.g. Flanders in Belgium, Brittany in France, the Po-valley in Italy, are designated as NVZs (Jakobsson et al., 2002). The total area of land designated as NVZ in England and Wales has increased under the 2008 Regulations, though some land has been removed from designation. About 62% of England and 3% of Wales are designated as NVZs (Environment Agency, 2011).

Fig. 2.1 Nitrate Vulnerable Zones in Europe (EC, 2011).
The new Groundwater Directive (2006) stipulates that nitrate concentrations must not exceed the trigger value of 50 mg/l (Fig. 2.2). Several Member States have set their own tighter limits, in order to reach good status.

The Nitrates Directive Action plan introduced by S.I. No.378 (2006) has imposed immense restrictions on the use of animal manure on grassland and cereals in Ireland. The action programmes include the maximum amount of animal manure that can be applied to land every year, which is equivalent to 210 kg N per ha until mid-December 2002, and then will be reduced to 170 kg N per ha. Many farms are no longer suitable for land spreading of pig manure because organic N loading from grazing livestock is already at or approaching the 170 kg N/ha limit (Jongbloed, 1999).

Increasing the amount of energy produced from renewable sources has been and is still an objective in EU countries. Ireland must increase its electricity production from renewable energy sources to greater than 20% by 2020. Anaerobic digestion of pig manure will partially contribute to this objective.
2.5 PIG MANURE MANAGEMENT

During storage and after field spreading, PM potentially leads to water pollution via runoff and nutrient leaching, to soil contamination by heavy metals and pathogens, and to gaseous emissions of odours, ammonia (NH$_3$), methane (CH$_4$), nitrous oxide (N$_2$O) and carbon dioxide (CO$_2$) (Mattila and Joki-Tokola, 2003; Dambreville et al., 2006; Sleutel et al., 2006). Several PM management strategies have been developed for different purposes, including ease in handling, energy production and soil amelioration. Among these strategies, mechanical separation of manure produces a large liquid fraction, and a small solid fraction, which may be used as a soil amendment, for anaerobic digestion and for composting (Section 2.4.1). This separation allows farmers to cost-effectively transport the solid fraction off-farm or to fields far from the farmstead, and thereby satisfying the nitrogen (N) supply threshold set by the Nitrates Directive (Bertora et al., 2008). The liquid fraction is subjected to further treatment, such as aerobic wastewater treatment and nutrition removal. Moreover, handling, transport, land spreading and soil infiltration of the liquid fraction is easier within suitable distance after the treatment. A typical pig waste treatment system is shown in Fig. 2.3.

Fig. 2.3 Illustration of collection and management options for piggery wastes (Chynoweth et al., 1998)
2.5.1 Solid-liquid separation

Separation of pig manure is undertaken to produce two fractions: phosphorus rich solid fraction and a nitrogen-rich liquid fraction. The solid fraction is cheaper to transport and can be transported off-farm or to fields far from the farmshed for application on tillage land, or used for composting, anaerobic digestion or thermal treatment. The nitrogen-rich liquid fraction can be applied on grassland in the proximity of the pig unit. Alternatively, the liquid fraction can be further treated and re-used as wash water.

Methods of solid-liquid separation of pig manure include: (i) natural methods like sedimentation; (ii) mechanical methods such as filtration, pressing, and centrifugation; and (iii) chemical methods by using coagulants and flocculants. Mechanical methods are widely used for on-farm manure separation. Chemicals (coagulants and flocculants) are often used with mechanical methods to enhance the separation process and the optimal dosage of chemicals will vary.

2.5.2 Land spreading

The conventional method of spreading slurry, surface broadcasting by splash plate applicators, is rapid and inexpensive (Chadwick et al., 2011). However, broadcasting of manure is typically uneven, especially under windy conditions (Huther, 1988). Another method of spreading slurry is the injection. The direct ground injection system forces finely separated manure under pressure into the soil with little soil disturbance (Morken and Sakshaug, 1998). Surface-banding slurry/manure with trailing-hose or trailing-shoe applicators is a compromise between injection and broadcasting. Band-spreading applies manure more uniformly than splash plates (Huther, 1988) and trailing-shoe machines place the manure beneath crop/grass canopies so that little adheres to and contaminates foliage. In Ireland, land spreading is the least expensive and most widespread disposal option for pig manure. However, it requires sufficient land area available in close proximity to the farm, especially considering restrictions imposed by the EU Nitrates Directive. Moreover, significant
amount of GHGs can be emitted through land application of manure (Chadwick et al., 2011).

### 2.5.3 Treatment of pig manure

Treatment technologies provide more flexible approaches than land spreading in dealing with specific problems. Treatment technologies include aerobic treatment, composting and anaerobic treatment.

Many processes consist of biological and physical methods and sometimes chemicals are used. Some of these systems are already in current use on large farms (e.g. separation and composting systems).

After separation, the liquid fraction may be treated using aerobic wastewater treatment technologies for nutrient removal prior to discharge into receiving waters. Aerobic treatment requires oxygen supply to support aerobic bacteria. The amount of oxygen required depends on whether it is desired to reduce odour, completely remove the organic matter, or to convert ammonium to nitrate (namely nitrification) (Table 2.7). Aerobic biological treatment of manure has been carried out for years with the purpose of reducing the organic load discharged into the environment and obtaining beneficial by-products that could be used as soil amendment. Due to the high-energy input for aeration, aerobic treatment is not recommended for high-strength organic wastes, wastes that are difficult to biologically degrade, or wastes that have disproportionate C/N and C/P ratios, since in these cases the performance of aerobic treatment is poor (Buendia et al., 2008).

Composting is an aerobic process in which manure organic matter is stabilised into a humus-like product called compost (Zhu et al., 2007). It has been defined as a controlled microbial aerobic decomposition process with the formation of stabilised organic materials that may be used as soil conditioners and/or organic fertilisers.
(Garcia et al., 1992; Negro et al., 1999). The main factors in the control of a composting process include environmental parameters (temperature, moisture content, pH, and oxygen), substrate parameters (C/N ratio, particle size, nutrient content) and operational parameters (turning efficiency and frequency, aeration rate, etc.) (Diaz et al., 2002; Kulcu and Yaldiz, 2004).

**Table 2.7.** Comparison of the three major approaches of manure treatment (Chan et al., 2009; Nelson, 2010; AD, 2011)

<table>
<thead>
<tr>
<th>Method</th>
<th>Benefits</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic treatment (pig manure or liquid fraction of manure)</td>
<td>Odour reduction; Nitrification of ammonium to nitrate; Reduction of greenhouse gases (especially methane)</td>
<td>High capital cost; High operating and maintenance cost</td>
</tr>
<tr>
<td>Composting (solid fraction of pig manure)</td>
<td>Saleable product (compost); Destruction of pathogens; Reduction of mass and volume; Improved handling; Improved transportability; Odour reduction</td>
<td>Loss of ammonia; Long operation period; High capital cost; Large land area; Marketing required for sale</td>
</tr>
<tr>
<td>Anaerobic digestion (pig manure or solid fraction of manure)</td>
<td>Less energy required; Less biological sludge produced; Lower nutrient demand; Methane production and energy recovery; Greenhouse gas emissions reduction</td>
<td>Significant capital and operational costs; Longer start-up time; No nutrient removal; Need for heating; May require alkalinity and/or specific nutrient addition; May require further treatment for digestate to meet discharge requirements</td>
</tr>
</tbody>
</table>

Directly or after separation, the manure is transported to an anaerobic digestion system whose configuration may be different in design. The operating temperature
varies in ambient, mesophilic (35 °C), or thermophilic (55 °C) ranges. Anaerobic digestion is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen. Anaerobic treatment of manure has been reported by several researchers (Bonmati et al., 2001; El-Mashad et al., 2004; Karim et al., 2005); a detailed review will be carried out in the next section. The produced biogas is used either for heating directly or for operation of internal combustion engines to run equipment or generate electricity. The digested manure from the AD system is stored and applied to land on a seasonal basis.

2.5.4 Utilisation

The solid fraction of pig manure can be utilised as a renewable source by thermal treatment, such as pyrolysis. However, these utilisation technologies are still at the research stage.

Pyrolysis is a process whereby a biomass feedstock, such as woodchips or livestock manure, is heated to very high temperatures ranging from 400-800 °C in the absence of air (Bulushev and Ross, 2011). During the heating of the biomass, gas, liquid and char are formed in proportions that depend on the mode of pyrolysis and the type of pyrolysis systems (Bulushev and Ross, 2011). Hemicellulose decomposes between 250 and 400 °C and produces up to 20 wt.% of char upon heating to 720 °C; cellulose requires slightly higher temperatures (310 – 430 °C), and produces about 8 wt.% of char; and lignin decomposes at 300 – 530 °C, yielding about 55 wt.% of char (Williams and Besler, 1996).

The different modes of pyrolysis have been classified in Table 2.8 (Bridgwater, 2006 and 2007). All the products are valuable. The most interesting product is pyrolysis oil as it can be converted to fuel additives in various ways. The yield of pyrolysis oil under optimised fast pyrolysis conditions may reach 70-80%, which includes up to 25% of water. However, slow pyrolysis, at lower temperature and with longer residence time, generates considerably higher water contents and the yield of pyrolysis oil decreases while char is formed in higher amounts. High pyrolysis temperature, i.e.,
800 °C, leads to the formation of gases.

**Table 2.8 Modes of pyrolysis (Bridgwater, 2006 and 2007)**

<table>
<thead>
<tr>
<th>Mode</th>
<th>Temperature</th>
<th>Residence time</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liquid</td>
</tr>
<tr>
<td>Slow</td>
<td>400</td>
<td>Very long</td>
<td>30</td>
</tr>
<tr>
<td>Intermediate</td>
<td>500</td>
<td>Moderate (10-20s)</td>
<td>50</td>
</tr>
<tr>
<td>Fast</td>
<td>600</td>
<td>Short (&lt;2s)</td>
<td>75</td>
</tr>
<tr>
<td>Gasification</td>
<td>800</td>
<td>long</td>
<td>5</td>
</tr>
</tbody>
</table>

**2.6 ENERGY CROPS, GRASS SILAGE AND POLICY COMPLIANCE**

Energy crops are crops cultivated in order to utilise biomass for energy production within a short time frame, excluding biomass extracted from existing (long-rotation) forestry (Styles and Jones, 2007). In the UK, legislation defines energy crops as “crops planted since 1989 and grown primarily for the purpose of being used as fuel” (McKay, 2006). The EU White Paper (EU, 1995) and Green Paper (EU, 2000) have targeted biomass as the major renewable source to contribute up to 74% of EU renewable energy. In December 2008, the European Parliament agreed to achieve the EU’s overall environmental target of a 20% reduction in GHG emissions and the renewable target of a 20% share of renewable in the EU’s gross final energy consumption by 2020 (EU, 2009a, 2009b). This requires the utilisation of energy crop biomass as a major renewable energy source.

Within Ireland, energy crops can contribute substantially towards achieving Kyoto and post-Kyoto EU GHG emission targets. Rapid energy-crop establishment is necessary to avoid high expenditure on national ETS (Emission Trading Scheme) allowances at the end of the Kyoto commitment period (2008-2012) (EPA, 2006). In addition, energy-crop utilisation could help Ireland to comply with the targets set in the EU Renewables Directive 2001/77/EC (RES-E: Renewable Energy). Under this Directive, Ireland agrees a national indicative target of 13.2% final electricity
consumption from renewables by 2010 (this target has since been increased to 15%, and 20% in 2020). These targets may be largely met by huge increases in wind generation in Ireland (Conlon et al., 2006), but it also requires the use of biomass for renewable electricity generation. The EU Nitrates Directive (91/676/EEC) requires a reduction of maximum N-application rates on land, which will encourage crops with a lower nutrient demand such as grass.

Approximately 91% of the agricultural land in Ireland is grasslands while there is only 9% of arable land (4 million hectares of grassland and 0.4 million hectares of arable land) (Hamelinck et al., 2004). Grass is an excellent perennial energy crop with a low input and a high yield (up to in excess of 15 t DM/ha/a) (King et al., 2011). The use of grass offers numerous benefits for the agriculture:

- no land use change, and preservation of habitat biodiversity;
- no new farming practices; and
- no annual tilling, thus helping carbon sequestration.

### 2.7 ANAEROBIC DIGESTION OF MANURE

A brief introduction of anaerobic digestion (AD) and different AD processes treating manure alone or co-digesting manure with other materials are reviewed in this section, with special attention being paid to the effects of operational parameters on the system performance.

#### 2.7.1 Anaerobic digestion

Anaerobic digestion converts organic matter to biogas for energy recovery (Tchobanoglous et al., 2003). Four key biochemical stages are included in anaerobic digestion: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 2.4).
Firstly in the hydrolysis stage, complex organic matter, like carbohydrates, lipids/fats, proteins and nucleic acids, are converted to sugars, fatty acids, amino acids, etc. Secondly in the acidogenesis stage, microorganisms convert the material produced in the first stage to volatile fatty acids (VFAs), hydrogen ($H_2$), and carbon dioxide ($CO_2$). Examples of different products from glucose fermentation are shown in Table 2.8.
Table 2.8. Examples of glucose fermentation products

<table>
<thead>
<tr>
<th>Products</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>$C_6H_{12}O_6 + 2H_2O → 2CH_3COOH + 2CO_2 + 4H_2$</td>
</tr>
<tr>
<td>Propionate + Acetate</td>
<td>$3C_6H_{12}O_6 → 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$</td>
</tr>
<tr>
<td>Butyrate</td>
<td>$C_6H_{12}O_6 → CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$</td>
</tr>
<tr>
<td>Lactate</td>
<td>$C_6H_{12}O_6 → 2CH_3CHOHCOOH$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$C_6H_{12}O_6 → 2 CH_3CH_2OH + 2CO_2$</td>
</tr>
</tbody>
</table>

(Batstone et al., 2002)

In the acetogenesis stage, volatile fatty acids are converted to acetic acid, $H_2$ and $CO_2$ (Salminen and Rintala, 2002). The conversion of typical volatile fatty acids to acetic acid is shown in Table 2.9.

Table 2.9. Conversion of volatile fatty acids to acetic acid

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acid</td>
<td>$C_3H_7COOH + 2H_2O → CH_3COOH + 3H_2 + CO_2$</td>
</tr>
<tr>
<td>$i$-butyric acid</td>
<td>$C_4H_9(CHCH_3)COOH + 2H_2O → 2CH_3COOH + 2H_2$</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>$C_4H_9CH_2CH_2COOH + 2H_2O → 2CH_3COOH + 2H_2$</td>
</tr>
<tr>
<td>$i$-valeric acid</td>
<td>$C_5H_{11}(CHCH_3)CH_2COOH + 2H_2O + CO_2 → 3CH_3COOH + H_2$</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>$C_5H_{11}CH_2CH_2CH_2COOH + 2H_2O + CO_2 → 3CH_3COOH + H_2$</td>
</tr>
</tbody>
</table>

(Batstone et al., 2002; Batstone et al., 2003)

Finally in the methanogenesis stage, two groups of methanogens – acetotrophic methanogens and hydrogenotrophic methanogens - convert acetic acid or hydrogen and $CO_2$ to methane, respectively (Tchobanoglous et al., 2003). The reactions in association with acetic acid and hydrogen consumptions are shown in Table 2.10.
Table 2.10. Reactions related to methanogenesis

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta G^\circ$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenotrophic methanogenesis</td>
<td>-135.0</td>
</tr>
<tr>
<td>Aceticlastic methanogenesis</td>
<td>-31.0</td>
</tr>
<tr>
<td>Acetate oxidation</td>
<td>+104.0</td>
</tr>
<tr>
<td>Homoacetogenesis</td>
<td>-104.0</td>
</tr>
</tbody>
</table>

(Batstone et al., 2002)

At the standard temperature (0 °C), the hydrogen consumption by hydrogenotrophic methanogenesis is more favorable than homoacetogenesis, and acetic acid consumption by aceticlastic methanogenesis is more favorable than acetate oxidation (Table 2.10). Hydrogenotrophic methanogenesis performs better at a high hydrogen partial pressure, while aceticlastic methanogenesis is independent from the hydrogen partial pressure (Schink, 1997).

2.7.2 Anaerobic digestion processes

Various AD processes are used in practice and these can be divided into two types: suspended- or attached- growth anaerobic systems. The attached growth anaerobic processes include upflow packed-bed reactor process, upflow expanded-bed reactor process and fluidised-bed reactor process, etc. In addition, covered anaerobic lagoons are used in some cases (Tchobanoglous et al., 2003). Anaerobic digesters can be operated in batch, semi-continuous or continuous modes. In semi-continuous or continuous operations, the maximum growth rate can be constantly achieved at steady-state by controlling the feed rate (Boe, 2006). In the batch operation mode, the steady-state cannot be achieved as the concentrations of components in the digester are not constant with time (Klass, 1984). Alternative ways to classify AD processes for high solids content feedstock are: (1) one-stage and two-stage digestion; and (2) wet and dry digestion.
2.7.2.1 One stage vs. two stage digestion

One - stage digestion

For anaerobic digestion of pig manure (PM), the conventional one-step continuously stirred tank reactor (CSTR) (Fig. 2.5) is simple to operate and widely used (Speece et al., 1997; Azbar et al., 2001). The high viscosity and particulate content of PM make the upflow anaerobic sludge blanket bed reactor (UASB) unsuitable. In recent years, a number of novel processes have been developed, through which a higher loading rate could be achieved.

![Fig. 2.5. One-stage CSTR (Boe and Angelidaki, 2009)](image)

Demirer and Chen (2005) adapted an anaerobic hybrid reactor (AHR) - a sludge bed in the lower part and an anaerobic filter (AF) in the upper part - to process dairy manure. The VS content in the feedstock varied from 1.83% to 11.5%. The achieved removal efficiencies for COD, BOD, TS, and VS were 48–63%, 64–78%, 55–65%, and 59–68%, respectively, and the methane yield was 0.191 l/g VS added.

Demirer and Chen (2008) investigated anaerobic digestion of undiluted dairy manure in leaching bed reactors (LBR). LBR is known as percolating anaerobic digestion or dry anaerobic digestion, where a high solid content above 15% is applied (Demirer and Chen, 2008). This is basically a column reactor operated in a batch mode; liquor collected at the bottom of the reactor is continuously recirculated to the top (Dogan et al., 2009). Around 25% improvement in biogas production was achieved in LBRs.
compared with conventional slurry anaerobic digesters which were operated in a batch mode with the same feedstock.

Mumme et al. (2010) evaluated the feasibility of a novel upflow anaerobic solid-state (UASS) reactor equipped with liquor recirculation when treating a mixture of maize silage and straw. It digests solid biomass while the particulate organic matter ascends in the form of a solid-state bed (SSB). As shown in Fig. 2.6, the total working volume of the UASS reactor consists of three sections: a lower liquid zone, an upper liquid zone and the SSB. New biomass is applied manually through an inclined feeding pipe to the reactor bottom. Solid residues are withdrawn manually from top after opening the reactor lid and removing the detached sieve. They found that the overall methane yield declined from 384 to 312 ml/g VS added in UASS and the yield contribution of the two anaerobic filters rose from 12% to 70%, when the OLR was increased from 7.1 to 17 g VS/l/d.

![Fig. 2.6 Schematic diagram of the experimental set-up of a laboratory scale UASS reactor (Mumme et al., 2010).](image)

Wen et al. (2007) used a sequential CSTR system to study anaerobic digestion (AD) of flush dairy manure and found that a higher OLR resulted in higher biogas production and chemical oxygen demand (COD)/solids removal. They also found that the plug-flow reactor and the CSTR had a similar AD performance.
Dareioti et al. (2010) explored anaerobic digestion of olive mill wastewater and liquid cow manure using two serial CSTRs with the overall HRT of 19 days under mesophilic conditions (35 °C), and they achieved 0.91 l CH\textsubscript{4}/l/day or 250.9 l CH\textsubscript{4} at standard temperature and pressure conditions (STP) per kg COD fed to the system.

A new configuration of cattle manure digesters for improving biogas production has been investigated in laboratory scale by Boe and Angelidaki (2009). A single thermophilic CSTR (Fig. 2.5) operated with a HRT of 15 days was compared to a serial CSTR configuration (Fig. 2.7) with volume distribution ratio of 80/20 and 90/10, and total HRT of 15 days. The results showed that the serial CSTR could obtain 11% higher biogas yields compared to the single CSTR.

Kaparaju et al. (2009) investigated the possibility of biogas production from manure in two methanogenic CSTRs connected in series. At 70/30% and 50/50% volume distributions, 13–17.8% more biogas and methane was produced, and low VFAs and residual methane potentials in the effluent were obtained compared to the one-step CSTR process. The applied hydraulic retention time (HRT) was equal to solid retention time (SRT).

**Two-stage AD**

In the two-stage AD process, acidogenic bacteria converting substrates such as carbohydrates to H\textsubscript{2}, CO\textsubscript{2} and fatty acids dominate in the first stage, while methanogens which convert the gaseous products and volatile fatty acids produced in
the first stage to CH₄ and CO₂ dominate in the second stage (Demirel and Yenigun, 2002). By means of separation of the two types of microorganisms in separate reactors or reactor zones, the growth of each type of microorganisms can be optimised by controlling the condition in the individual stage. The acetogenic bacteria grow at a lower pH (e.g. 5–6) (Pohland and Ghosh, 1971) and a shorter HRT (typically 1–2 days) in the first stage, while the slower growing methanogens, require a neutral pH and prefer a much longer HRT (typically 10–20 days) in the second stage (Blonskaja et al., 2003; Demirel and Yenigun, 2002). The advantages of the two-stage AD process over the one-stage process include increased stability, smaller reactor size, cost efficiency, shorter total detention time, higher gas conversion efficiency and higher methane content in the produced biogas (Yu et al., 2002). The two-stage process can also reduce the effect of the hydrogen partial pressure on the methanogenesis reaction. A small amount of H₂ (>0.1%) in the gas phase could cause a cessation of CH₄ production (Cooney et al., 2007). However, the two-stage process is considered by some researchers to be sensitive to high OLRs, complicated to operate and costly (Sachs et al., 2003; Babel et al., 2004).

Ahn et al. (2004) developed an anaerobic digestion elutriated phased treatment (ADEPT) process - consisting of an acid elutriation slurry reactor for hydrolysis and acidification and an upflow anaerobic sludge blanket (UASB) reactor for generation of CH₄ - for pig slurry treatment. They found that the optimum pH and HRT were 9 and 5 days, respectively, at both 35 °C and 55 °C in the first reactor, and the CH₄ yield and the CH₄ content in biogas in the ADEPT were 0.3 l CH₄/g VS fed (0.67 l CH₄/g VS destroyed) and 80%, respectively.

Feng et al. (2008) developed a two-stage digestion system to treat swine wastes and garbage consisting of an acidification reactor (0.4 m³), a methane fermentation reactor (2.5 m³) which was divided into five compartments in a configuration of anaerobic baffled reactors (ABR), and a digested effluent tank (Fig.2.8). A biogas holder (1.0 m³) was combined with the methane fermentation reactor. The acidification reactor also served as a buffering tank because the material was fed once a day. The biogas production of 865-930 l/ kg VS added was obtained at the OLR of 5.0 - 5.3 kg VS/m³/day and the HRT of 9-13 days.
Yilmaz and Demirer (2008) compared a one-stage process with a two-stage process, which consisted of an acidogenic reactor and a methanogenic reactor for dairy manure treatment. Operated at a HRT of 8.6 days in the methanogenic stage, the biogas yield at an OLR of 3.5 kg VS/m$^3$/day was 42% higher than that of the one-stage process operated at a HRT of 20 days.

2.7.2.2 Wet vs. dry digestion

Wet digestion

The wet anaerobic digestion process works with a total solid concentration less than 15% (Erickson et al., 2004). The wet process for manure and energy crops can be operated in a single-stage or two-stage mode under mesophilic or thermophilic conditions depending on the waste input and the site conditions. Some reactors reinject biogas to the bottom of the reactor tank to create a loop in the digester and to obtain better homogenisation; other reactors use simple mechanical mixing (Erickson et al., 2004).

A two-stage wet anaerobic digestion process has been patented by STRABAG Umweltanlagen GmbH Company in Germany (Fig. 2.9). In addition to a hydrolysis
tank the wet digestion technology is characterised by the wet treatment concept using a waste dissolver and a rotary screen. The pre-treated waste is mashed in the waste dissolver to produce a pumpable pulp. This is transferred into the downstream rotary screen to remove both heavy materials and light matter (like plastic sheets) which are not separated from the pulp by sedimentation in the waste dissolver. The pulp is then delivered to a buffer and hydrolysis tank before digested in a digestion reactor (STRABAG Umweltanlagen GmbH, 2011). The main process features and advantages are: (1) high flexibility and adjustment capability for a wide range of wastes; (2) high decomposition performance and biogas production rate by optimizing process parameters; and (3) high waste disposal reliability and process stability via the two-stage concept.

![Fig. 2.9 Patented two-stage wet anaerobic digestion process (STRABAG Umweltanlagen GmbH, 2011)](image)

*Dry digestion*

Dry anaerobic digestion takes place at solids contents in the digester of about 20-45%. Energy crops, organic fraction of municipal waste are ideal feedstock for dry anaerobic digestion.
A Dranco-Farm continuous thermophilic dry digestion process can be used for digestion of energy crops and organic fraction of municipal solid waste (Fig. 2.10). The process consists of a vertical plug flow digester with a short pass-through time, and the digester consists of two separate zones. The upper intensive fermentation zone is maintained by constantly recycling the digestate and mixing with the fresh feedstock. The second zone is the postfermentation zone, where the fermentation of the digestate happens to complete biogas generation. Fresh feedstock material is mixed with digestate and the mixture is pumped back to the top of the digester. The digesting material flows from top to bottom by gravity and no mixing is needed inside the dry digester. The digestate is extracted from the bottom of the digester about every 2-3 days. The mixing of digestate with the fresh feedstock also prevents the accumulation of VFAs.

### 2.7.3 Operation parameters

#### 2.7.3.1 Temperature

Temperature markedly affects the biogas yield during anaerobic digestion of manure by affecting the thermodynamics of acetogenic reactions (Table 2.9) and methanogenic reactions (Table 2.10). At higher temperatures, the formation of H₂ from organic acids oxidation becomes more energetic, while the consumption of H₂ by hydrogenotrophic
methanogenesis becomes less energetic (Fig. 2.10). The organic acids degradation is expected to be faster (De Bok et al., 2004) and acetate oxidation becomes more favourable at higher temperatures (>30°C) (Schink, 2002). Aceticlastic methanogens such as Methanosarcina thermophila also activate at high temperature to compete for acetate (Fig. 2.10; Schink, 1997). The syntrophic acetate oxidation pathway dominates in extreme thermophilic conditions (>65 °C) which is beyond the optimum temperature (63 °C) of aceticlastic methanogens (Lepistö and Rintala, 1999). Under psychrophilic conditions (<15 °C), the activity of hydrogen-utilising methanogens becomes very low. Homoacetogenesis is the main hydrogen removal function, and methane formation from aceticlastic methanogens becomes dominant (Kotsyurbenko et al., 2001). Under such conditions, Kotsyurbenko (2005) thinks that methane formation through homoacetogenesis contributes up to 95% of the total methane production.

![Fig. 2.10 Temperature dependence of the free energy change in anaerobic hydrogen and acetate metabolism; solid lines, standard conditions (1 M concentrations, 1 atm pressure); dashed lines, the same for 10^-4 atm of hydrogen; □: aceticlastic methanogenesis; ○: acetate oxidation; △: hydrogenotrophic methanogenesis (Schink, 1997)](image)

Masse et al. (2003) observed in psychrophilic anaerobic sequencing batch reactors treating swine manure that, the methane yield decreased from 0.266 l/g total COD (TCOD) fed at 20 °C to 0.218 and 0.080 l/g TCOD fed at 15 and 10 °C, respectively. In mesophilic conditions treating swine manure in batch experiments, Chae et al. (2008) found that the methane yield was reduced slightly by 3% at 30 °C, in
comparison with that at 35 °C, while a 17.4% reduction was observed when the digestion was performed at 25 °C. Goberna et al. (2010) co-digested cattle excreta and olive mill wastes in a pilot-scale CSTR and derived a specific methane yield (SMY) of 179 ml CH₄/g VS added when mesophilic digestion (37 °C) was used, while there was an increase in the methane production of about 17% under thermophilic conditions (55 °C). Ge et al. (2011) applied temperature phased anaerobic digestion as a pre-treatment method treating waste activated sludge. An experimental thermophilic (50-70 °C)-mesophilic (35 °C) system was compared against a control mesophilic-mesophilic system. The SMY after thermophilic pre-treatment increased by 42.9%-88.9% (from 0.07-0.11 l/g VS added to 0.10-0.17 l/g VS added) compared with that after mesophilic pre-treatment. Nielsen et al. (2004) used two serial digesters to treat cattle manure, which consisted of a digester operating at 68 °C with a HRT of 3 days and a digester operating at 55 °C with a 12-day HRT. When an OLR of 3 g VS/L/day was applied, the setup had a 6%-8% higher SMY and a 9% more effective VS-removal than the conventional single reactor.

2.7.3.2 Organic loading rate (OLR)

Normally, at a unit digester volume, biogas production firstly rises with increasing OLRs, and then declines due to the inhibition of by-products (VFAs or free ammonia) formed in the digester when OLRs are too high. A number of experiments have been carried out to evaluate the proper OLRs for anaerobic digestion of manure with or without co-digestion material. Demirer and Chen (2005) found that in a conventional one-stage reactor with HRT of 20 days, when the OLR was increased to 6.3 g VS/l/day, the biogas yield decreased; while two-stage reactors could tolerate an elevated OLR of 12.6 g VS/l day. Lindorfer et al. (2008) investigated an increase of the OLR from 2.11 to 4.25 kg VS/m³/d in a two-stage agricultural biogas plant and observed that the biogas yield based on VS added was on the same level, while the volumetric biogas productivity almost doubled. Comino et al. (2010) found, when increasing the OLR from 4.45 to 7.78 kg VS/m³/d, when feeding fresh whey up to 70% VS in the feedstock, up to 109% higher SMYs were obtained than during the start up
phase (only manure) at the OLR of 5.15 kg VS/m$^3$/d. Further increase of the OLR to 7.78 kg VS/m$^3$/d led to a decrease of the SMY to only 61 l CH$_4$/kg VS and the methane content in biogas was down to 39.9%.

2.7.3.3 HRT/SRT

Effective anaerobic digestion of organic matter requires a HRT longer than 25 days at 31 °C (Salminen and Rintala, 2002). Linke (2006) described the biogas yield, $y$, as a function of the maximum biogas yield, $y_m$, the reaction rate constant, $k$, and the HRT on the basis of a mass balance in a CSTR treating solid potato processing wastes and first order kinetics:

$$y = \frac{HRT \cdot k \cdot y_m}{HRT \cdot k + 1}$$  \hspace{1cm} (2.1)

Biogas yield $y$ can be expressed as an absolute proportion $p$ of $y_m$ ($y/y_m$). The relationship between $p$ of $y_m$ and HRT under different reaction rate constant, $k$, is shown in Fig. 2.11. It can be seen that about 45 days of HRT is needed to obtain 80% of the maximum biogas yield at the $k$ value of 0.09 d$^{-1}$. Zhu et al. (2007) examined continuous production of hydrogen through fermentation with liquid swine manure as the substrate at the HRT of 16, 20 and 24 h and found that a high HRT would result in fluctuation of the hydrogen content in the biogas.

![Fig. 2.11 Absolute proportion p of y_m for different values of HRT and k (Linke, 2006).](image)
2.7.3.4 Mixing

Suitable mixing is essential for optimum performance of the AD system. Mixing provides intimate contact between the digested material and active biomass, yielding uniformity of chemical (such as substrate, intermediate and final products) and physical (such as temperature) conditions throughout the digester, and preventing the formation of surface scum layers and the deposition of solids on the bottom of the tank (Appels et al., 2008). Natural mixing occurs to some extent in the digestion tank, due to the rise of gas bubbles. However, this is not sufficient for optimum performance; therefore, auxiliary mixing is needed. Prasad et al. (2008) evaluated the effect of mixing on anaerobic digestion of manure in laboratory-scale and pilot-scale experiments at 55 °C. They found that in comparison with continuous mixing, intermittent and minimal mixing strategies improved methane production by 1.3% and 12.5%, respectively. Methods of auxiliary mixing include external pumped recirculation, internal mechanical mixing and internal gas mixing (Igoni et al., 2008), as illustrated in Fig. 2.12.

Using external pumped recirculation, a large amount of the digestate withdrawn from the digester is firstly pumped through external heat exchangers where it is blended with the raw feedstock and heated. Then it is pumped back into the digester through nozzles at the base of the digester or at the top to break the scum (Lue-Hing, 1998). The recirculation flow rate should be large enough for ensuring complete mixing, which limits the use of this mixing method. The minimum power required is 0.005–0.008 kW/m$^3$ of digester volume and may be higher. Other disadvantages are clogging of the pumps by solids, impeller wear and bearing failures (Lue-Hing, 1998).

Mechanical stirring systems generally use low-speed flat-blade turbines. The sludge is transported by the rotating impeller(s), thereby mixing the content of the digester. These systems are suitable for digesters with fixed covers (Appels et al., 2008).

In gas mixing systems, the gas is collected at the top of the digestion tank, compressed and then released through a pattern of diffusers or a series of radially placed lances suspended from the digester cover (Appels et al., 2008). The digester
content is mixed, releasing gas bubbles that rise and push the digested material to the surface, so scum has to be specifically controlled. The lance system is successful against the build-up of scum but does not prevent solids from settling to the digester bottom. The diffuser system, which is installed at the bottom of the reactor tank, is effective against solids settlement, but is not adequate to control scum build-up. There is a possibility of diffuser clogging, resulting in digester drainage for tank cleaning. The unit gas flow requirement for unconfined systems is 0.0045–0.005 m$^3$/m$^3$ digester volume/min (Turovskiy and Mathai, 2006).

Fig. 2.12 Types of digester mixing: (a) external, pumped recirculation; (b) internal, mechanical mixing; and (c) external, gas recirculation (Igoni et al., 2008).

2.7.3.5 VFAs

VFAs are important intermediate products and most of the CH$_4$ produced is derived from VFAs. Before being converted to CH$_4$, VFAs are transformed to acetic acid (HAc), and the conversion rates vary in the order of butyric acid (HBu) > propionic acid (HPr). It has been suggested by Marchaim and Krause (1993) that the HPr to HAc ratio can be used as an indicator to digester imbalance. Hill et al. (1987) has proposed that an HPr to HAc ratio greater than 1.4 could indicate impending digester failure. As reported by Wang et al. (2009), when HPr and total VFA concentrations reached about 2850 mg/l and 10.0 g/l, respectively, the activity of methanogens was
inhibited to a significant extent, but this inhibitory effect was weakened when the total VFAs concentration fell to 6.2–8.5 g/l.

2.7.3.6 Ammonium/free ammonia

Ammonia is produced during the degradation of nitrogenous matter, mainly proteins and urea (Chen et al., 2008). Ammonium (NH$_4^+$-N) and free ammonia (NH$_3$) are the two most predominant forms of inorganic nitrogen present in anaerobic digesters. It has been indicated that NH$_3$ is more toxic to methagens than NH$_4^+$-N, due to the fact that it can pass through the cell membrane and into the cell, causing proton imbalance and potassium deficiency (Sung and Liu, 2003). The concentrations of NH$_4^+$-N and NH$_3$ that cause inhibition of AD vary in different AD systems. For example, 50% reductions in methane production have been found when NH$_4^+$-N concentrations increased from 1.7 to 14 g/l (Chamy et al., 1998; Hansen et al., 1998; Sung and Liu, 2003). The inhibition of NH$_3$ and NH$_4^+$-N on AD is reversible. Wu et al. (2009) found that during AD of meat and bone meal, inhibition of methanogens by NH$_3$ was reversible when the NH$_3$ concentration was as high as 998 mg/l. The varying inhibition concentrations of NH$_3$ and NH$_4^+$-N are attributed to the differences in substrates and inocula, environmental conditions (temperature, pH, etc.), and acclimation periods. Strik et al. (2006) proved that NH$_3$ existed in biogas and reduction of the pH level in the reactor can control the NH$_3$ content in biogas, although this practice resulted in severe decrease in the CH$_4$ yield.

2.7.4 Co-digestion

Co-digestion of animal manure with biomass will results in a higher methane yield than mono-digestion due to the synergistic effects of the co-substrates (Mata-Alvarez et al., 2000). The advantages of co-digestion of manures with co-substrates are: (1) due to the increase in the buffering capacity and the possibility in accumulation of volatile fatty acids (VFAs) during digestion (Campos et al., 1999), pH value can be constantly suitable within the methanogenesis stage (Brummeler and Koster, 1990); (2) it can avoid high concentrations of NH$_3$ in association with mono manure.
digestion; (3) it can provide a wide range of nutrient contents (C:N ratios) required by the methanogens (Angelidaki and Ahring, 1997); and (4) it can provide organisational and economical benefits by bringing an energy surplus, which will provide additional income to the biogas plants (Brolin and Kattstrom, 2000).

The co-digestion concept has been studied and applied to treat substrates such as municipal solid waste, sewage sludge, cow manure and energy crops (Mata-Alvarez et al., 2011), with Denmark and Germany as the leading countries in the world (Raven and Gregersen, 2007). Recent interest in producing renewable electricity through AD has rapidly increased the use of co-digestion of crops (such as maize silage) in farm-scale manure digesters (Neureiter et al., 2005). Co-digestion of cattle manure with off-farm waste is also a common practice in the United States (Frear et al., 2011). Co-digestion of crops with manure results in a higher methane yield than mono-digestion of manure. For example, co-digestion of 40% of sugar beet tops with dairy manure in batch and continuously fed laboratory reactors yielded about 1.5 times higher methane yields than mono-digestion of dairy manure (Umetsu et al., 2006). Laboratory continuously stirred tank reactors (CSTRs) operated with 30% of feedstock VS consisting of crops (grass, sugar beet tops, and straw) resulted in 16–65% higher methane yields than from dairy manure alone, but the yields decreased at the crop fraction of 40% of feedstock VS (Lehtomäki et al., 2007). Cavinato et al. (2010) reported that biogas production improved from 0.45 to 0.62 m³/kg VS operating at proper thermophilic conditions treating cattle manure and energy crops in pilot-scale studies. There are a number of publications reporting co-digestion of manure with other agricultural and industrial materials, as summarised in Table 2.11.
Table 2.11 Examples of co-digestion of animal manures with agro-residues and wastes

<table>
<thead>
<tr>
<th>Manure</th>
<th>Co-digestion substrate</th>
<th>Reactor</th>
<th>Conditions</th>
<th>Performance</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>chicken manure</td>
<td>fruit and vegetable wastes (FVW)</td>
<td>18 l CSTRs</td>
<td>T: 35 °C</td>
<td>HRT: 21 days</td>
<td>Increasing the proportion of FVW from 20% to 50% improved the SMY from 0.23 to 0.45 l CH₄/g VS added, but slightly decreased the VS reduction</td>
<td>Callaghan et al. (2002)</td>
</tr>
<tr>
<td>pig manure</td>
<td>sewage sludge, slaughterhouse waste, vegetable waste and various industrial wastes</td>
<td>3-litre jacketed glass reactor</td>
<td>T: 35 °C</td>
<td>OLR: 2.6 kg VS/m³/d HRT:36 days</td>
<td>Having a high buffering capacity due to the manure, the processes worked well with high SMYs, 0.56–0.68 l CH₄/g VS, although not all VFAs were converted.</td>
<td>Murto et al. (2004)</td>
</tr>
<tr>
<td>pig manure</td>
<td>herbal-extraction residues (HER)</td>
<td>7 l CSTRs</td>
<td>T:35 °C</td>
<td>OLR: 2.9 g VS/l/d HRT: 30 days</td>
<td>Biogas production was efficiently enhanced</td>
<td>Yan et al. (2011)</td>
</tr>
<tr>
<td>pig manure</td>
<td>potato tuber and its industrial</td>
<td>5 l CSTR</td>
<td>T: 35 °C</td>
<td>OLR: 2 kg VS/m³/d</td>
<td>Post-digestion (for 60 days) of the digested materials in batch</td>
<td>Kaparaju and Rintala (2005)</td>
</tr>
<tr>
<td>by-products fraction: 80% of pig manure reactors produced 0.12–0.15 l CH₄/g VS added at 35 °C</td>
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<tr>
<td>pig manure glycerine Batch reactor T: 35 °C Reactor volume of 250 ml with 230 ml being the working volume 0.215 l CH₄/g COD The mixture of 80% PM produced the highest methane with 0.215 l CH₄/g COD, almost 125% more than mono-digestion of pig manure. In contrast, the system with 20% PM was clearly inhibited by VFAs due to the low nitrogen concentration of the mixture Astals et al., (2011)</td>
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<tr>
<td>pig manure vegetable waste (green peas, maize, carrots and leeks) 5 l CSTR T: 37 °C OLR: 0.4 and 0.6 kg VS/m³/d fraction: 50% of pig manure and 50% of vegetable waste 0.277 and 0.285 l CH₄/g VS added The addition of vegetable wastes (50% dw/dw) resulted in an improvement of 3 and 1.4 folds in methane yields compared with mono-digestion at HRTs of 25 and 15 days, respectively. Molinuevo-salces et al. (2012)</td>
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<tr>
<td>cow manure OFMSWb 4.5 l lab-scale reactors T: 55 °C OLR: 3.3–4.0 kg VS/m³/d 0.63–0.71 biogas /g VS added There was no sign of inhibition at the free ammonia Hinrich et al. (2005)</td>
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<tr>
<td>Cow manure</td>
<td>Grass silage, sugar beet tops and oat straw</td>
<td>VS: 50% of manure HRT: 14–18 d T: 35 °C VS: 30% of crop in the feedstock OLR: 2 kg VS/m³/d</td>
<td>0.268, 0.229 and 0.213 l CH₄/g VS added, respectively</td>
<td>Concentration of 0.45 - 0.62 g-N/l</td>
<td>Including 30% of crop in the feedstock increased methane production per digester volume by 16-65% in comparison with that obtained from digestion of manure alone. Increasing the proportion of crops further to 40% decreased the SMYs by 4-12%</td>
<td>Lehtomaki et al. (2007)</td>
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<tr>
<td>Cow manure</td>
<td>OFMSW, and cotton gin waste (CGW)</td>
<td>two-phase anaerobic digestion system</td>
<td>OFMSW + cow manure CGW + cow manure</td>
<td>0.172 and 0.087 l CH₄/g dry waste, respectively</td>
<td>Co-digestion resulted in higher methane gas yield, higher mass conversion and lower weight and volume of digested residual</td>
<td>Macias-Corral et al. (2008)</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Whey</td>
<td>Pilot-scale 128 l anaerobic digester</td>
<td>Operate under batch and fed-batch conditions</td>
<td>0.211 l CH₄/g VS added</td>
<td>There is a good potential to use a cow manure and whey biomass mix for biogas production</td>
<td>Comino et al. (2009)</td>
</tr>
<tr>
<td>Cow manure</td>
<td>By-products from sugar production: sugar beet leaves</td>
<td>3 l CSTRs</td>
<td>T: 37 and 55 °C HRT: 20 days OLR: 1.76-6.75 g VS/l/d</td>
<td>0.17-0.28 l CH₄/g VS added</td>
<td>Co-digestion of SBP and DM was only successful at high dilution with manure or water.</td>
<td>Fang et al. (2011)</td>
</tr>
</tbody>
</table>
(SBL), sugar beet top (SBT), sugar beet pulp (SBP) and desugared molasses (DM) were used as substrates for biogas production. SBP was a good substrate for biogas production and the methane yield was 0.28 l CH₄/g VS added at 55°C and 50% of SBP in the feedstock.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Methane yield (l CH₄/g VS added)</th>
<th>Temperature (°C)</th>
<th>VS Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow manure (chicken litter, sheep manure, barley straw, food waste and fresh leaves)</td>
<td>0.133-0.268</td>
<td>T: 37-55°C</td>
<td>55°C</td>
</tr>
</tbody>
</table>

Methane yield from multi-component substrates was significantly higher than what would be expected based on the methane yields from single-component substrates.

Ashekuzzaman and Poulsen, 2011

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*a: OFMSW: organic fraction of the municipal solid waste*
2.8 GREENHOUSE GASES MITIGATION

Greenhouse gases (GHGs) include carbon dioxide (CO\textsubscript{2}), methane (CH\textsubscript{4}), nitrous oxide (N\textsubscript{2}O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulphur hexafluoride (SF\textsubscript{6}), etc. CO\textsubscript{2}, CH\textsubscript{4}, and N\textsubscript{2}O contribute 49%, 15–20%, and 6% of GHGs to global warming, respectively (van’t Klooster et al., 1997). Globally, 360 Mt of CH\textsubscript{4} and 10-17.5 Mt of N\textsubscript{2}O are released from anthropogenic sources annually (Olsen et al., 2003). CH\textsubscript{4} has a global warming potential (GWP) 21 times that of CO\textsubscript{2}, and N\textsubscript{2}O has a GWP 310 times that of CO\textsubscript{2} (IPCC, 2007). The atmospheric mixing ratio of CO\textsubscript{2}, confirmed by a wide range of direct and indirect measurement, has increased globally by about 100 ppm (36%) since pre-industrial era (AD 1000 – 1750); the average CO\textsubscript{2} increase rate determined by direct instrumental measurements over the period 1960 to 2005 is 1.4 ppm/yr (IPCC, 2007). The consequences of the increase in GHG emissions are a rise in average global temperature (by 0.5–2.5 °C by 2030) and a rise in the global mean sea level, predicted to be 17–26 cm by 2030, which would affect water supplies, flood frequency, the range and number of pests, biodiversity, etc (Moss et al., 2000).

2.8.1 Greenhouse gases emissions in the agricultural sector

GHG emissions from the agricultural sector relevant to animal production mainly comprise direct CH\textsubscript{4} emission from livestock, CH\textsubscript{4} and N\textsubscript{2}O emission from manure storage and grazed lands, and N\textsubscript{2}O emission from soils after application of manure (Kebreab et al., 2006b).

The IPCC (IPCC, 2007) estimates that agriculture’s share of total anthropogenic
emissions amounts to 10-12% (5.1-6.1 Gt CO$_2$-eq/yr) in 2005; agriculture contributes to about 47% and 58% of total anthropogenic emissions of CH$_4$ and N$_2$O, respectively. However, there is a wide range of uncertainty in estimating the agricultural contribution. US-EPA (2006) estimated net CO$_2$ emissions of 40 Mt CO$_2$-eq from agricultural soils in 2000, less than 1% of global anthropogenic CO$_2$ emissions. CH$_4$ and N$_2$O emissions from the agriculture sector in the EU (15 states) amounted to 374 Mt CO$_2$-eq in 2007, which were 11% below 1990 levels (the baseline for the Kyoto Agreement), corresponding to approximately 10% of total EU (15 states) GHG emissions (EEA, 2009).

In Ireland, emissions from the agricultural sector accounted for 35% of all GHG emissions in 1990, the highest of all sectors. According to the National Inventory Report (McGettigan et al., 2006), GHG emissions from the agricultural sector amounted to 18.96 Mt of CO$_2$ equivalent (Mt CO$_2$-eq.), or 27.7% of total recorded GHG emissions from Ireland in 2004. In 2011 this figure stood at 29.1% and it is projected to fall by 30% by 2020 (Teagasc, 2011). The GHG emissions in the agricultural sector are dominated by CH$_4$. In 2004, livestock enteric fermentation accounted for 81% of agricultural CH$_4$ emission, which in turn accounted for 91% of the national CH$_4$ emissions; whilst manure management accounted for the remaining 19% (Bullock and Styles, 2006).

Given the relative strength of the agriculture sector in the Irish economy and its high GHG emissions, the Irish government is targeting large reductions of CH$_4$ emission from this sector as one of the main approach to meet its Kyoto commitments (Hynes et al., 2009). As part of the EU target under the Kyoto Protocol, Ireland has agreed to limit the growth in its GHG emissions to 13% above 1990 levels by the first commitment period of 2008-2012 (SEAI, 2011). Under EU Commission’s ‘Energy and Climate Package’, Ireland is required to deliver a 20% reduction in non-ETS
greenhouse gas emissions (from agriculture, transport, residential and waste activities, and excluding main industrial activities which are covered under the EU Emissions Trading Scheme) by 2020 (relative to 2005 levels) and keep emissions below annual limits over the period 2013-2020 (EPA, 2011).

Emission factors of livestock are presented in Table 2.12, which represent the quantity of gas produced by an animal over a specific period of time. For instance, a dairy cow is estimated to release 100 kg of CH$_4$ per annum through enteric fermentation and 15.9 kg CH$_4$ through its waste. In the pig industry, the current manure management practice emits more GHG emissions than enteric fermentation (Table 2.12) (Chadwick et al., 2011).

**Table 2.12. Emission Factors of Livestock by the Source Category**

<table>
<thead>
<tr>
<th>Source category</th>
<th>Emission factors (kg of CH$_4$ per livestock per annum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enteric fermentation</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>100</td>
</tr>
<tr>
<td>Non-dairy cattle</td>
<td>50</td>
</tr>
<tr>
<td>Sheep</td>
<td>8</td>
</tr>
<tr>
<td>Horses</td>
<td>18</td>
</tr>
<tr>
<td>Mules/asses</td>
<td>10</td>
</tr>
<tr>
<td>Swine</td>
<td>1.5</td>
</tr>
<tr>
<td>Poultry</td>
<td>0</td>
</tr>
</tbody>
</table>


Most CH$_4$ of agricultural origin arises from enteric fermentation, with rice paddies
also being a large source (Chadwick et al., 2011). Methane generation during manure management is due to anaerobic decomposition of organic matter in manure and bedding material (Batstone and Keller, 2003; Møller et al., 2004).

Nitrous oxide (N\textsubscript{2}O) is generated by nitrification and denitrification, which occur in soil following application of manure (Fangueiro et al., 2008, 2010; Singurindy et al., 2009). Transformation of NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{−} via nitrification is a source of N\textsubscript{2}O, which is a by-product; during the biological reduction of NO\textsubscript{3}\textsuperscript{−} to N\textsubscript{2} gas, N\textsubscript{2}O is an important intermediate product of incomplete denitrification (Fig. 2.13) (Chadwick et al., 2011). Livestock bedding and solid manure storage are other sources of N\textsubscript{2}O emissions (Blanes-Vidal et al., 2008).

\[
\begin{align*}
\text{Nitrification} & \quad \text{Denitrification} \\
\text{NH}_4^+ & \xleftrightarrow{} \text{NH}_2\text{OH} \xrightarrow{} \text{NO}_2^- \\
\text{NO}_3^- & \xrightarrow{} \text{NO}_2^- \xrightarrow{} \text{NO} \xrightarrow{} \text{N}_2 \\
\end{align*}
\]

Fig. 2.13 Schematic chemical representation of N\textsubscript{2}O production pathways (Chadwick et al., 2011).

### 2.8.2 GHG emissions mitigation during manure management

A variety of techniques have been studied for GHG reduction from manure management, such as covered storage, diet manipulation, composting, solid-liquid separation and anaerobic digestion.
Storage

Storage of solid manure provides both aerobic and anaerobic conditions within close proximity, causing N₂O production, consumption and emission to occur (Hansen et al., 2006). Emissions of N₂O range from less than 1% to 4.3% of the total N in stored cattle and pig manure heaps, but emissions up to 9.8% have been reported by Webb et al. (in press). In Europe, covers have been built in an attempt to reduce odours or capture biogas so that it cannot escape into the atmosphere. The covers also prevent wind from removing gas (Miner et al., 2000).

Very little is known about the effects of diet on GHG emissions from stored manure. Clark et al. (2006) reviewed the effects of diet manipulation on odour and GHG reductions from swine and suggested that diet manipulation is related to GHG emissions reduction.

There is a need to determine the relationship between CH₄ production in slurry storage and temperature. Sommer et al. (2007) found that CH₄ production from stored pig and cattle slurry was not significant at temperatures below 15 °C, while CO₂ was the main product of decomposition processes. In contrast, CH₄ production was high and significant relative to CO₂ production at 20 °C. Peak emissions of CH₄ averaging 0.012 and 0.02 g C h⁻¹ kg⁻¹ VS were reached within about 10 days at 10 and 15 °C, respectively. At 20 °C, the emission of CH₄ from pig slurry storage was about 0.01 g C h⁻¹ kg⁻¹ for 10 days, and thereafter emissions increased to about 0.10 g C h⁻¹ kg⁻¹ VS. For cattle slurry storage a peak emission of 0.08 g C h⁻¹ kg⁻¹ VS was measured after 180 days.
Separation/Composting

The agricultural practice of solid-liquid separation of manure is able to reduce GHG emissions. Separated solids can be used in conjunction with anaerobic digestion for biogas production (Holmberg et al., 1983). Using only the solid portion of the manure for anaerobic digestion can increase the volatile solids concentration of the substrate and allow for a greater volumetric CH$_4$ yield than from the whole manure (Moller et al., 2004). The more volatile solids transfer from the liquid to the solid portion of the manure, the more CH$_4$ can be produced.

The solid fraction emits more N$_2$O during storage than untreated slurry (Hansen et al., 2006). Hansen et al. (2006) showed that 4.8% of the initial total N content of separated solids from pig slurry was lost as N$_2$O over a 4-month period.

Manure treatment via composting can be active with forced aeration, or passive with turning. After active composting of liquid hog manure with wheat straw, Thompson et al. (2004) found that GHG emissions were reduced to 30% of those from composting of untreated manure. However, air and water pollution during composting through ammonia volatilisation and nitrate leaching may reduce the compost’s fertiliser value and thereby its desirability as a mitigation measure (Peigné and Girardin, 2004).

A study conducted by Su et al. (2003) measured GHG emissions from three pig farms and three dairy farms in northern, central, and southern Taiwan. Average emissions rates for CH$_4$ from selected pig and dairy farms were lower than the limits imposed by the IPCC, because animal manure was diluted before being treated with a solid/liquid separator and an anaerobic wastewater treatment system in Taiwan. Analysis of GHG samples from in situ anaerobic wastewater treatment systems of pig and dairy farms
revealed average emissions of 0.768 and 4.898 kg CH$_4$ per head per year, 0.714 and 4.200 kg CO$_2$ per head per year, and 0.002 and 0.011 kg N$_2$O per head per year during three temperature periods, respectively.

*Land spreading*

A number of publications have addressed the effects of AD on N$_2$O emissions after land application of animal manure. Amon *et al.* (2006) indicated that AD could be an effective way to reduce GHG emissions from dairy manure slurries, as AD treated dairy manure emitted 28% less N$_2$O than raw manure after field application. Chantigny *et al.* (2007) compared N$_2$O emissions from mineral fertiliser, raw liquid swine manure and anaerobically digested swine manure applied on loam and sandy loam soils planted with timothy. Raw liquid swine manure led to the highest N$_2$O emissions, and AD reduced N$_2$O emissions by 50% compared to raw liquid swine manure and by more than 24% compared to mineral fertiliser. However, other publications have showed that N$_2$O emission is highly variable, and did not differ among treatments before land application (Bertora *et al.*, 2008; Thomsen *et al.*, 2009).

Chiara *et al.* (2008) examined the effect of the slurry composition on N$_2$O and CO$_2$ emissions during a 58-day mesocosm study. They tested raw pig slurry (NT), the solid fraction (SF) and the liquid fraction (LF) of pig slurry, and the anaerobically digested liquid fraction of pig slurry (DG). The treatment type strongly affected slurry composition (like its C, fibre and NH$_4^+$ content), and hence N$_2$O and CO$_2$ emission patterns. They estimated that amending the soil with 100 kg of NT would produce approximately 0.98 kg of CO$_2$-C and 18.7 g of N$_2$O-N in 58 days; separating the slurry and applying to the soil with SF and LF would produce approximately 1.22 kg of CO$_2$-C and 8.1 g of N$_2$O-N; the total emissions after the soil application with DG
would be 0.99 kg CO$_2$-C and 5.8 g N$_2$O-N. Therefore the separation alone or the combination of separation and digestion can reduce the N$_2$O emissions in comparison with application with raw slurry.

*Anaerobic digestion*

Kaparaju and Rintala (2011) investigated the impact of AD technology on mitigating GHG emissions during manure management on typical dairy, sow and pig farms in Finland. Results show that enteric fermentation (CH$_4$) and manure management (CH$_4$ and N$_2$O) accounted for 231.3, 32.3 and 18.3 Mg of CO$_2$ eq. yr$^{-1}$ on typical dairy, sow and pig farms, respectively. An estimated renewable energy of 115.2, 36.3 and 79.5 MWh of heat yr$^{-1}$ and 62.8, 21.8 and 47.7 MWh of electricity yr$^{-1}$ could be generated in a CHP plant on these farms with biogas produced via anaerobic digestion, respectively. The total GHG emissions that could be offset on the studied dairy, sow and pig farms were 177, 87.7 and 125.6 Mg of CO$_2$ eq. yr$^{-1}$, respectively. They conclude that the impact of AD technology on mitigating GHG emissions was mainly through replaced fossil fuel consumption followed by reduced emissions due to reduced fertiliser use and production, and from manure management (storage and manure treatment).

In order to investigate mitigation measures, Weiske *et al.* (2006) implemented four measures (optimised lifetime efficiency of dairy cows; frequent removal of manure and scraping systems; biogas production by anaerobic digestion; improved manure application techniques) in a whole-farm model and found that scraping systems and a frequent removal of manure from animal housing into a covered storage do not always have the expected reduction potential when evaluated at the farm level, while anaerobic digestion could be a very efficient and cost-effective option to reduce GHG
emissions. The GHG reduction efficiency depends on the amount and quality of organic matter used for co-digestion, and the extent to which the energy contained in biogas produced is exploited. A reduction of GHG emissions by up to 96% would be achievable when all biogas energy produced is used to substitute fossil fuels.

Amon et al. (2006) quantified NH$_3$, CH$_4$ and N$_2$O emissions from dairy cattle and pig slurries. 10 m$^3$ of differently treated slurry (no treatment, slurry separation - liquid fraction and composting of the solid fraction, anaerobic digestion, slurry aeration and straw cover) were stored in slurry tanks for 80 days, followed by application to permanent grassland. They found that GHG emissions from slurry were mainly caused by CH$_4$ emissions during storage and by N$_2$O emissions after field application. Untreated slurry emitted 226.8 g NH$_3$ /m$^3$ and 92.4 kg CO$_2$ eq. /m$^3$ (storage and field application). Slurry separation resulted in NH$_3$ losses of 402.9 g/m$^3$ and GHG emissions reduction of 58.5 kg CO$_2$ eq./ m$^3$. Anaerobic digestion was a very effective means to reduce GHG emissions (reduction of 37.9 kg CO$_2$ eq./ m$^3$). NH$_3$ emissions were similar to those from untreated slurry. Covering the slurry with a layer of chopped straw instead of a wooden cover increased NH$_3$ emissions to 320.4 g/m$^3$ and GHG emissions to 119.7 kg CO$_2$ eq./ m$^3$. Slurry aeration nearly doubled NH$_3$ emissions compared to untreated slurry and GHG emissions were reduced to 53.3 kg CO$_2$ eq. /m$^3$. They concluded that anaerobic digestion was an effective method to reduce GHG emissions but straw cover and slurry aeration showed negative environmental effects.

Cornejo and Wilkie (2010) investigated GHG emissions and biogas potential from livestock in Ecuador. They found that the total direct N$_2$O emissions from manure management (manure storage, solid - liquid separation and land application) were 172 Gg CO$_2$ eq, and total direct and indirect N$_2$O emissions from manure deposited on soils by grazing livestock were 2176 Gg CO$_2$ eq. A further 103 Gg CO$_2$ eq of direct N$_2$O emissions came from the application of confined-livestock manure to soils. They
estimated the total potential reductions in GHG emissions by capturing CH$_4$ from manure anaerobic digestion and substituting CH$_4$ for liquefied petroleum gas (LPG) at 308 Gg CO$_2$ eq.

Manure pre-stored before AD may be a significant source of CH$_4$, causing the reduction in the CH$_4$ production potential in the AD reactor. Moller et al. (2004) found that the losses in potential CH$_4$ yield from manure by AD after 5 d of storage were 1.8 - 3.8%, where the lowest loss was observed in pig manure stored at 15 °C and the highest loss was observed in cattle manure storage at 15 °C. After 15 d storage, the loss was 4.3 - 6.6%, where the highest loss was observed in cattle manure storage at 20 °C, and after 30 d, the loss was 7.7 - 11.9%. During long-term storage (50 d), the losses from pig manure stored at 20°C increased dramatically, whereas losses from cattle manure and pig manure stored at 15°C only increased moderately.

GHG mitigation from different livestock farms by using AD technology is summarised in Table 2.13.

**Table 2.13 GHG mitigation through the adoption of AD technology**

<table>
<thead>
<tr>
<th>Livestock farm</th>
<th>Mitigation of GHG emissions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy farms</td>
<td>177 Mg of CO$_2$ eq. yr$^{-1}$</td>
<td>Kaparaju and Rintala (2011)</td>
</tr>
<tr>
<td>Sow farms</td>
<td>87.7 Mg of CO$_2$ eq. yr$^{-1}$</td>
<td>Kaparaju and Rintala (2011)</td>
</tr>
<tr>
<td>Pig farms</td>
<td>125.6 Mg of CO$_2$ eq. yr$^{-1}$</td>
<td>Kaparaju and Rintala (2011)</td>
</tr>
<tr>
<td>Dairy cattle and pig slurries</td>
<td>37.9 kg CO$_2$ eq. m$^{-3}$</td>
<td>Amon <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>Livestock in Ecuador</td>
<td>308 Gg CO$_2$ eq. yr$^{-1}$</td>
<td>Cornejo and Wilkie (2010)</td>
</tr>
</tbody>
</table>
2.9 SUMMARY

This chapter presented a brief overview of pig industry in Ireland and worldwide, generation, characteristics and management of PM, the associated policies and its relevance in the context of the generation and treatment of PM and energy crops. Previous research on AD of PM and energy crops in terms of AD process, operational parameters and the technology of co-digestion have been reviewed. Mitigation of GHG emissions during manure management has also been discussed.
CHAPTER THREE

Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage

3.1. INTRODUCTION

To date, there is little information on the effects of the PM to GS ratio on the methane production potential and the stability of anaerobic co-digestion of PM and GS. This information would be beneficial to determine the maximum amount of GS to co-digest with PM.

In the present study, anaerobic co-digestion of GS and PM was investigated in batch experiments at various PM to GS ratios to examine: (i) the process stability, (ii) the system performance in terms of specific methane yield (SMY) and VS reduction, and (iii) kinetics of hydrolysis.

3.2. MATERIALS AND METHODS

3.2.1 Materials

Pig manure was obtained from a pig farm in Co. Galway, Ireland, and GS was obtained from Teagasc Athenry Research Centre, Co. Galway. After delivery to the laboratory, PM was sieved through a 2-mm sieve to remove coarse materials thus ensuring that laboratory tubing would not be blocked. The collected PM was dilute due to rain water
and settlement in the storage pond, with the total solids (TS) content of 3.7%, volatile solids (VS) content of 2.5% and soluble COD concentration of 33,200 mg/l. The PM was then concentrated by sieving through a 0.5-mm sieve. The PM fraction passing the sieve was settled in a container for 2 hours before some supernatant was removed from the container. The solid fraction remaining on the sieve was then added to the container and mixed evenly with the mixed liquor, to form concentrated PM. The concentrated PM had a TS content of 12.6% and VS content of 9.3%. This PM was used to simulate PM with high TS contents and PM concentrated with the separation process.

GS was manually cut to less than 20 mm by a knife. The sieved PM and cut GS were then frozen to prevent biological decomposition. To freeze GS was in accordance with the protocol used by Lehtomaki et al. (2008). Prior to commencement of the experiment, the frozen PM and cut GS were transferred to a refrigerator at 4 ºC for one day. The characteristics of PM and GS are given in Table 3.1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Grass silage</th>
<th>Pig manure</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5</td>
<td>7.4</td>
<td>7.9</td>
</tr>
<tr>
<td>TS (% fresh weight)</td>
<td>21.4</td>
<td>12.6</td>
<td>2.5</td>
</tr>
<tr>
<td>VS (% fresh weight)</td>
<td>20.2</td>
<td>9.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Neutral detergent fiber, NDF (%) DM</td>
<td>68.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein (% DM)</td>
<td>14.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble sugars (%) DM</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble COD (mg/l)</td>
<td>-</td>
<td>31200</td>
<td>5570</td>
</tr>
<tr>
<td>Total COD (mg/l)</td>
<td>-</td>
<td>126000</td>
<td>22420</td>
</tr>
<tr>
<td>Total COD (mg/mg VS)</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen, TKN (%) DM</td>
<td>1.6</td>
<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>NH₄-N (mg/l)</td>
<td>-</td>
<td>1650</td>
<td>1930</td>
</tr>
<tr>
<td>Lactic acid (%) DM</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile fatty acids, VFA (%) DM</td>
<td>4.9</td>
<td>3.1</td>
<td>-</td>
</tr>
</tbody>
</table>

3.2.2 Biological methane production potential (BMP) tests

The biological methane production potentials (BMPs) of the PM-GS mixtures were examined at five PM/GS VS ratios - 1:0 (Treatment A), 3:1 (Treatment B), 1:1
(Treatment C), 1:3 (Treatment D) and 0:1 (Treatment E) - in 1-litre digesters made from glass bottles. Each digester had two ports on the cap, one for liquid sampling and the other for gas sampling. The masses of VS of PM/GS added to each 1-litre digester for ratios A, B, C, D and E were respectively 28 g/0 g, 21 g/7 g, 14 g/14 g, 7 g/21 g and 0 g/28 g. Each digester was inoculated with 500 ml of mixed liquor (inoculums) taken from laboratory-scale continuously stirred digesters treating mixtures of PM and GS at a PM to GS ratio of 4:1. The inoculum contained 24.5 g/l of total suspended solids (TSS) and 15.6 g/l of volatile suspended solids (VSS). The control digesters had no PM and GS added but 500 ml of inoculum added. Tap water was added to each digester to give a working volume of 800 ml. The initial pH of the mixed liquor in each digester was adjusted to 7.5±0.1 by using 1 M HCl or 1 M NaOH. Finally, the digesters were flushed with N₂, and then sealed with the caps. The digesters were placed in a shaker incubator at 35 °C. The methane content in the head space and the methane volume produced from each digester were measured once daily. The specific methane yield (SMY) of each mixture was calculated by dividing the cumulative volume of methane produced after anaerobic degradation was complete by the total mass of VS initially added. Complete anaerobic degradation was assumed when there was no methane production observed for 15 days. No supplemental nutrients were added to the substrate. There were two replicates for each PM to GS ratio.

### 3.2.3 Analytical methods

The liquid samples were taken from digesters once every three days using 5-ml syringes. After immediate measurement of pH, the samples were then centrifuged at 1,700 g for 10 min and then at 21,912 g for 20 min at 4 °C. The supernatants were tested for soluble COD. For analysis of volatile fatty acids (VFAs), the supernatants were further filtered through 0.45 μm cellulose nitrate membrane filter paper (Whatman, England), and then VFAs were measured with high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) using a UV index detector and an Aminex HPX-87H column (Bio-Rad, USA). Separation during HPLC measurement was achieved using a mobile phase of 1% H₂SO₄ at a flow rate of 0.6 ml/min and the column temperature of 65 °C. The detector temperature was 40 °C. The
VFA mix containing acetic, propionic, isobutyric, butyric, isovaleric and valeric acids, each of 10 mM (Sigma–Aldrich, USA) was used for HPLC calibration.

Total solids, VS, soluble COD and alkalinity were analysed according to standard methods (APHA, 1995). The NH$_4$-N concentration in the liquid samples was analysed using a nutrient analyser (Konelab, Thermo Clinical Labsystems, Vantaa, Finland). The volume of biogas was measured by displacement of water, and was then converted to the biogas volume under standard temperature and pressure (STP) conditions of 0 °C and 1 atmosphere. The CH$_4$ content in biogas was measured using a 7890A gas chromatograph (GC, Agilent Technology, USA) with a thermal conductivity detector and a 45–60 mesh, matrix molecular sieve 5A column (Sigma–Aldrich, USA). Helium gas was the carrier gas at a flow rate of 30 ml/min. The temperature of the injection inlet, oven and detector was 100 °C, 60 °C and 105 °C, respectively. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc; Chicago, IL, USA).

3.3. RESULTS AND DISCUSSIONS

3.3.1 Process stability

Key factors measured to assess AD process stability were pH, VFA/alkalinity ratio, and concentrations of ammonium/free ammonia.

3.3.1.1 pH

In Treatment E (PM:GS = 0:1), pH fell immediately after the commencement of the experiment and reached a pH value of 5.9 on Day 27 (Fig. 3.1). The low pH value in Treatment E brought methane production to a complete halt. In Treatments A, B, C and D, pH values over 90 days were in the range of 6.4 - 8.0. The lowest values in Treatments A, B, C and D were 7.55 (Day 9), 7.14 (Day 21), 6.93 (Day 18) and 6.45 (Day 18), respectively. After the lag phase (about 20 days) of biogas production, pH
values in Treatments A, B, C and D rose and remained in the range of 7.0 to 7.8 till the end of the experiment. These findings are compatible with the normal growth of anaerobic microorganisms (Raposo et al., 2009). Higher pH values during the lag phase reflected the higher proportion of PM in the feedstock since the pH value of raw PM material was 7.4 and of raw GS material was 4.5. While the pH values at the four PM/GS ratios (Treatments A, B, C and D) were very close after the lag phase.

![Graph showing pH variation with time at different PM/GS ratios](image)

**Fig. 3.1 Variation of pH with time at different pig manure to grass silage ratios (1:0 (A), 3:1 (B), 1:1 (C), 1:3 (D) and 0:1 (E)).**

### 3.3.1.2 VFA/alkalinity

The maximum total VFA (TVFA) concentrations were obtained on Days 6, 24, 21 and 24 in Treatments A, B, C and D, and were 13.5 g/l, 17.1 g/l, 17.3 g/l and 16.2 g/l, respectively. The accumulation of TVFA demonstrates the inhibition of the methanogenesis process (Siles et al., 2008). TVFA concentrations were almost zero after Day 87, Day 81, Day 75 and Day 69 in Treatments A, B, C and D, respectively (Fig. 3.2). In Treatment E, the maximum TVFA concentration was 15.9 g/l occurring on Day 27 and then levelled off (data not shown).

When the VFA/alkalinity ratio was less than 0.3–0.4, the AD process was stable.
without an acidification risk (Borja et al., 2004). On Day 12, the ratios were 2.25, 2.30, 2.00, 2.88 and 2.23 in Treatments A, B, C, D and E, respectively, which were quite high and considered to inhibit the activity of methanogens (Borja et al., 2004). By Day 33, the ratios were 0.65, 0.37, 0.37, 0.82 and 5.15 in Treatments A, B, C, D and E, respectively. Hence, after 30 days from the commencement of the experiment, the systems under all PM to GS ratios except Treatment E were stable. Digestion of pure GS (Treatment E) was not stable and failed to produce methane after Day 18. This shows that for successful AD of GS it is necessary to add a source of external alkalinity to increase the buffering capacity. Otherwise, the digestion system would be unstable and even fail.

Fig. 3.2 Total volatile fatty acids (TVFA) profiles under different pig manure to grass silage ratios (1:0 (A), 3:1 (B), 1:1 (C) and 1:3 (D)).

3.3.1.3. Ammonium/free ammonia

The ammonium (NH$_4^+$-N) concentrations in Treatments A, B, C and D decreased with increasing the fraction of GS in the feedstock (Table 3.2). This clearly shows that co-digestion of GS with animal manure can prevent the probable adverse effects of NH$_4^+$-N on the system stability. The concentration of free ammonia (NH$_3$) in the
liquid phase was dependent on pH and its concentrations during the experiment at the five PM/GS ratios are shown in Table 3.2. In this study, free ammonia concentrations were high in Treatments A and B in comparison with Treatment C and D, with the highest levels of 246 and 210 mg/l, respectively. However, no significant inhibition was observed. The concentrations of $\text{NH}_4^+$-N and free ammonia that cause inhibition of AD vary in different AD systems. For example, 50% reductions in methane production have been found for $\text{NH}_4^+$-N concentrations from 1.7 to 14 g/l (Chamy et al., 1998; Hansen et al., 1998; Sung and Liu, 2003). The inhibition of free ammonia and $\text{NH}_4^+$-N on AD is reversible. Wu et al. (2009) found that during AD of meat and bone meal, inhibition of methanogens by free ammonia was reversible when the free ammonia concentration was as high as 998 mg/l. The varying inhibition concentrations of free ammonia and $\text{NH}_4^+$-N are attributed to the differences in substrates and inocula, environmental conditions (temperature, pH, etc.), and acclimation periods.

### Table 3.2. Anaerobic digestion at different pig manure to grass silage ratios.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM/GS ratio</td>
<td>1:0</td>
<td>3:1</td>
<td>1:1</td>
<td>1:3</td>
</tr>
<tr>
<td>Total methane production (ml)</td>
<td>7833</td>
<td>8517</td>
<td>8478</td>
<td>7484</td>
</tr>
<tr>
<td>lag phase*, $\lambda$ (d)</td>
<td>29.5±0.3</td>
<td>28.1±0.3</td>
<td>24.6±0.3</td>
<td>21.3±0.3</td>
</tr>
<tr>
<td>T80**</td>
<td>52.5</td>
<td>46.0</td>
<td>41</td>
<td>37.1</td>
</tr>
<tr>
<td>Rmax* (ml CH$_4$/d)</td>
<td>210±4</td>
<td>287±5</td>
<td>309±7</td>
<td>280±7</td>
</tr>
<tr>
<td>P* (ml)</td>
<td>8385±78</td>
<td>8829±52</td>
<td>8587±46</td>
<td>7497±42</td>
</tr>
<tr>
<td>R squared</td>
<td>0.997</td>
<td>0.997</td>
<td>0.997</td>
<td>0.995</td>
</tr>
<tr>
<td>SMY (ml CH$_4$/g VS)</td>
<td>279.8</td>
<td>304.2</td>
<td>302.8</td>
<td>267.3</td>
</tr>
<tr>
<td>VFA yields (g/g VS)</td>
<td>0.25±0.01</td>
<td>0.32±0.02</td>
<td>0.34±0.03</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>7.5-8.0</td>
<td>7.1-8.0</td>
<td>6.9-7.9</td>
<td>6.5-7.8</td>
</tr>
<tr>
<td>$\text{NH}_4$-N (mg/l)</td>
<td>1562-2368</td>
<td>1430-2240</td>
<td>1288-1850</td>
<td>1160-1330</td>
</tr>
<tr>
<td>Free $\text{NH}_3$ range (mg/l)</td>
<td>55-246</td>
<td>22-210</td>
<td>12-136</td>
<td>4-93</td>
</tr>
<tr>
<td>Free $\text{NH}_3$ at pH=7.8***</td>
<td>158</td>
<td>150</td>
<td>124</td>
<td>89</td>
</tr>
<tr>
<td>VS removal rate (%)</td>
<td>60.5</td>
<td>63.8</td>
<td>64.7</td>
<td>59.5</td>
</tr>
</tbody>
</table>

*Calculated from the Gompertz equation with 95% confidence interval.
** Technical digestion time: the time needed to produce 80% of the maximum gas production
*** Calculated after Anthonisen et al. (1976) for comparison
3.3.2 BMP of the PM-GS mixtures at different PM to GS ratios

In Treatments A, B, C and D, the initial methane production was low during the period from Day 1 to Day 20. This was probably due to the low addition of inoculum (VS = 7.8 g) relative to the substrate (VS = 28 g), resulting in low initial concentrations of methanogens in the reactors. After Day 20, methane production increased sharply, and consequently pH rose to 7.6 ± 0.2, indicating the enrichment of methanogens in the reactors. At the end of the experiment, methane production declined due to the lack of soluble biodegradable organic substances. In Treatment E, methane production ceased on Day 18 due to the accumulation of VFAs and a low pH.

In Treatment A, there were two peaks of the daily methane yield, occurring on Day 31 (223 ml) and on Day 55 (459 ml). In Treatments B, C and D, peaks of the daily methane yield occurred on Day 33 (368 ml), Day 29 (449 ml) and Day 29 (353 ml), respectively (Fig. 3.3).

The cumulative methane yields in Treatments A, B, C, D and E were 7833, 8517, 8479, 7484 and 298 ml, respectively. Thus, the SMYs of the PM-GS mixture in Treatments A, B, C, D and E were calculated as 279.8, 304.2, 302.8, 267.3 and 10.6 ml CH₄/g VS added, respectively. Treatments B and C had the highest SMY values and the ANOVA treatment showed that there were no significant differences between Treatments B and C (P>0.05).

There was no significant difference in the CH₄ content in biogas at different PM/GS ratios. The methane contents rose from Day 20 to reach a peak of 65%, 70%, 67% and 60% in Treatments A, B, C and D, respectively. Over the periods of Days 40 - 60, the methane contents at all Treatments ranged 55-65%. In the following 30 days, the methane contents decreased steadily to 54%, 54%, 53% and 52% in Treatments A, B, C and D, respectively.

Apart from the SMY and the cumulative methane yield, the duration of the lag phase
is also an important factor in determining the efficiency of anaerobic digestion. The lag phase ($\lambda$) can be calculated with the modified Gompertz model as described by Lay et al. (1998) as follows:

$$
M = P \cdot \exp \left\{ \exp \left[ \frac{R_{\text{max}}}{P} (\lambda - t) + 1 \right] \right\}
$$

(3.1)

Where, $M$, cumulative methane yield (ml CH$_4$); $P$, methane production potential (ml CH$_4$); $R_{\text{max}}$, maximum methane production rate (ml CH$_4$/day); $\lambda$, lag phase (day); and $t$, time (day).

The experimental data (Fig. 3.3) were used to fit Eq. (3.1) to estimate the values of $P$, $R_{\text{max}}$ and $\lambda$, given in Table 3.2. The methane production potential $P$ was 8385, 8829, 8587 and 7497 ml in Treatments A, B, C and D, respectively. The cumulative methane yields measured in the experiment were up to 94%-99% of corresponding $P$. $\lambda$ was 29.5, 28.1, 24.6 and 21.3 days in Treatments A, B, C and D, respectively. The digestion time is a key indicator to substrate biodegradability and the utilisation rate, and was thus investigated in this study. The technical digestion time, described with T80, is defined as the time needed to produce 80% of the maximum gas production (Palmowski and Muller, 2000). In Treatments A, B, C and D, according to Eq.(3.1), T80s calculated were 52.5, 46.0, 41.0 and 37.1 days, respectively. After subtracting the lag time ($\lambda$) from T80, the effective biogas production period in Treatments A, B, C and D lasted 23.0, 18.0, 16.4 and 15.8 days, respectively. Increasing the GS fraction in the feedstock resulted in a shorter effective biogas production period. In practice, the digestion time, in terms of hydraulic retention time (HRT) and solid retention time (SRT), can be shortened according to the effective biogas production period.
Fig. 3.3 Profiles of the daily methane yield and cumulative methane yield over 90 days at different pig manure to grass silage ratios (1:0 (A), 3:1 (B), 1:1 (C), 1:3 (D) and 0:1 (E)).

Co-digesting animal manure that has a low C/N ratio along with feedstock containing low levels of nitrogen (high C/N ratio) gives more stable operation performance and a higher methane yield than digesting manure only (Callaghan et al., 2002). It is likely that the low carbon content and high free ammonia concentrations resulted in relatively low SMY in Treatment A. The reasons for low SMY in Treatment D include: (1) during the first 20 days, the system reached a pH value as low as 6.45; this possibly inhibited the activity of methanogens; and (2) additional nutrients are required for anaerobic digestion of silage (Scherer et al., 2009). Therefore, when the fraction of GS in the feedstock is too high, it is hard to reach the optimum system performance in terms of the methane production potential.

Lehtomäki et al. (2007) investigated anaerobic co-digestion of cattle manure with GS, sugar beet tops and oat straw, and found that the highest BMPs were obtained when the crops were up to 30% of the feedstock. This optimal ratio was in the range of PM to GS ratios in Treatments B and C.

VS removals were 60.5%, 63.8%, 64.7% and 59.5% in Treatments A, B, C and D, respectively (Table 3.2). AD of GS can only remove 37%-67% of VS (Yu et al., 2002;
Lehtomaki and Bjornsson, 2006; Cirne et al., 2007; Lehtomaki et al., 2008), depending on the reactor configuration, temperature, GS type, pre-treatment methods, etc. VS removals during AD of PM alone or with various agro-industrial wastes range from 42% to 82% (Monou et al., 2009; Panichnumsin et al., 2010). In this study, the highest VS removals were achieved in Treatments B and C due to the positive synergism between GS and PM, resulting from the provision of balanced nutrients and reduction of inhibitory materials. Thus, the PG to GS ratio of 1:1 is recommended for application, under the context of the concentrated PM, because of its high methane production potential, short effective biogas production period, and as a result, utilisation of a high amount of GS in co-digestion with PM. The optimum VS ratio would depend on the type of animal manure, characteristics of the manure, species and characteristics of co-digested energy crops.

Up to the commencement of methane production there was an accumulation of acetic acid in the reactors. Once obvious methane production started (Day 31, Day 33, Day 29 and Day 29 in Treatments A, B, C and D, respectively), acetic acid began to decrease. Acetic acid was not detected after Day 90, Day 84, Day 78 and Day 72 in Treatments A, B, C and D, respectively.

During AD, methane is derived from acetoclastic methanogenesis or from hydrogenotrophic methanogenesis. Accumulation of acetic acid in a digester is the result of a greater production of acetic acid than its conversion to methane and carbon dioxide. It is difficult to quantify how much methane is originated from acetoclastic methanogenesis and from hydrogenotrophic methanogenesis (Mottet et al., 2009). In the present study, the daily methane production ($\nu$) was positively related to the acetic acid concentration ($x$) at all PM to GS ratios ($P<0.01$) (Fig. 3.4); this may indicate that methane was mainly produced via acetoclastic methanogenesis.
3.3.3 Hydrolysis process

At all PM/GS ratios, soluble COD concentrations increased dramatically in the first 10 days and decreased after Day 25. The highest soluble COD concentrations were 23,960, 23,180, 23,160 and 23,780 mg/l, and declined to 3,220, 2,360, 2,300 and 4,600 mg/l at the end of biogas production in Treatments A, B, C and D, respectively. The soluble COD removals were 86.6%, 89.8%, 90.1% and 80.7% in Treatments A, B, C and D, respectively.

It is often assumed that the rate-limiting step in anaerobic digestion of energy crops and crop by-products is hydrolysis of particulate matter to soluble matter. The hydrolysis yield, $Y_H$, is defined as liquefaction or solubilisation of organic matter and it is calculated according to Eq.(3.2):

$$Y_H = \frac{S_y}{S_I}$$  \hspace{1cm} (3.2)

Where, $S_I$ is the initial total substrate concentration expressed as mg/l total COD and $S_y$
is the soluble COD concentration in the liquid samples due to hydrolysis.

Increasing the GS fraction in the feedstock resulted in increasing soluble COD concentrations from 8678.6 to 14958.3 mg/l. However, the hydrolysis yields decreased from 59.5% to 50.1% as the GS fraction in the feedstock rose. The soluble COD removal rate increased from 86.6% to 90.1% with the increase of GS fractions up to 50%, and a further increase in the GS fraction resulted in a negative effect and a lower soluble COD removal rate of 80.7% in Treatment D.

The hydrolysis can be described with a first-order equation:

\[
S_I(t) = S_{\text{max}}[1 - \exp(-k_H t)]
\]  
(3.3)

Where, \( S_I(t) \) is the soluble COD concentration at time \( t \) due to hydrolysis; \( S_{\text{max}} \) is the maximum soluble COD concentration due to hydrolysis; and \( k_H \) is the first-order hydrolysis rate constant (d\(^{-1}\)). Soluble COD concentrations in individual lag phases were used to fit into Eq. (3.3) to obtain the parameter of \( k_H \).

In this study, \( k_H \) decreased with decreasing PM/GS ratios (0.56, 0.46, 0.44 and 0.34 d\(^{-1}\) in Treatments A, B, C and D, respectively) (Table 3.3). Because the hydrolysis rate constant of cellulose is as low as 0.09 d\(^{-1}\) (Myint et al., 2007) and the main component of grass silage is cellulose, the trend of decreasing \( k_H \) with the high fraction of GS in the feedstock was to be expected:

\[
k_H = -0.26f + 0.55 \quad (R^2=0.94)
\]  
(3.4)

Where, \( f \) is the fraction of GS in the PM-GS mixture.

The hydrolysis rate constant, \( k_H \), is different for each energy crop and crop by-product, and varies with temperature. Veeken and Hamelers (1999) found \( k_H \) of 0.266 d\(^{-1}\) for
grass at 40 °C. Lübken et al. (2007) simulated anaerobic digestion of manure and cow fodder under mesophilic conditions (38 °C) and calculated $k_H$ of 0.31 d$^{-1}$. The hydrolysis of GS depends on the contents of cellulose, hemicellulose and lignin in the grass structure. The hydrolysis of cellulose can be enhanced by applying cultures that have the cellulolytic activity. Song and Clarke (2009) found $k_H$ of 0.45 d$^{-1}$ for cellulose in a mixed culture enriched from landfill waste in a continuous reactor at 38 °C. Hu and Yu (2005) used rumen microorganisms to enhance anaerobic digestion of corn stover and estimated $k_H$ to be 0.94 d$^{-1}$ at 40 °C, which was quite high due to the high cellulolytic activity of the rumen microorganisms.

Table 3.3. Hydrolysis process at different pig manure/grass silage ratios (±standard error).

<table>
<thead>
<tr>
<th>PM:GS ratio</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM:GS ratio</td>
<td>1:0</td>
<td>3:1</td>
<td>1:1</td>
<td>1:3</td>
</tr>
<tr>
<td>Total COD (mg/l)</td>
<td>40600</td>
<td>43225</td>
<td>45850</td>
<td>48475</td>
</tr>
<tr>
<td>Initial soluble COD (mg/l)</td>
<td>15550</td>
<td>13600</td>
<td>11700</td>
<td>9760</td>
</tr>
<tr>
<td>$S_{\text{max}}$ (mg/l)</td>
<td>8679±96</td>
<td>10082±173</td>
<td>12257±362</td>
<td>14958±422</td>
</tr>
<tr>
<td>$k_H$ (d$^{-1}$)</td>
<td>0.56±0.03</td>
<td>0.46±0.03</td>
<td>0.44±0.06</td>
<td>0.34±0.04</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.996</td>
<td>0.975</td>
<td>0.991</td>
<td>0.980</td>
</tr>
<tr>
<td>Soluble COD removal rate</td>
<td>86.6%</td>
<td>89.8%</td>
<td>90.1%</td>
<td>80.7%</td>
</tr>
<tr>
<td>Hydrolysis yield, $Y_H$</td>
<td>59.5%</td>
<td>54.8%</td>
<td>52.2%</td>
<td>50.1%</td>
</tr>
</tbody>
</table>

3.4 SUMMARY

The co-digestion systems were stable in operation at PM to GS ratios of 1:0, 3:1, 1:1 and 1:3, while the digestion systems digesting pure GS failed. The highest SMYs were achieved at PM to GS ratios of 1:3 and 1:1. The efficient methane production period lasted 23.0, 18.0, 16.4 and 15.8 days at PM to GS ratios of 1:0, 3:1, 1:1 and 1:3, respectively. The hydrolysis rate constants were 0.56, 0.46, 0.44 and 0.34 d$^{-1}$ at PM to GS ratios of 1:0, 3:1, 1:1 and 1:3, respectively. The PG/GS VS ratio of 1:1 is recommended for commercial application.
CHAPTER FOUR

Methane Production from Anaerobic Co-Digestion of the Separated Solid Fraction of Pig Manure with Dried Grass Silage

4.1. INTRODUCTION

On mainland Europe, AD systems are common. For example, in Germany there are more than 4,000 on-farm AD systems and in Denmark there were more than 20 large scale centralised AD systems in 2009 (Raven and Gregersen, 2007; Wilkinson, 2011). However, as biomass to feed large digesters must be transported from the farms, transport costs can be high. Reducing the cost of transportation, which is one of the important factors determining whether or not anaerobic digestion of PM is economically feasible, can be achieved by separating the solid and liquid fractions of PM and then only transporting the solid fraction for digestion (Raven and Gregersen, 2007). The solid fraction of PM produces more biogas per unit volume than raw PM due to its higher organic matter content. For these reasons, AD of the solid fraction of separated pig manures is carried out in some countries such as Denmark (Møller et al., 2007).

Compared to AD of PM on its own, co-digestion of manure with GS can increase biogas yields as found in Chapter 3. Therefore, when PM and GS are co-digested increasing the fraction of GS in the feedstock should increase the methane yields. The organic loading rate (OLR) is an important operational parameter for AD. Whilst increasing the OLR could possibly result in an increase in the volumetric methane
production it could also increase the risk of system failure due to accumulation of VFA$s$, $\text{NH}_4^+$-$\text{N}$ and free ammonia ($\text{NH}_3$) in the system (Sánchez et al., 2005). However, there is limited information available on the concurrent effects of PM to GS ratio and OLR on methane production and the operation stability of AD systems.

In the present study, anaerobic co-digestion of the solid fraction of mechanically separated pig manure (SPM) and dried grass silage (DGS) was investigated using continuously stirred tank reactors (CSTRs) with different OLRs at various SPM to DGS VS ratios. The purpose of this experiment was to examine: (i) process stability; (ii) system performance in terms of methane production: volumetric methane production, SMY, post methane production potential and VS reduction; and (iii) the energy yield.

4.2. MATERIALS AND METHODS

4.2.1 Feedstocks and inoculum

Grass silage was obtained from Teagasc, Athenry Research Centre, Co. Galway. Pig manure solids were produced by separation of liquid PM using a decanter centrifuge (GEA Westfallia Separator UCD 205, Germany). Alum at 3 litres/m$^3$ liquid manure and polyacrylamide flocculant (PAM) diluted with water to 0.4% by volume and added at 17% by volume to the liquid PM were used during the process to increase the separation efficiency. Campos et al. (2008) did not observe PAM toxicity on anaerobic digestion for PAM concentrations below 415 g/kg total solids, so the addition of PAM in this study did not affect the following anaerobic digestion. After delivery to the laboratory, the SPM was stored in a cold room where temperature was set at 11±1 °C until required. Because feeding fresh GS would have caused difficulties with the stirrers in the laboratory-scale digesters and with non homogeneity of the digestate, the GS was dried at 60 °C for 24 hours and then chopped in a blender (Philips HR2000/50 Silver Food Blender, Netherlands) to ~5 mm in length. Some losses of VFA}s from the
GS during drying were expected though these were not measured. The dried and chopped grass silage (DGS) was stored at ~4 °C in a sealed container. One day before use the DGS was transferred to ambient temperature (~20 °C). The characteristics of fresh GS, DGS and SPM measured before the experiments are shown in Table 4.1. Total solids and VS of SPM were 28.2% and 20.1% of fresh weight, respectively; TS and VS of GS were 26.6% and 25.0% of fresh weight, respectively.

### 4.2.2 Operation of continuously stirred tank reactors

Anaerobic co-digestion of SPM and DGS was carried out in three identical glass CSTRs (CSTR1, CSTR2, and CSTR3), each having a working volume of 3 litres. The top of each digester had three outlets which were used for feeding the feedstock, withdrawing digestate and collecting biogas, respectively. The CSTRs were placed on a three-position stirrer hotplate (SB162-3, Stuart, UK) which maintained the temperature in each reactor at 35±1 °C. The magnetic stirrers were used to ensure the homogeneity of the liquid phase in the reactors. Digesters were seeded with 2.5 l of sludge taken from a mesophilic digester (Mutton Island Waste Water Treatment Plant, Galway, Ireland), one day before commencement of the experiment. Additionally, 400 g of SPM and 20 g of DGS were added to the digesters at this time. Tap water was added to the reactors to reach the working volume of 3 l so as to obtain a SPM/DGS-inoculum mixture with a solid concentration suitable for anaerobic digestion, and then the reactors were flushed with N₂ gas for 5 minutes. The characteristics of the seed sludge are shown in Table 4.1.

The digesters were syringe-fed daily with 100 ml of feedstock (SPM, DGS and tap water mixture) below the liquid level to achieve a nominal HRT of 30 days. The feedstock for each digester had different proportions of DGS and SPM. The proportions used were characterised by the quantities of VS supplied. In CSTR1, 20% of the VS was supplied by DGS and 80% by SPM; in CSTR2, 30% of the VS was supplied by DGS and 70% by SPM; and in CSTR3, 40% of the VS was supplied by DGS and 60%
by SPM. These ratios were chosen based on a previous batch study on co-digestion of grass silage and pig manure, which shows that the PM to GS ratio should be no lower than 1:1 in order to achieve a stable digestion system (Xie et al., 2011).

**Table 4.1.** Characteristics of fresh grass silage, separated solid pig manure and seed sludge used as inoculum

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fresh GS</th>
<th>DGS</th>
<th>SPM</th>
<th>Seed sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5</td>
<td>-</td>
<td>7.4</td>
<td>7.9</td>
</tr>
<tr>
<td>TS (% fresh weight)</td>
<td>26.6</td>
<td>28.2</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>VS (% fresh weight)</td>
<td>25.0</td>
<td>20.1</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>Soluble COD (mg/l)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5570</td>
</tr>
<tr>
<td>Total COD (mg/l)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22420</td>
</tr>
<tr>
<td>Total COD (mg/mg VS)</td>
<td>1.41</td>
<td>1.40</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>TKN (mg/g TS)</td>
<td>16.1</td>
<td>-</td>
<td>83.1</td>
<td>-</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/g TS)</td>
<td>-</td>
<td>-</td>
<td>16.7</td>
<td>28.3</td>
</tr>
<tr>
<td>NH$_4^+$-N/TKN</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>VFA (% TS)</td>
<td>4.9</td>
<td>0.3</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>94.0</td>
<td>94.0</td>
<td>71.3</td>
<td>64</td>
</tr>
</tbody>
</table>

The operation of the three CSTRs is shown in Table 4.2. Initially, the OLR, defined as the VS mass of the feedstock fed to the digesters per m$^3$ of the digester volume per day, was equivalent to 1 kg VS/m$^3$/d, and it was increased stepwise through 1.5 kg VS/m$^3$/day and 2.0 kg VS/m$^3$/day to 3.0 kg VS/m$^3$/day (since the nominal HRT was constant at 30 days, the OLR was increased by increasing the feedstock mass). It was observed that there was little methane production from CSTRs in the first 13 days, so it was decided to stop feeding the three reactors from Day 14 to Day 24. The feeding was resumed on Day 24 when production of biogas increased.
Table 4.2. Operational and environmental parameters in CSTRs (average of 10 days data in pseudo steady state)

<table>
<thead>
<tr>
<th>OLRs</th>
<th>Parameters</th>
<th>CSTR1</th>
<th>CSTR2</th>
<th>CSTR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPM/DGS ratio</td>
<td>80%/20%</td>
<td>70%/30%</td>
<td>60%/40%</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>1 kg VS/m³/d</td>
<td>pH</td>
<td>7.60±0.01</td>
<td>7.58±0.01</td>
<td>7.55±0.01</td>
</tr>
<tr>
<td>(Days 24-84)</td>
<td>TVFAs (mg HAc/l)*</td>
<td>462±17</td>
<td>516±47</td>
<td>509±15</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N (mg/l)</td>
<td>1454±13</td>
<td>1341±10</td>
<td>1314±16</td>
</tr>
<tr>
<td></td>
<td>TKN (mg/l)</td>
<td>2561±39</td>
<td>2340±43</td>
<td>2240±47</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N/TKN (%)</td>
<td>56.8</td>
<td>57.3</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td>TS of digestate (%)</td>
<td>1.99±0.05</td>
<td>1.84±0.04</td>
<td>1.74±0.03</td>
</tr>
<tr>
<td></td>
<td>VS of digestate (%)</td>
<td>0.99±0.04</td>
<td>0.94±0.02</td>
<td>0.95±0.03</td>
</tr>
<tr>
<td>1.5 kg VS/m³/d</td>
<td>pH</td>
<td>7.52±0.01</td>
<td>7.48±0.01</td>
<td>7.44±0.01</td>
</tr>
<tr>
<td>(Days 85-109)</td>
<td>TVFAs (mg HAc/l)</td>
<td>728±31</td>
<td>754±25</td>
<td>768±56</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N (mg/l)</td>
<td>1704±21</td>
<td>1525±12</td>
<td>1472±19</td>
</tr>
<tr>
<td></td>
<td>TKN (mg/l)</td>
<td>3161±42</td>
<td>2808±47</td>
<td>2666±29</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N/TKN (%)</td>
<td>53.9</td>
<td>54.3</td>
<td>55.2</td>
</tr>
<tr>
<td></td>
<td>TS of digestate (%)</td>
<td>3.17±0.08</td>
<td>3.00±0.06</td>
<td>2.85±0.03</td>
</tr>
<tr>
<td></td>
<td>VS of digestate (%)</td>
<td>1.66±0.06</td>
<td>1.65±0.03</td>
<td>1.65±0.01</td>
</tr>
<tr>
<td>2 kg VS/m³/d</td>
<td>pH</td>
<td>7.51±0.01</td>
<td>7.47±0.01</td>
<td>7.45±0.01</td>
</tr>
<tr>
<td>(Days 110-134)</td>
<td>TVFAs (mg HAc/l)</td>
<td>987±60</td>
<td>989±19</td>
<td>1022±11</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N (mg/l)</td>
<td>1829±65</td>
<td>1672±58</td>
<td>1544±34</td>
</tr>
<tr>
<td></td>
<td>TKN (mg/l)</td>
<td>4387±44</td>
<td>3849±49</td>
<td>3481±38</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N/TKN (%)</td>
<td>41.7</td>
<td>43.4</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td>TS of digestate (%)</td>
<td>4.66±0.03</td>
<td>4.43±0.03</td>
<td>4.21±0.04</td>
</tr>
<tr>
<td></td>
<td>VS of digestate (%)</td>
<td>2.65±0.02</td>
<td>2.63±0.01</td>
<td>2.62±0.03</td>
</tr>
<tr>
<td>3 kg VS/m³/d</td>
<td>pH</td>
<td>7.46±0.01</td>
<td>7.41±0.01</td>
<td>7.36±0.01</td>
</tr>
<tr>
<td>(Days 135-172)</td>
<td>TVFAs (mg HAc/l)</td>
<td>812±12</td>
<td>790±11</td>
<td>873±12</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N (mg/l)</td>
<td>2288±16</td>
<td>2065±28</td>
<td>1830±21</td>
</tr>
<tr>
<td></td>
<td>TKN (mg/l)</td>
<td>7084±66</td>
<td>6300±58</td>
<td>5692±53</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺-N/TKN (%)</td>
<td>32.3</td>
<td>32.9</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>TS of digestate (%)</td>
<td>8.07±0.03</td>
<td>7.76±0.04</td>
<td>7.42±0.03</td>
</tr>
<tr>
<td></td>
<td>VS of digestate (%)</td>
<td>5.07±0.01</td>
<td>5.05±0.01</td>
<td>5.01±0.01</td>
</tr>
</tbody>
</table>

* TVFAs: total volatile fatty acids. TVFA was estimated as the sum of acetic acid equivalents (mg/l) of each gram volatile fatty acid: acetic acid, 1; propionic acid, 1.418; isobutyric and butyric acid, 1.704; and isovaleric and valeric acids, 1.910 (Yilmaz and Demirer, 2008).
4.2.3 Measurement of post methane production potentials of digestate

The post-methane production potentials of the digestates were measured in batch experiments using 118 ml serum vials incubated at 35 °C. Digestate (50 ml, which contained active biomass), was taken on Day 84, Day 109, Day 134 and Day 164 from each of the three CSTRs and thoroughly mixed before addition to the vials. The vials were then flushed with N₂ gas for 5 min, sealed with butyl rubber stoppers and aluminium crimps to ensure the air-tight condition, and incubated until there was no further increase in methane production for 7 consecutive measurements. No supplemental nutrients were added to the substrate. The post methane production potential tests were conducted in triplicate. The volume of biogas was measured twice daily using a 100-ml syringe.

4.2.4 Analytical methods

Each day, prior to feeding, 100 ml of digestate was removed from each reactor and its pH was immediately measured. The analytical methods for soluble COD, VFAs, TS, VS, alkalinity, NH₄-N concentration, volume of biogas, and CH₄ content in the biogas are described in Chapter 3 (Section 3.2.3). Values for methane production and digestate characteristics are presented as averages of data obtained over 10 consecutive days when the reactor performance was considered to have been adapted to the respective operational conditions and to have reached pseudo steady state. Statistical analysis was performed via one-way analysis of variance (ANOVA) using PASW statistics 18 for Windows (SPSS Inc., Chicago, IL, USA) for the significant difference.

4.2.5 Calculations

SMY, Yᵥ, VP and PP are calculated as:
\[
SMY = \frac{V_m}{W_{VS}} \quad (4.1)
\]

\[
Y_v = \frac{V_m}{V_D}
\] (4.2)

\[
VP = \frac{V_{cm}}{V_d} \quad (4.3)
\]

\[
PP = \frac{V_{cm}}{V_0} \quad (4.4)
\]

\( \eta \) is calculated as follows:

\[
\eta \ (\%) = \frac{V_E \times C_{EVS} \times PP}{V_E \times C_{EVS} \times PP + V_I \times C_{WS} \times SMY} \quad (4.5)
\]

or

\[
\eta \ (\%) = \frac{V_E \times VP}{V_E \times VP + V_I \times C_{WS} \times SMY} \quad (4.6)
\]

**4.3. RESULTS AND DISCUSSIONS**

**4.3.1 Performance of CSTRs**

**4.3.1.1 Methane production**

After inoculating the CSTRs, there was a sharp decrease in pH from 7.45 (Day 1) to 6.42-6.53 (Day 10) and only small quantities of methane were produced (Fig. 4.1 a). For this reason, feeding of the feedstock was suspended on Day 14 until methane
production increased significantly on Day 24. At this point feeding was resumed at a loading rate of 1 kg VS/m$^3$/day. Thereafter, pH in the digesters and the methane content in the biogas remained stable (Fig. 4.1 a). The mean SMYs calculated over 10 days of steady state operation (Days 73-84) were 252.5, 261.6 and 271.2 ml CH$_4$/ g VS added for CSTR1, CSTR2 and CSTR3, respectively (Fig. 4.1 b, Table 4.3). The mean volumetric methane production was 252.5, 261.6 and 271.2 ml/l/d for CSTR1, CSTR2 and CSTR3, respectively.

![Fig. 4.1 pH, methane content and daily methane production during anaerobic co-digestion of solid fraction of pig manure and dried grass silage in CSTRs.](a)

![Fig. 4.1 pH, methane content and daily methane production during anaerobic co-digestion of solid fraction of pig manure and dried grass silage in CSTRs.](b)
The OLR was then increased to 1.5 kg VS/m$^3$/d on Day 85. The pH values decreased to 7.45, 7.38 and 7.33 on Day 91, Day 94 and Day 94 for CSTR1, CSTR2 and CSTR3, respectively, and increased slightly thereafter (Fig. 4.1a). The methane content in the biogas decreased during this period and increased to above 54% again for all CSTRs (Fig. 4.1a). The average SMY during the 10 days of pseudo steady-state (Days 99-109) for CSTR1, CSTR2 and CSTR3 were lower than those at the OLR of 1 kg VS/m$^3$/d (P<0.01) (Table 4.3). The mean volumetric methane production was 372.3, 382.5 and 400.5 ml/l/d for CSTR1, CSTR2 and CSTR3, respectively.

The OLR was increased to 2 kg VS/m$^3$/d on Day 110. The lowest pH values observed were 7.33, 7.31 and 7.28 on Day 115 for CSTR1, CSTR2 and CSTR3, respectively. It then increased and levelled at 7.51, 7.46 and 7.45 (Fig. 4.1a). The average SMYs in the 10 days of pseudo steady-state for CSTR1, CSTR2 and CSTR3 were 15.4%, 14.9% and 16.4% less than those at the OLR of 1.5 kg VS/m$^3$/d (Table 4.3), respectively. The mean volumetric methane production was 419.8, 434.0 and 446.6 ml/l/d for CSTR1, CSTR2 and CSTR3, respectively.

The OLR was increased to 3 kg VS/m$^3$/d on Day 135. The lowest pH values observed were 7.39, 7.33 and 7.29 on Day 142 in CSTR1, CSTR2 and CSTR3, respectively. It increased and levelled at 7.45, 7.41 and 7.36 (Fig. 4.1a). The average SMYs during the 10 days of pseudo steady-state for CSTR1, CSTR2 and CSTR3 were 35.2%, 36.0% and 37.5% less than those under the OLR of 1.5 kg VS/m$^3$/d, respectively (Table 4.3). The volumetric methane production was 482.1, 489.6 and 501.0 ml/l/d for CSTR1, CSTR2 and CSTR3, respectively.

Increasing the OLR from 1 to 3 kg/m$^3$/d increased the volumetric methane production from an average (of data of all three CSTRs) of 0.26 to 0.49 litres CH$_4$ /litre of digester per day (88% increase), while the SMYs decreased by an average of 38% (from 262 to 164 ml CH$_4$/g VS added). The lower SMY as the OLR increased was reflected by lower VS removals. At each OLR there was a tendency for SMY to increase as the proportion of VS provided by DGS in the feedstock increased.
It was shown in the study that a DGS fraction of 40% (VS) in the feedstock is feasible to AD without any decrease in the methane yield (Table 4.3). Similarly, Xie et al. (2011) obtained a methane production potential of 303 ml CH₄/ g VS added under the optimum PM/GS VS ratio of 1:1. However, Lehtomaki et al. (2007) observed a reduction in the SMY at an energy crop (grass silage, sugar beet tops and oat straw) ratio equal to or higher than 40% in co-digestion with cow manure, which was possibly due to the lower HRT applied (16-20 days) than in the present study (30 days). A long HRT can ensure sufficient degradation and utilisation of the organic matter in biomass, and as a result, a high SMY. The benefit from optimising the OLR and the ratio of DGS to SPM during co-digestion was shown by the fact that during feeding with 40% VS of DGS in the feedstock at the OLR of 3 kg VS/m³/d, up to 98.4% higher volumetric methane production was obtained than that obtained at the lower proportions (20%) of DGS at OLR of 1 kg VS/m³/d. Furthermore, the highest SMYs were obtained at the OLR of 1 kg VS/m³/d with 40% VS of DGS in the feedstock, while increasing the OLR resulted in linear decrease in the SMY (Fig. 4.2 a).
Table 4.3. Performance of CSTRs at the mesophilic condition (average of 10 days in steady state)

<table>
<thead>
<tr>
<th>OLRs</th>
<th>Parameters</th>
<th>CSTR1</th>
<th>CSTR2</th>
<th>CSTR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPM/GS ratio</td>
<td>80%/20%</td>
<td>70%/30%</td>
<td>60%/40%</td>
</tr>
<tr>
<td>1 kg VS/m^3/d</td>
<td>SMY (ml CH₄/g VS)</td>
<td>252.5</td>
<td>261.6</td>
<td>271.2</td>
</tr>
<tr>
<td></td>
<td>Days 24-84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VS removal (%)</td>
<td>67.0</td>
<td>68.7</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>Volumetric methane production (ml CH₄/l digester/day)</td>
<td>252.5</td>
<td>261.6</td>
<td>271.2</td>
</tr>
<tr>
<td></td>
<td>PP (ml CH₄/g VS)</td>
<td>115.2</td>
<td>111.3</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>VP (ml CH₄/ml digestate)</td>
<td>1.14</td>
<td>1.05</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Post/total methane production (%)</td>
<td>13.1</td>
<td>11.8</td>
<td>10.7</td>
</tr>
<tr>
<td>1.5 kg VS/m^3/d</td>
<td>SMY (ml CH₄/g VS)</td>
<td>248.2</td>
<td>255.0</td>
<td>267.0</td>
</tr>
<tr>
<td></td>
<td>Days 85-109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VS removal (%)</td>
<td>63.1</td>
<td>63.3</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>Volumetric methane production (ml CH₄/l digester/day)</td>
<td>372.3</td>
<td>382.5</td>
<td>400.5</td>
</tr>
<tr>
<td></td>
<td>PP (ml CH₄/g VS)</td>
<td>114.4</td>
<td>109.3</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>VP (ml CH₄/ml digestate)</td>
<td>1.90</td>
<td>1.80</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>Post/total methane production (%)</td>
<td>14.5</td>
<td>13.6</td>
<td>12.1</td>
</tr>
<tr>
<td>2 kg VS/m^3/d</td>
<td>SMY (ml CH₄/g VS)</td>
<td>209.9</td>
<td>217.0</td>
<td>223.3</td>
</tr>
<tr>
<td></td>
<td>Days 110-134</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VS removal (%)</td>
<td>55.8</td>
<td>56.2</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>Volumetric methane production (ml CH₄/l digester/day)</td>
<td>419.8</td>
<td>434</td>
<td>446.6</td>
</tr>
<tr>
<td></td>
<td>PP (ml CH₄/g VS)</td>
<td>145.6</td>
<td>139.4</td>
<td>134.7</td>
</tr>
<tr>
<td></td>
<td>VP (ml CH₄/ml digestate)</td>
<td>3.86</td>
<td>3.67</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td>Post/total methane production (%)</td>
<td>23.5</td>
<td>22.0</td>
<td>20.8</td>
</tr>
<tr>
<td>3 kg VS/m^3/d</td>
<td>SMY (ml CH₄/g VS)</td>
<td>160.7</td>
<td>163.2</td>
<td>167.0</td>
</tr>
<tr>
<td></td>
<td>Days 135-172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VS removal (%)</td>
<td>43.7</td>
<td>43.9</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td>Volumetric methane production (ml CH₄/l digester/day)</td>
<td>482.1</td>
<td>489.6</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td>PP (ml CH₄/g VS)</td>
<td>196.5</td>
<td>187.4</td>
<td>182.6</td>
</tr>
<tr>
<td></td>
<td>VP (ml CH₄/ml digestate)</td>
<td>9.96</td>
<td>9.46</td>
<td>9.15</td>
</tr>
<tr>
<td></td>
<td>Post/total methane production (%)</td>
<td>40.8</td>
<td>39.2</td>
<td>37.8</td>
</tr>
</tbody>
</table>
The positive linear relationship between increasing DGS VS ratio in the feedstock and the SMY is shown in Fig. 4.2 b. This has been reported previously with different crops and crop by-products. For example, Lehtomaki et al. (2007) obtained an increase in the SMY from 143 to 268 ml CH₄/g VS added when the proportion of grass silage in the feedstock increased from 10% to 30% at a VS basis when co-digested with cow manure; Callaghan et al. (2002) reported that increasing the proportion of fruit and vegetable wastes from 36% to 69% at a VS basis when
co-digested with cattle slurry improved the SMY from 230 to 450 ml CH\textsubscript{4}/g VS added; and Kaparaju and Rintala (2005) observed increasing the proportion of potato tuber and its industrial by-products (potato stillage and potato peels) from 0% to 20% at a VS basis when co-digested with pig manure improved the SMY from 130-150 to 300-330 ml CH\textsubscript{4}/g VS added. The increase in the SMY when the proportion of crops/crop by-products increased was due to the fact that crops/crop by-products usually contains more easily biodegradable carbohydrates and provides higher C/N ratios than manure. Compared with these studies, the less increase in the SMY when increasing the DGS VS ratio in the feedstock in the present study may be due to the loss of soluble organic matter like VFAs during drying fresh GS.

The effects of the OLR and r\textsubscript{DGS} on the SMY can be expressed with the equation as follows:

\begin{equation}
\text{SMY} = -52.2 \times \text{OLR} + 71.5 \times r_{\text{DGS}} + 301.1 \quad (R^2=0.96, \, P=0.00)
\end{equation}  

(4.7)

4.3.1.2 VFAs

During anaerobic digestion, VFAs are important intermediate products. Most CH\textsubscript{4} produced is derived from VFAs. The addition of 400 g SPM and 20 g DGS in the first day of the experiment, equating an instant OLR of 19.7 kg VS/m\textsuperscript{3}/d, would result in an accumulation of VFAs due to the relatively high hydrolysis and acidification rate, thereby inhibiting the activity of methanogens (Siles \textit{et al.}, 2008). As reported by Wang \textit{et al.} (2009), when propionic acid (HPr) and total VFA concentrations reached about 2850 mg/l and 10.0 g/l, respectively, the activity of methanogens was inhibited to a significant extent, but this inhibitory effect was weakened when the total VFA concentration fell to 6.2 - 8.5 g/l. From Day 13 to Day 25, the total VFA concentrations were in the range of 10.3 - 10.5, 10.7 - 11.0 and 10.9 - 11.4 g/l in CSTR1, CSTR2 and CSTR3, respectively (Fig. 4.3). The feeding stopped from Day 14 to Day 24 to allow methanogens to gradually recover from the high concentrations of VFAs. Biogas production was found to rise from Day 25. Therefore, the feeding was resumed at this time as methanogens were thought to have been adapted to the high concentrations of
VFAs; the concentrations of VFAs began to decrease on Day 31. Similarly, Xie et al. (2011) observed the accumulation of VFAs with the total VFA concentration reaching as high as 17.3 g/l when pig manure was anaerobic co-digested with grass silage and the concentrations of VFAs decreased following the production of biogas. The total VFA concentrations decreased to less than 1.1 g/l in the three CSTRs on Day 58. The concentrations of VFAs increased slightly in the first few days after each increase in the OLR. For instance, total VFA concentrations increased to 1.4-1.6 g/l when the OLR increased from 1 to 1.5 kg VS/m$^3$/d and subsequently decreased to 0.7-0.8 g/l. The gradual increase of the OLR avoided sharp accumulations of VFAs and the total VFA concentrations remained below 2 g/l after Day 58 in the three CSTRs indicating they were not operated under a stress-overloading state and there was no inhibition of VFAs.

![Fig. 4.3 Total VFA concentrations during anaerobic co-digestion of pig manure and grass silage in CSTRs](image)

4.3.1.3. $NH_4^+$/TKN

The $NH_4^+$-N and TKN concentrations at different OLRs are summarised in Table 4.2. As the concentration of TKN in the TS of SPM was more than four times greater than that in GS, the TKN concentration decreased when the DGS ratio in the feedstock increased from 20% to 40%. Therefore, the lowest $NH_4^+$-N concentration was found in CSTR3 at each OLR. However, increasing the OLR from 1 to 3 kg VS/m$^3$/d
resulted in higher concentrations of TKN in all CSTRs, due to higher NH$_4^+$-N concentrations in the feedstock (from 407-542 mg/l to 1220-1626 mg/l). The NH$_4^+$-N concentration in CSTRs continued to increase throughout the experiment from an initial 0.8 g/l to 1.8-2.3 g/l (Table 4.2). The linear relationship between the NH$_4^+$-N concentration and the OLR is shown in Fig.4.4 a. The NH$_4^+$-N concentrations in the present study did not reach toxic levels of >4 g/l which would induce free ammonia inhibition to methane production (De Baere et al., 1984). The calculated free ammonia concentrations, corresponding to the above-mentioned NH$_4^+$-N values, ranged 4.3-74.1 mg/l, 3.7-59.7 mg/l and 3.1-51.7 mg/l in CSTR1, CSTR2 and CSTR3, respectively. Studies with pure cultures suggest that excess ammonia can act in two ways, by inhibiting the enzyme which is involved in methane production or by diffusing into the cells and causing a proton imbalance (Kayhanian, 1999); while, Wu et al. (2009) reported inhibition of methanogens by free ammonia was reversible even though free ammonia concentrations were as high as 998 mg/l during anaerobic digestion of meat and bone meal. The digestate contained a higher NH$_4^+$-N to TKN ratio than the raw SPM (0.20); this indicates that nitrogen availability to plants would increase if landspreading the digestate as a fertiliser (Table 4.2). However, when increasing the OLR from 1 to 3 kg VS/m$^3$/d, the NH$_4^+$-N to TKN ratio decreased from 56.8%-58.7% to 32.1%-32.9%.
Fig. 4.4 Dependence of NH$_4^+$-N concentrations (a), VS removal (b) and PP (c) on the OLR (because VS removal rates were very close in the three CSTRs, the mean VS removal rate of the three CSTRs were plotted).
4.3.1.4 VS removal

The highest VS removal efficiencies achieved were in the range of 67.0%-68.3% at the OLR of 1 kg VS/m$^3$/d and the VS removal efficiencies decreased to 43.7%-44.3% at an OLR of 3 kg VS/m$^3$/d (Table 4.3). AD of GS can only remove 37%-67% VS (Cirne et al., 2007; Lehtomaki and Bjornsson, 2006; Lehtomaki et al., 2008; Yu et al., 2002), depending on the reactor configuration, temperature, GS type, pre-treatment methods, etc. In general, VS removals during AD of PM alone or with various agro-industrial wastes range from 42% to 82% (Monou et al., 2009; Panichnumsin et al., 2010). In this study, the highest VS removals were achieved at the OLR of 1 kg VS/m$^3$/d and the VS removal was negatively related to the OLR for the three CSTRs ($P < 0.05$) (Fig. 4.4 b).

4.3.1.5 Post-methane production potentials of digestate

When increasing the OLRs, the volumetric methane productivity increased (Table 4.3), but the efficiency of organic matter degradation decreased, leading to an increase in the PP and VP (Table 4.3, Fig. 4.4 c). The highest PP was 196.5 ml CH$_4$/g VS, corresponding to VP of up to 9.96 ml CH$_4$/ml digestate. This suggests that the digested materials (digestate) still contained biodegradable material. Similarly, Kaparaju and Rintala (2003) obtained 30% additional methane during 6 months’ storage of digestate at 35 °C compared to that obtained during anaerobic digestion of dairy cow manure. The PP values increased with increasing the OLR (Fig. 4.4 c) and when the OLR was increased from 1 to 3 kg VS/m$^3$/d, the post methane potential of the effluent at 35 °C increased from 10.7%-13.1% to 37.8%-40.8% of the total methane production from the CSTRs (Table 4.3). The relatively short HRT and the high OLR in conventional CSTRs could be the main reason for the high post methane production potential. A high post methane production potential indicates that proper
management (storage, land application, etc) of the digestate should be carefully considered so as to avoid the waste of the biogas and to reduce greenhouse gas emissions. Therefore, Kaparaju et al. (2009) suggested adopting anaerobic digestion of cow manure in two CSTRs that were connected in series and obtained 13 -17.8% more methane compared with the previous one-step process.

4.3.2 Energy yield

In Ireland, the average herd size is currently 654 sows (Teagasc, 2011). Assuming that 20.7 m$^3$ of liquid manure is produced per sow and progeny per annum (S.I. 610, 2010) the liquid manure generated from 654 sows would be 13538 m$^3$ per year. On average, this manure is assumed to contain 4.3% TS (Hackett, 1997), which means that 431 tonnes of VS (74% of TS, Table 4.1) is generated annually on an average Irish pig farm. Assuming there is 20% loss of VS during the process of handling, pumping, separation and natural decomposition during storage, etc, the total amount of SPM VS is 345 tons for digesters. The required mass of fresh GS (25% of VS) to be co-digested at GS VS ratios in the feedstock of 20%, 30% and 40% will be 345, 591 and 920 tons, which requires a land area of 6.6, 11.4 and 17.7 hectare, respectively, based on the annual yield of 13 tons dry matter of GS per hectare (Braun et al., 2008).
The Combined Heat and Power (CHP) process produces both usable heat energy and electric power from the same fuel source. The energy yield estimated at the OLR of 3 kg VS/m$^3$/d from a farm with 654 sows using the CHP technology is given in Table 4. The estimation is based on the assumptions as follow: a pig farm has 654 sows and (or has access to) at least 17.7 hectares grassland; the energy content of methane gas is 11.04 kWh/m$^3$ methane or 890.3 kJ/ mole methane (Duerr et al., 2005); and energy input for manure separation and transport, and for mixing in the digester is not considered. Increasing the OLR from 1 to 3 kg VS/m$^3$/d nearly doubles the energy yield (85%-91%) in terms of both electricity and heat. The amount of energy generated from a farm with 654 sows would be equivalent to up to 530 MWh/annual of heat and 371 MWh/annual of electricity from CHP.

### Table 4.4. Energy yield calculation at the OLR of 3 kg VS/m$^3$/d from an Irish farm with 654 sows

<table>
<thead>
<tr>
<th></th>
<th>GS20%</th>
<th>GS30%</th>
<th>GS40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated reactor working volume (m$^3$)$^a$</td>
<td>394</td>
<td>450</td>
<td>525</td>
</tr>
<tr>
<td>Hydraulic retention time (day)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Area of grassland (hectare)</td>
<td>6.6</td>
<td>11.4</td>
<td>17.7</td>
</tr>
<tr>
<td>Methane production (m$^3$/year)</td>
<td>69302</td>
<td>80434</td>
<td>96025</td>
</tr>
<tr>
<td>Energy yield @11.04 kwh/m$^3$ methane (MWh/year)$^b$</td>
<td>765</td>
<td>888</td>
<td>1060</td>
</tr>
<tr>
<td>Combustion engine electricity CHP production @ 35% (MWh/year)$^*$</td>
<td>268</td>
<td>311</td>
<td>371</td>
</tr>
<tr>
<td>Heat generation @ 50% (MWh/year)$^b$</td>
<td>383</td>
<td>444</td>
<td>530</td>
</tr>
<tr>
<td>Increase of energy yield compared with OLR 1 kg VS/m$^3$/d</td>
<td>90.9%</td>
<td>87.2%</td>
<td>84.7%</td>
</tr>
</tbody>
</table>

$^a$ Estimated reactor working volume = VS treated daily ÷ OLR; VS treated daily = total weight of the feedstock (in VS) treated annually ÷ 365

$^b$ Duerr et al. (2005)
4.4 SUMMARY

When DGS and SPM were co-digested in CSTRs at 35 oC with a HRT of 30 days, digestion was successful at all OLRs examined. Digestion was successful at all DGS ratios that were examined (20%, 30% and 40% of VS in the feedstock). Tripling the OLR (1.0 to 3.0 kg VS/m3/d) increased the volumetric methane yield by an average of 88% and decreased the specific methane yield by an average of 38%. The methane yield at each OLR was little affected by the proportion of DGS in the feedstock. Under the current situation investigated, the energy yields showed promising.
CHAPTER FIVE

Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion

5.1 INTRODUCTION

Dry matter of grass silage (GS) comprises up to 75% cellulose, hemi-cellulose and lignin. The crystalline structure of cellulose and the non-water soluble nature of lignin are barriers to the penetration of microbes and enzymes during anaerobic digestion. Hydrolysis is often assumed to be a rate-limiting step in anaerobic digestion particularly with materials like GS that contain high structural carbohydrates (Koch et al., 2009). Treating ligno-cellulosic biomass prior to anaerobic digestion can accelerate hydrolysis and improve biogas yields. Treatments include physical, biological, thermal and chemical processes.

Physical pre-treatment aimed at reducing the particle size and crystallinity of lingo-cellulosic, such as milling processes (ball milling, two-roll milling, hammer milling, colloid milling and vibro energy milling) and high-energy radiation (such as microwave irradiation at different oven’s irradiation power), is expensive (Alvira et al., 2010). Biological pre-treatment with lignin-degrading micro-organisms is energy-saving (fossil fuel saving compared with physical and thermal pre-treatment) but it takes a long time to enrich sufficient lignin-degradation microorganisms; additionally, the cellulose and hemi-cellulose are partially consumed during pre-treatment (Rodgers et al., 2009). Chemical pre-treatment with alkali at ambient
temperature is readily achievable and may have commercial potential (Rodgers *et al.*, 2009). Sodium hydroxide (NaOH) is typically used in alkaline pre-treatment. Lin *et al.* (2009) investigated NaOH pre-treatment of pulp and paper sludge prior to anaerobic digestion and found that the methane productivity was improved by up to 83.5% at a NaOH loading rate of 8% by weight of volatile solids (VS) in pulp and paper sludge. Zhu *et al.* (2010) applied NaOH pre-treatment to corn stover at an alkaline VS loading rate of 5% by weight of VS in corn stover and found enhanced biogas production by up to 37% compared with that without pre-treatment. Thermal pre-treatment with steam or hot water is also effective in hydrolysis of crops and crop by-products. Thermal pre-treatment at temperatures of 160 °C or above results in the solubilisation of hemicellulose and lignin. However, phenolic compounds can be produced, which have inhibitory or toxic effects on methanogenic bacteria (Gossett *et al.*, 1982). Chang *et al.* (2001) proposed thermal pre-treatment in combination with alkaline pre-treatment at temperatures of between 100-150 °C to improve the pre-treatment efficiency for switchgrass, corn stover and poplar wood. Wu *et al.* (2009) found that CH$_4$ production potential of meat and bone meal was increased from 389 to 503 ml CH$_4$/g, after alkaline (NaOH loading of 25% by weight of VS in meat and bone meal) and heat (55 °C) pre-treatments. Rafique *et al.* (2010) achieved an increase of 60% in methane production from dewatered pig manure after pre-treatment at 70 °C and a calcium hydroxide loading rate of 5% by weight of VS in pig manure. There have been few studies conducted on thermal alkali pre-treatment of GS in order to improve subsequent methane production.

Therefore, the objective of this study was to examine (1) the effects of combined thermal (ambient, 60 °C, 100 °C and 150 °C temperatures) and alkali (NaOH loading rates of 1%, 2.5%, 5% and 7.5% by weight of VS in GS) pre-treatment of GS, on the hydrolysis yield, kinetics of hydrolysis process and degradation of ligno-cellulosic materials, and (2) the effects of pre-treatment on the methane production potential of GS.
5.2. MATERIALS AND METHODS

5.2.1. Grass silage

GS was obtained from Teagasc, Athenry Research Centre, Co. Galway, Ireland. The GS was harvested in June, 2010 from a perennial ryegrass (Lolium perenne) dominant sward. The herbage was field-wilted for 24 hours before being baled and wrapped with 6 layers of polythene stretch film. 2 kg of the baled GS was obtained from each of 5 bales and mixed in a plastic bag after 5 weeks of ensilage. After transport to the laboratory, it was oven dried at 60 °C until no further weight loss and comminuted in a blender (Philips HR2000/50 Silver Food Blender) to an average length of approximately 10 mm. In order to prevent biological decomposition, the dried and comminuted GS (DCGS) was stored in an air tight container at 4 °C until one day before it was used; and then it was transferred to the laboratory at an ambient temperature of about 20 °C. The characteristics of fresh GS are given in Table 5.1.

Table 5.1. Characteristics of fresh grass silage

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (% fresh weight)</td>
<td>25.5</td>
</tr>
<tr>
<td>VS (% TS)</td>
<td>94.4</td>
</tr>
<tr>
<td>Cellulose (% TS)</td>
<td>34.3</td>
</tr>
<tr>
<td>Hemicellulose (% TS)</td>
<td>29.6</td>
</tr>
<tr>
<td>Lignin (% TS)</td>
<td>8.60</td>
</tr>
<tr>
<td>COD (mg/mg VS)</td>
<td>1.40</td>
</tr>
<tr>
<td>TKN (% TS)</td>
<td>1.61</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>26</td>
</tr>
</tbody>
</table>
5.2.2. Alkali-thermal pre-treatment of GS

The pre-treatment experiment was carried out in 50 ml digestion vials. In each vial, 2 g VS of DCGS (2.1 g DCGS) and 8 ml of NaOH solution were added. The NaOH solutions had NaOH concentrations of 2.5 g/l, 6.25 g/l, 12.5 g/l and 18.75 g/l, corresponding to NaOH loading rates of 1 g per 100 g VS from DCGS (1%), 2.5 g per 100 g VS from DCGS (2.5%), 5.0 g per 100 g VS from DCGS (5%) and 7.5 g per 100 g VS from DCGS (7.5%), respectively. The vials were sealed with caps and placed in a temperature controlled oven where the temperature was set at 20°C, 60°C, 100°C or 150°C. Under each pre-treatment condition, 12 parallel digestion vials were used. Two vials were used as controls, in which only 2 g VS of DCGS and 8 ml of de-ionised water were added. The vials were taken out of the oven at intervals depending on the time to reach equilibrium which varied from 4 - 48 hours. After cooling, samples were taken from the vials for measurement of soluble COD in the liquid phase, and cellulose, hemi-cellulose and lignin contents in the biomass. The results from the pre-treatments were obtained after subtracting the results from the control treatments. The experiment was conducted in duplicate.

5.2.3. Measurement of biological methane production potential of untreated and pre-treated grass silage samples

The biological methane production potentials (BMPs) of the untreated and pre-treated DCGS were tested in 1-litre batch glass digesters as described in Section 3.2.2, Chapter 3. Each digester had two ports on the lid, one for liquid sampling and the other for gas sampling. Liquid samples were taken using a 5-ml syringe and gas samples were taken using a 50-ml syringe.

Firstly, 20 g VS DCGS (untreated DCGS or DCGS pre-treated at 100°C and NaOH
VS loading rates of 1%, 2.5%, 5% and 7.5% by weight of VS in DCGS) was added to each digester. Secondly, 600 ml of inoculum (33.3 g VS/l) was added, which was taken from laboratory-scale continuously stirred digesters treating mixtures of pig manure and GS. The VS-based feedstock to inoculum ratio was 1:1. For the control digesters, only 600 ml of inoculum was added. Thirdly, tap water was added to each digester to make a final working volume of 800 ml so as to obtain a DCGS-inoculum mixture with a solid concentration suitable for anaerobic digestion and to reduce the headspace of the digesters. Finally, the digester headspaces were flushed with N₂, and then the digesters were sealed with caps and placed in a shaker incubator at 35 °C.

The experiment was carried out in duplicate. The methane content in the headspace and the biogas volume produced from each digester were measured daily. The biological methane production potential of each sample, in terms of the SMY, was calculated by dividing the total volume of methane produced up to the point when anaerobic degradation was complete by the total mass of VS initially added. During the experiment no additional nutrients were added to the digesters.

5.2.4. Analytical methods

The analytical methods for soluble COD (sCOD), VFAs, TS, VS, alkalinity, NH₄-N concentration, volume of biogas, and CH₄ content in the biogas are described in Section 3.2.3, Chapter 3. The concentrations of cellulose, hemi-cellulose and lignin in DCGS before and after pre-treatment were measured by the modified method of Wang and Xu (1987), which is outlined as follows: 1 g of biomass sample was heated with 100 ml of neutral detergent (made from sodium lauryl sulfate, disodium EDTA, sodium borate, sodium phosphate and water) at the boiling point for 1 hour, forming Residue 1; the hemi-cellulose content was measured as the weight loss after heating Residue 1
with 100 ml of 2 M HCl at 100 °C for 50 minutes (forming Residue 2); the cellulose content was measured as the weight loss after adding 10 ml of 72% H₂SO₄ to Residue 2, keeping the mixture at 20 °C for 3 hours, adding 90 ml of tap water and then keeping the mixture at ambient temperature overnight (forming Residue 3); and the lignin content was measured as the weight loss after ashing Residue 3 at 550 °C. The physical structures of DCGS were observed with a scanning electron microscope (SEM; Hitachi S-4700, Japan). The DCGS samples before and after the pre-treatment were dried at 60°C for 24 hours, ground in a mill (IKA Grinder, Germany) fitted with a 0.25 mm sieve. The changes in functional groups of the samples were recorded from 4000 to 550 cm⁻¹ by a FT-IR/FT-NIR spectrometer (PerkinElmer Spectrum 400, USA) with 32 accumulations at a resolution of 4 cm⁻¹. Every sample was measured in triplicate and for each of the three measurements a fresh (unused) sample was used (Hu et al., 2010).

5.3. RESULTS AND DISCUSSION

5.3.1. Thermal-chemical pre-treatment of DCGS

In this study, the pre-treatment efficiency was evaluated with respect to the hydrolysis yield, kinetics, and degradation of ligno-cellulosic materials in DCGS.

5.3.1.1. Hydrolysis yield and kinetics

The thermal-chemical pre-treatment resulted in the solubilisation of ligno-cellulosic materials and the release of sCOD. The increase in sCOD under each pre-treatment condition, referred to the total COD of fresh grass silage, is shown in Fig. 5.1.
At a constant temperature and pH, the hydrolysis rate followed a first-order equation for the conversion of particulate biomass to soluble substrates by chemicals (Rajan et al., 1989). The hydrolysis of DCGS by thermo-chemical pre-treatment can therefore be described with a first-order equation:

\[
S_I(t) = S_{I_{\text{max}}} [1 - \exp(-k_H t)]
\]  

(5.1)

where, \(S_I(t)\) is the soluble COD concentration at time \(t\) due to hydrolysis; \(S_{I_{\text{max}}}\) is the maximum soluble COD concentration due to hydrolysis; and \(k_H\) is the first-order hydrolysis rate constant (d\(^{-1}\)).

The equilibrium time is defined as the time at which \(S_I\) was equal to 99\% of \(S_{I_{\text{max}}}\). The experimental data of sCOD was used to fit Eq. (5.1) to estimate the equilibrium time by non-linear regression using SPSS 17.0 for Windows (SPSS Inc; Chicago, IL, USA). The equilibrium time was 8.8-36.3 hours, 2.0-3.9 hours, 1.9-3.6 hours and 2.3-3.5 hours at 20 °C, 60 °C, 100 °C and 150 °C, respectively. Solely increasing the NaOH loading rates shortened the time to reach the equilibrium, particularly at 20 °C (\(P<0.01\)), while there was little difference in the time to reach equilibrium when the temperatures were between 60 °C -150 °C (\(P>0.05\)).
As shown in Fig. 5.1, increasing both the pre-treatment temperature and the NaOH loading rate increased the release of sCOD at the equilibrium state. However, temperature had a much greater effect than NaOH concentration. For example, increasing temperature from 20 °C to 150 °C resulted in 8.3, 7.7, 7.2 and 6.2 fold increases in sCOD at NaOH loading rates of 1%, 2.5%, 5% and 7.5%, respectively. In contrast, increasing the NaOH loading rate from 1% to 7.5% increased sCOD by 90%, 38%, 42% and 36%, respectively, at pre-treatment temperatures of 20 °C, 60 °C, 100 °C and 150 °C.

5.3.1.2. Degradation of lingo-cellulosic materials

In biomass containing ligno-cellulose, such as grass silage, cellulose and hemicellulose are densely packed by layers of lignin, which protects them against
enzymatic hydrolysis; therefore, the digestibility of biomass mainly depends on the degree of solubilisation of lignin (Leonowicz et al., 1999). Alkali treatment breaks alkali-labile linkages between lignin monomers, or between lignin and polysaccharides, because alkaline solution can ionise the carboxylic and phenolic groups, increase the solubility of individual fragments or induce the swelling of the cell wall (Gierer, 1985). Increasing the NaOH loading rate increased the reduction of the lignin content in DCGS, causing the solubilisation of lignin from DCGS to the liquid phase (Fig. 5.2). In each pre-treatment condition, the reduction percentage of lignin in DCGS was the highest among the three ligno-cellulosic materials: lignin, cellulose and hemicellulose. At each temperature, increasing the NaOH loading rate resulted in the increased lignin reduction. This result is confirmed by Zhu et al. (2010), who found that increasing the NaOH loading rate from 1% to 7.5% resulted in increased lignin reduction in corn stover from 9.1% to 46.2% at ambient temperature.

Fig. 5.2. Profiles of reduction of lignocellulosic materials with thermo-chemical pretreatment.

The reduction of the hemicellulose content in the DCGS biomass was lower than that
of lignin, but higher than that of cellulose. Xiao et al. (2001) attribute solubilisation of hemicellulose by alkaline solution to the disruption and breaking of hydrogen bonds; they have observed that nearly all of the ester-linked structures of hemicellulose can be cleaved by alkali. The reduction of hemicelluloses increased as the NaOH loading rate and temperature increased (Fig. 5.2).

The reduction of the cellulose content in DCGS was much lower than that of lignin and hemicellulose. At 20 °C, 60 °C, 100 °C and 150 °C, with the increase of NaOH loading rate from 1% to 7.5%, the reduction of cellulose increased from 3.6% to 5.4%, from 9.6% to 18.9%, from 10.3% to 21.2%, and from 17.6% to 35.2%, respectively. The relatively low cellulose reduction was possibly due to (1) the physical protection afforded by lignin and hemicellulose, and (2) the modification of cellulose crystallinity during alkaline treatment, rather than direct solubilisation (Hendriks and Zeeman, 2009).

Temperature proved to be a crucial factor in the pre-treatment of ligno-cellulosic biomass. Increasing temperature can result in a higher reduction rate of cellulose, hemicellulose and lignin in DCGS. However, high pre-treatment temperatures need increased energy input, increase the complexity of the process operation and may generate possible inhibitors (such as phenolic compounds) to enzymatic hydrolysis and fermentation (Galbe and Zacchi, 2007).

As mentioned previously, the solubilisation of lignin is critical to the digestibility of GS. Zhu et al. (2010) reported the solubilisation of lignin needed to be at least 31% to allow corn stover to be efficiently digested. In addition, the methane production rate and the methane yield from anaerobic digestion depend on the hydrolysis process in terms of concentrations of sCOD (Xie et al., 2011). At 20°C, the solubilisation of
lignin was less than 31%. At 60°C, the solublisation of lignin was 38% and 57% at the NaOH loading of 5% and 7.5% by the weight of VS in GS. When the temperature was increased to 100 °C, the reduction of lignin at NaOH loading rates of 5% and 7.5% was improved by 13%-16% in comparison with that at 60 °C. In addition, the sCOD concentration in the equilibrium state was 50% - 54% higher. When the temperature increased from 100 °C to 150 °C, the solubilisation of lignin was more than 31% at all NaOH loading rates. The sCOD production was 25%-38% higher than that at 100 °C; however, the solubilisation of lignin only increased by 4.0% at the NaOH loading rate of 7.5% compared to that at 100 °C. Therefore, 100 °C is considered to be an appropriate pre-treatment temperature at these perspectives. However, further research on energy balancing, in consideration of the energy input due to thermo-chemical pretreatment and the energy output due to the enhanced biogas yield, should be carried out.

5.3.1.3. Changes of structure of and functional groups in pre-treated DCGS

Changes of the physical structure of DCGS before and after thermo-chemical pre-treatment were imaged by a scanning electron microscope SEM at the magnification of 100 and 2500 (Fig.5.3). As shown in Fig. 5.3 a, the texture of untreated DCGS was compact and smooth, which is due to the crystalline structure of lignin and cellulose. After thermo-chemical treatment, the overall structure of the DCGS samples was still relatively intact, whereas DCGS treated at 100 °C and the NaOH loading rates of 5% and 7.5% became thinner (Figs. 5.3b, 3c, 3d and 3e). The surface of all treated DCGS samples became rough with small particulates attached, which resulted from partial breakdown of the lignin and cellulose structure (Figs. 5.3b’, 3c’, 3d’ and 3e’). With increasing the NaOH loading rate up to 7.5%, it was observed that more particulates were attached to the surfaces.
The solubilisation of lignin, cellulose and hemicellulose is also confirmed by the Fourier transform infrared spectroscopy (FTIR) analysis. The fingerprint regions of the FTIR spectra of untreated and treated DCGS samples were compared and analysed. The banks in the fingerprint regions of the FTIR spectra are assigned as follow (Pandey and Pitman, 2003): $1730 \text{ cm}^{-1}$ for unconjugated C=O in xylans (a hemicellulose structure), $1512 \text{ cm}^{-1}$ for aromatic skeletal vibration C=C in lignin, $1375 \text{ cm}^{-1}$ for C–H deformation in cellulose and hemicellulose, $1158 \text{ cm}^{-1}$ for C–O–C vibration in cellulose and hemicellulose, and $895 \text{ cm}^{-1}$ for C–H deformation in cellulose. The four spectra of DCGS treated at $100^\circ$C and NaOH loading rates of 1%, 2.5%, 5% and 7.5% show apparent difference from that of untreated DCGS (Table 5.2). The intensity of absorption for banks in each spectrum is calculated from the decrease of the transmittance (%) of the banks compared with the baseline (the maximum transmittance (%)) in the spectrum. The intensity of absorption was decreased at $1730 \text{ cm}^{-1}$ and $1512 \text{ cm}^{-1}$ for pre-treated DCGS compared with that for
untreated DCGS, illustrating the reduction of hemicellulose and lignin. The intensity decreased with the increasing NaOH loading rates. The intensity of absorption at 1375 cm\(^{-1}\) and 1158 cm\(^{-1}\) decreased with increasing the NaOH loading, illustrating that cellulose and hemicellulose were partly decomposed. The band intensities at 895 cm\(^{-1}\) of all treated samples were lower than those of untreated samples, indicating the solubilisation of cellulose and disassociation of lignin with carbohydrates.

Table 5.2. Intensity of absorption (%) in untreated DCGS and DCGS treated at 100 °C and NaOH loading rates of 1%, 2.5%, 5% and 7.5%

<table>
<thead>
<tr>
<th>Samples</th>
<th>Wavelength (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1730</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.38</td>
</tr>
<tr>
<td>NaOH loading rate 1%</td>
<td>1.82</td>
</tr>
<tr>
<td>NaOH loading rate 2.5%</td>
<td>1.74</td>
</tr>
<tr>
<td>NaOH loading rate 5%</td>
<td>1.36</td>
</tr>
<tr>
<td>NaOH loading rate 7.5%</td>
<td>0.85</td>
</tr>
</tbody>
</table>

These structural changes in treated DCGS are beneficial, because the solubilisation of lignin can liberate the remaining solid-phase carbohydrate substrates, increase its porosity and consequently, improve its bio-degradability (Pavlostathis and Gossett, 1985a).

5.3.2. Anaerobic digestion of pre-treated DCGS
5.3.2.1. Methane production potential of pre-treated DCGS

The daily methane yield and cumulative methane yield of untreated DCGS and DCGS pre-treated at NaOH loading rates of 1%, 2.5%, 5% and 7.5% at 100°C are presented in Fig. 5.4. Results show that there were significant differences ($P<0.01$) between treated DCGS samples and untreated DCGS samples and among DCGS samples treated at the four NaOH loading rates. Similar trends were observed for the daily methane yield of DCGS pre-treated at NaOH loading rates of 1%, 2.5% and 5%, but the trend for DCGS treated at the NaOH loading rate of 7.5% was different. The main differences included: (1) the peak value of the daily methane yield; (2) duration of the lag phase; and (3) the duration of the effective biogas production period.

The daily methane yield peaked on Day 8 (500 ml/day), Day 9 (540 ml/day), Day 12 (560 ml/day), Day 21 (600 ml/day) and Day 17 (420 ml/day) for DCGS treated at NaOH loading rates of 1%, 2.5%, 5% and 7.5%, and untreated DCGS, respectively. The cumulative methane yields were 7190, 8035, 8989, 9050 and 6516 ml for DCGS treated at the NaOH loading rates of 1%, 2.5%, 5% and 7.5% and untreated DCGS respectively. Thus, the SMYs were calculated as 359.5, 401.8, 449.5, 452.5 and 325.8 ml CH$_4$/g VS added for DCGS treated at the four NaOH loading rates and untreated DCGS, respectively. The results show that thermal-alkali pre-treatment resulted in higher SMYs, namely higher biological methane production potentials. At the NaOH loading rates of 1%, 2.5%, 5% and 7.5%, in comparison with untreated DCGS, the increase in the SMY was 10%, 23%, 38% and 39%, respectively. The higher SMYs of pre-treated DCGS was attributed to the opening of the ‘acetyl valve’ and partial opening of the ‘lignin valve’, making the substrate more accessible for hydrolysis (Chang and Holtzapple, 2000). According to Kaar and Holtzapple (2000), lime (calcium hydroxide) pre-treatment (with heating from 100 to 140 °C) increased the
digestibility of low-lignin containing biomass, such as corn stover.

Fig. 5.4. Daily methane production and cumulative methane production of untreated grass silage and grass silage treated at 100 °C and NaOH loading rates of 1%, 2.5%, 5% and 7.5%.

The methane production can be simulated with the modified Gompertz model (Eq. (3.1) in Chapter 3):

\[
M = P \cdot \exp \left\{ -\exp \left[ \frac{R_{\text{max}} \cdot e}{P} \left( \lambda - t \right) + 1 \right] \right\} \quad (5.2)
\]

Simulated with the modified Gompertz model, as to the lag phase (\(\lambda\)), 1% and 2.5% NaOH loading rate treated DCGS showed relatively lower \(\lambda\) values, which were 3.9 days and 3.7 days, respectively, followed by 5% NaOH loading rate treated DCGS (5.5 days), while 7.5% NaOH loading rate treated DCGS and untreated DCGS had higher \(\lambda\), which were 13.2 days and 9.1 days, respectively (Table 5.3).
Table 5.3. Methane production simulation using the Modified Gompertz equation.

<table>
<thead>
<tr>
<th>NaOH loading rate</th>
<th>1%</th>
<th>2.5%</th>
<th>5%</th>
<th>7.5%</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag phase, $\lambda$ (d)</td>
<td>3.9</td>
<td>3.7</td>
<td>5.5</td>
<td>13.2</td>
<td>9.1</td>
</tr>
<tr>
<td>$R_{max}$ (ml CH₄/d)</td>
<td>531.6</td>
<td>570.9</td>
<td>565.5</td>
<td>556.8</td>
<td>353.9</td>
</tr>
<tr>
<td>$P$ (ml)</td>
<td>7264.3</td>
<td>8126.9</td>
<td>9149.3</td>
<td>9390.3</td>
<td>6669.9</td>
</tr>
<tr>
<td>T80 (d)</td>
<td>16.5</td>
<td>16.8</td>
<td>20.4</td>
<td>28.8</td>
<td>26.5</td>
</tr>
<tr>
<td>R²</td>
<td>0.998</td>
<td>0.998</td>
<td>0.998</td>
<td>0.994</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The technical digestion time, T80, is defined as the time needed to produce 80% of the maximum gas production (Palmowski and Muller, 2000) and can be used as a guideline in design of the hydraulic retention time (HRT) and solid retention time (SRT) for anaerobic digesters (Section 3.2, Chapter 3). According to the Modified Gompertz Equation, T80s simulated were 16.5, 16.8, 20.4, 28.8 and 26.5 days for DCGS treated at NaOH loading rates of 1%, 2.5%, 5% and 7.5% and untreated DCGS, respectively. After subtracting the lag time, the effective biogas production periods were 12.6, 13.1, 14.9, 15.6 and 17.4 days, respectively. Increasing the NaOH loading rate resulted in a longer effective biogas production period. However, the effective biogas production period for untreated DCGS was the longest. The long lag phase for DCGS treated at the NaOH loading rate of 7.5% was possibly due to inhibitory compounds such as phenolic compounds that were produced in the pre-treatment process. For untreated DCGS, the limited hydrolysis and acidification degree resulted in a longer lag phase compared to DCGS treated at NaOH loading rates of 1%, 2.5% and 5%.
5.3.2.2 VS removal during anaerobic digestion of pre-treated DCGS

VS removals following anaerobic digestion were up to 67.6%, 76.9%, 85.3%, 95.2% and 96.7% for untreated DCGS and DCGS treated at the NaOH loading rates of 1%, 2.5%, 5% and 7.5%, respectively. Anaerobic digestion of GS can remove 37%-67% of VS (Lehtomaki and Bjornsson, 2006; Cirne et al., 2007; Lehtomaki et al., 2008), depending on the reactor configuration, temperature, GS type, pre-treatment methods, etc. The VS removals of treated DCGS obtained in this study were much higher, indicating the enhancement of biodegradability of DCGS by means of thermal-alkali pre-treatment.

5.3.2.3 Characteristics of VFA profiles and composition

The longest lag phase during AD of DCGS pre-treated at 100 °C and 7.5% NaOH loading rate was related to high concentrations of propionic acid and butyric acid in the digesters. During anaerobic digestion, VFAs are important intermediate products and most of the CH₄ produced is derived from VFAs. Before being converted to CH₄, all VFAs are transformed to acetic acid (HAc), and the conversion rates vary in the order of butyric acid (HBu) > propionic acid (HPr). Therefore, HPr levels have been found to rise prior to or during failure of anaerobic digesters (Ren et al., 2005). It has been suggested by Marchaim and Krause (1993) that the HPr to HAc ratio can be used as an indicator of digester imbalance. Hill et al. (1987) has proposed that an HPr to HAc ratio greater than 1.4 could indicate impending digester failure. However, Pullammanappallil et al. (2001) suggest that HPr accumulation may be an effect of and not a cause of inhibition of the anaerobic digestion process.
Fig. 5.5. Concentrations of VFAs (g HAc/l) during anaerobic digestion of treated and untreated grass silage. (a), acetic acid; (b), propionic acid; (c), butyric acid; and (d), total VFAs.

The HPr to HAc ratios were below 1.4 in all digesters. As shown in Fig. 5.5, the peak concentrations of HPr in the digester with DCGS pre-treated at NaOH loading rates of 1%, 2.5% and 5% were 2.1 g/l, 2.2 g/l and 2.5 g/l on Day 12, respectively, and the concentrations decreased afterwards. The peak concentration of HPr in the digesters with untreated DCGS was 1.8 g/l on Day 15 and its degradation took place quickly afterwards. In digesters with DCGS treated at the NaOH loading of 7.5%, from Day 1 to Day 15, the HPr concentration increased to 3.4 g/l and peaked on Day 24 at the concentration of 3.5 g/l. Then, HPr concentration began to decrease from Day 24; however, the degradation rate of HPr in the digester was quite low, and it was not less than 0.2 g/l until Day 33 (Fig. 5.5b). The high HPr concentration over the period of biogas production indicates the existence of possible inhibition. Pre-treatment at higher NaOH loading rates (5% and 7.5%) solubilised more ligno-cellulosic materials especially lignin, which resulted in high concentrations of HPr and total VFAs. In all
digesters, HPr was degraded more rapidly when HAc and HBu concentration dropped to 3.5 g/l and 1.1 g/l, respectively. As reported by Wang et al. (2009), when HPr and total VFAs concentrations reached about 2850 mg/l and 10.0 g/l, respectively, the activity of methanogens was inhibited to a significant extent, but this inhibitory effect was weakened when the total VFAs concentration fell to 6.2 - 8.5 g/l. High concentrations of total VFAs, up to 10.2 g/l and 11.0 g/l, occurred in the digesters with DCGS pre-treated at NaOH loading rates of 5% and 7.5%, respectively. Compared with pre-treatment at the NaOH loading rate of 5%, pre-treatment at the NaOH loading rate of 7.5% had higher concentrations of total VFAs, HPr and HBu. This could explain the longer T80 time for digestion of DCGS pre-treated at the NaOH loading rate of 7.5%.

5.4 SUMMARY

Pre-treatment at increasing temperature and NaOH loading rates enhanced the solubilisation and the biodegradability of DCGS biomass and thereafter, increased the biological methane production potential, in terms of SMYs, which were increased by 10%-38.9%. However, pre-treatment at the NaOH loading rate of 7.5% resulted in the longest lag phase. The VS removals achieved were in the range of 76.9%-96.7%. It is recommended that pre-treatment condition of 100 °C and the NaOH loading rate of 5% be used.
CHAPTER SIX

Hydrolysis and acidification of grass silage in leaching bed reactors

6.1 INTRODUCTION

The conversion of GS to biogas via AD consists of three steps: hydrolysis, acidogenesis and methanogenesis, in which hydrolysis is the rate-limiting step (Shin-ya et al., 2001). Enhancement of hydrolysis will lead to faster anaerobic digestion. To maximise methane production, two-stage anaerobic digestion has been studied and adopted in practice (Lehtomäki et al., 2007).

Leaching bed reactors (LBRs) have been recently developed as the first-stage reactor to treat solid wastes and biomass such as energy crops (the operation of LBRs has been detailed in Chapter 2).

Recirculation of leachate plays an important role in the operation of LBRs and can accelerate the hydrolysis of the solid feedstock by increasing the moisture content, promoting mass diffusion, and distributing the enzymes and microbes (Lu et al., 2008). The function of leachate recirculation in extracting organic matter from food waste has been confirmed by Stabnikova et al. (2008). Media such as woodchips and pistachio-half-shells sometimes are loaded into LBRs either to increase the leachability (Demirer and Chen, 2008) or to enhance the transport of enzymes and leachate within LBRs by increasing the porosity (Myint et al., 2009). Therefore,
LBRs with leachate recirculation and loaded media to increase the leachability are designed especially to treat high solids waste.

A number of strategies, such as pH adjustment (Cysneiros et al., 2008), use of hot water (40 °C) as the leaching medium (Nizami et al., 2010), and mechanical reduction of the particle size of digested material (Mshandete et al., 2006) have been adopted to enhance the hydrolysis of solid organic matter. However, there is little information available regarding the combined effect of operational conditions on the hydrolysis/acidification of GS in LBRs.

Therefore, the aim of this study was to investigate various factors affecting hydrolysis and acidification of GS on the performance of LBRs. Performance parameters studied were hydrolysis rate, formation of fermentation products (volatile fatty acids), reduction of lignocellulosic materials in GS and the cellulase activities. The factors included organic loading rates (OLRs), addition of inoculum, adjustment of pH and dilution of leachate. In addition, the biogas production during the LBRs operation was also evaluated.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Grass silage was collected from the Teagasc Athenry Research Centre, Ireland. It was harvested in June from a homogeneous perennial ryegrass (Lolium perenne) dominant plot. The herbage was field-wilted for 24 hours before being baled. The baled GS was obtained after 5 weeks of ensilage in a wrapped 6 layers of polythene stretch film. In the laboratory, GS was cut to approximately 2 - 3 cm using scissors and stored in a freezer until further use. The characteristics of the fresh GS are given in Table 6.1.
Table 6.1. Characteristics of fresh grass silage

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>25.5</td>
</tr>
<tr>
<td>VS (% TS)</td>
<td>94.4</td>
</tr>
<tr>
<td>Cellulose (% TS)</td>
<td>34.3</td>
</tr>
<tr>
<td>Hemicellulose (% TS)</td>
<td>29.6</td>
</tr>
<tr>
<td>Lignin (% TS)</td>
<td>8.60</td>
</tr>
</tbody>
</table>

6.2.2. Reactor set-up

Six identical column-type stainless steel LBRs were set up. Each LBR had an effective volume of 2 litres, consisting of a lid, a main reactor zone and a funnel bottom made from stainless steel. The height of the reactor zone and the funnel were 300 mm and 100 mm, respectively (Fig. 6.1). The diameter of the main body was 100 mm. A mesh with an opening size of 200 µm was placed between the main body and the funnel and another mesh was placed between the main body and the lid to avoid (1) loss of GS and (2) blockage of the circulation pipes by GS particles or media particles. The HDPE (high density polyethylene) 2H-BCN random media (GEA 2H Water Technologies GmbH, Germany) was added to the reactors at 30 g media/l of the effective reactor volume, as a drainage layer helping leachate collection. The media had a surface area 859 m²/m³ and a specific weight of 150 kg/m³. The LBRs were operated in a room where temperature was controlled at 30±2 °C. Maintaining such temperatures could benefit the hydrolysis rate. Since any successive anaerobic digesters such as UASB will be used to digest the soluble organic matter produced in the LBRs at mesophilic temperature ranges, the temperature used in the present study...
was reasonable.

There were three ports on each reactor: top, middle and bottom ports (Fig. 6.1). The liquid inside each LBR was circulated from the bottom of the funnel to the top port via a peristaltic pump (Watson-Marlow 323 E/D, USA) at a flow rate of 4 l/day during leachate circulation. The pump was controlled by a timer and circulated the leachate for 10 minute every hour. The liquid leachate samples were collected from the middle ports for subsequent analysis. The biogas produced in each LBR was collected with a 1-litre biogas bag attached to the top port on the lid.

Fig. 6.1 Schematic diagram of a leaching bed reactor (unit: mm)
The six LBRs were operated at three OLRs of GS (OLR1, OLR2 and OLR3) with two replicate of each treatment over five batch tests; the operation strategies for these 5 batch tests are detailed in Table 6.2. The OLRs for LRB1, LBR2 and LBR3 were 2.08, 3.13 and 4.17 g fresh GS /l/d calculated based on a solid retention time of 24 days, respectively. In all batch tests, leachate was circulated at a flow rate of 4 l/day during the leachate circulation period. The procedures for the five batch tests are outlined as follow. In Batch Test 1, 100, 150 and 200 g of fresh GS was loaded into LBR1, LBR2 and LBR3 on the top of the media. Tap water was then added to each reactor to reach 2-litre of the working volume, followed by continuous leachate circulation until the completion of this batch test. For Batch Test 2, 1 litre of the leachate and all treated GS from Batch Test 1 were removed from each reactor; 1 litre of inoculum was added, in addition with 100, 150 and 200 g of fresh GS for LRB1, LBR2 and LBR3, respectively. The initial pH of the liquor in LBRs was then adjusted to 6.5 by adding 1M HCl in LRB1, LBR2 (because pH was higher than 6.5 in the two LBRs) and 1M NaOH in LBR3 (because pH was lower than 6.5 in this LBR), before the leachate circulation commenced. In Batch Test 3, the same procedure of inoculum and fresh GS addition and pH adjustment was carried out as in Batch Test 2, followed by the leachate circulation. Then dilution of the leachate was carried out on Day 6, 12 and 18 for LBR 1, LBR2 and LBR 3, on Day 24 for LBR3 due to its higher OLR; each time, the dilution was carried out by removing 1 litre of leachate from the circulation line and adding 1 litre of inoculum. The pH was then adjusted after each dilution to 6.5 by adding 1M NaOH to the leachate in LRBs. The same experiment procedure was used in Batch test 4 as in Batch test 3, except that tap water was used to dilute the leachate (v:v=1:1) rather than the inoculum. In Batch test 5, 1 litre of the leachate and all digested GS were removed from LBRs, and 1 litre of tap water was added to each LBR, in addition with 100, 150 and 200 g of fresh GS for LRB1, LBR2 and LBR3, respectively. Leachate dilution was carried out as in Batch Test 4, followed by leachate pH adjustment to 6.5 by adding 1 M NaOH. The duration of the batch tests was 24, 24, 24-32, 24-32 and 24-30 days in Batch Tests 1-5 (Table 6.2), respectively.
The duration of each batch test was decided due to the experiment purposes but results measured from Day 0 to Day 24 in each batch test were used for consistent comparison. In each batch test, the experiment ceased when the production of sCOD was no longer obvious (less than 0.25% of total COD of GS - tCOD) over two consecutive measurements.

### Table 6.2. Operation strategies of LBRs during the 5 batch tests

<table>
<thead>
<tr>
<th>Batch tests</th>
<th>Duration in LBR1, 2 and 3 (days)</th>
<th>Inoculum</th>
<th>pH adjustment</th>
<th>Leachate dilution</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24, 24 and 24</td>
<td>No</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24, 24 and 24</td>
<td>Yes</td>
<td>adjusted to 6.5 on Day 1</td>
<td>No</td>
<td>effect of inoculum addition</td>
</tr>
<tr>
<td>3</td>
<td>24, 28 and 32</td>
<td>Yes</td>
<td>adjusted to 6.5 every 6 days with inoculum (v:v=1:1)</td>
<td>effect of leachate dilution with inoculum</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24, 28 and 32</td>
<td>Yes</td>
<td>adjusted to 6.5 every 6 days with tap water (v:v=1:1)</td>
<td>effect of leachate dilution with tap water</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>24, 26 and 30</td>
<td>No</td>
<td>adjusted to 6.5 every 6 days with tap water (v:v=1:1)</td>
<td>effect of inoculum addition</td>
<td></td>
</tr>
</tbody>
</table>

The inoculum, which contained 2.3% TS, 1.6% VS and sCOD of 580 mg/l, was the digestate taken from laboratory-scale digesters co-digesting PM and GS. Before use, the inoculum was stored in a tank at 35 °C without feeding until little methane production was observed. This would ensure that methane produced in the LBRs was due to the decomposition of GS rather than the inoculums itself.
6.2.3. Analytical methods

Leachate samples (5 ml) were taken from the LBRs every two days using a 10 ml syringe. pH was immediately measured. The liquid samples (2 ml) were centrifuged at 1,700 g for 10 minutes and at 21,912 g for 20 min at 4 °C. The cellulase activity was measured according to the IUPAC method (Ghose, 1987). The analytical methods for sCOD, VFAs, TS, VS, alkalinity, NH$_4$-N concentration, volume of biogas, and CH$_4$ content in the biogas are described in Section 3.2.3, Chapter 3. The analytical methods for cellulose, hemi-cellulose and lignin contents in GS are described in Section 5.2.4, Chapter 5.

The hydrolysis yield, $\eta_h$, is defined as the degree of solubilisation of GS and is calculated according to Eq. (6.1) (Demirel and Yenigun, 2004):

$$\eta_h = \frac{S_s}{S_I} \times 100\%$$  \hspace{1cm} (6.1)

Where, $S_I$ is the initial total COD of GS, and $S_s$ is the cumulative sCOD in the leachate obtained after a batch test ended.

The acidification yield, $Y_H$, is defined as the ratio between the cumulative total volatile fatty acids (tVFAs) and $S_s$, is calculated using Eq. (6.2):

$$Y_H = \frac{S_{tVFAs}}{S_s} \times 100\%$$  \hspace{1cm} (6.2)
Where, $S_{t\text{VFAs}}$ is the cumulative tVFAs expressed as mg COD using the theoretical COD equivalents for each VFA. The theoretical COD equivalents of each gram of VFAs are: acetic acid, 1.066; propionic acid, 1.512; butyric acid, 1.816; and valeric acid, 2.036 (Yilmaz and Demirer, 2008).

In order to determine conversion of particulate biomass into sCOD and tVFAs, initial concentrations of tVFAs and sCOD contained in fresh GS were subtracted from the final cumulative tVFAs and sCOD values, giving net tVFA and sCOD production, respectively. Statistical analysis was by one-way analysis of variance (ANOVA) using PASW statistics 18 for Windows (SPSS Inc., Chicago, IL, USA).

6.3. RESULTS

6.3.1 pH profiles

The pH values in the leachate of LBRs varied throughout the batch tests (Fig. 6.2). In Batch Test 1, pH dropped to 6.24, 5.90 and 5.63 in LBR1, LBR2 and LBR3, respectively due to the generation of VFAs and low buffering capacities of the feedstock. In Batch Test 2, with the initial addition of inoculum and adjustment of pH to 6.5, the final pH was 6.25, 5.99 and 5.80, which was slightly higher compared with Batch Test 1. In Batch Tests 3, 4 and 5, due to pH adjustment every 6 days and leachate dilution, there was no continuous decrease of pH values in the LBRs. The patterns of pH profiles in Batch Tests 3, 4 and 5 were similar, and pH varied in the range of 6.24-6.50, 6.20-6.50 and 6.18-6.50 for LBR1, LBR2 and LBR3, respectively. A slight increase in pH in Batch Tests 3 and 4 was observed after Day 30 at the end of the
experiment. This was due to the low VFA generation at the end of the experiment, and consumption of VFAs.

Fig. 6.2 Leachate pH profiles in the experiment (B1, B2, B3, B4 and B5 correspond to Batch tests 1, 2, 3, 4 and 5, respectively; R1, R2 and R3 correspond to LBR1, LBR2 and LBR3, respectively.)
6.3.2 Hydrolysis yield, VFA production and composition

The hydrolysis yields in each batch test are summarised in Table 6.3. In Batch Test 1, low hydrolysis yields of 6.8% - 7.2% were obtained compared with those obtained in Batch Test 2 (16.5% - 22.4%). The hydrolysis yields in Batch test 2 were lower than those in Batch Test 3 (45.7% - 57.1%). Hydrolysis yields in Batch Tests 3 and 4 were similar ($p>0.10$). In Batch Test 5, hydrolysis yields, 43.4% - 55.4%, were slightly lower than in Batch Tests 3 and 4 ($p<0.05$). If not specified elsewhere, the description below is assigned to Batch Test 4.

Based on profiles of pH values of the leachate and VFA concentrations, it was found that acidification took place just after each batch test commenced. In Batch Tests 3, 4 and 5, dilution of the leachate and pH adjustment was applied to accelerate the hydrolysis and acidification process. For instance, in Batch Test 4, pH decreased from 6.50 to 6.18-6.25 after Day 5 (Fig. 6.2). With the highest hydrolysis rate achieved in Batch Test 4, the cumulative tVFA concentrations in the leachate (the VFAs contained in the feedstock had been excluded) were 6.1, 8.7 and 10.3 g/l in LBR1, LBR2 and LBR3 by the end of the experiment, respectively. TVFA concentrations reached 4.6 g/l (pH=6.2) by Day 6. In order to reduce or alleviate the inhibition of tVFA on the hydrolysis process, dilution of leachates was carried out immediately on Day 6. The acidification yield in Batch Test 4 was 57.1% - 60.3%, which was slightly lower than that in Batch Test 3 (59.1% - 67.1%) in which the medium used to dilute the leachate was inoculum ($P<0.05$).
Table 6.3. Performance of hydrolysis/acidification of grass silage in LBRs (standard deviation in parentheses where applicable)

<table>
<thead>
<tr>
<th>Batch tests</th>
<th>Reactors</th>
<th>Hydrolysis yield (%) a</th>
<th>Hydrolysis yield (%) b</th>
<th>Acidification yield (%) b</th>
<th>VFA yield (gVFA COd/g tCOD) b</th>
<th>Cellulose degradation (%) b</th>
<th>Hemicellulose degradation (%) b</th>
<th>Lignin degradation (%) b</th>
<th>VS removal (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LBR1</td>
<td>7.4 (0.2)</td>
<td>7.4 (0.2)</td>
<td>65.9 (0.9)</td>
<td>0.048</td>
<td>2.9 (0.1)</td>
<td>8.5 (0.4)</td>
<td>1.1 (0.2)</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>LBR2</td>
<td>7.1 (0.2)</td>
<td>7.1 (0.2)</td>
<td>64.0 (1.3)</td>
<td>0.043</td>
<td>3.1 (0.2)</td>
<td>9.3 (0.4)</td>
<td>1.6 (0.2)</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>LBR3</td>
<td>6.8 (0.1)</td>
<td>6.8 (0.1)</td>
<td>62.6 (1.1)</td>
<td>0.037</td>
<td>3.2 (0.1)</td>
<td>9.4 (0.5)</td>
<td>1.5 (0.2)</td>
<td>15.4</td>
</tr>
<tr>
<td>2</td>
<td>LBR1</td>
<td>22.4 (0.3)</td>
<td>22.4 (0.3)</td>
<td>64.8 (1.6)</td>
<td>0.145</td>
<td>9.9 (0.4)</td>
<td>27.4 (0.8)</td>
<td>6.0 (0.3)</td>
<td>28.1</td>
</tr>
<tr>
<td></td>
<td>LBR2</td>
<td>18.9 (0.2)</td>
<td>18.9 (0.2)</td>
<td>60.5 (1.4)</td>
<td>0.114</td>
<td>7.1 (0.5)</td>
<td>21.3 (0.6)</td>
<td>4.6 (0.2)</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>LBR3</td>
<td>16.5 (0.2)</td>
<td>16.5 (0.2)</td>
<td>55.1 (1.2)</td>
<td>0.091</td>
<td>5.1 (0.3)</td>
<td>16.4 (0.5)</td>
<td>3.4 (0.3)</td>
<td>24.9</td>
</tr>
<tr>
<td>3</td>
<td>LBR1</td>
<td>57.1 (1.2)</td>
<td>57.1 (1.2)</td>
<td>67.1 (0.8)</td>
<td>0.381</td>
<td>28.8 (0.8)</td>
<td>73.0 (3.0)</td>
<td>9.2 (0.6)</td>
<td>64.8</td>
</tr>
<tr>
<td></td>
<td>LBR2</td>
<td>53.3 (1.0)</td>
<td>53.8 (1.1)</td>
<td>61.7 (0.9)</td>
<td>0.332</td>
<td>25.3 (1.2)</td>
<td>64.9 (1.8)</td>
<td>8.7 (0.8)</td>
<td>62.3</td>
</tr>
<tr>
<td></td>
<td>LBR3</td>
<td>45.7 (0.9)</td>
<td>51.2 (1.4)</td>
<td>59.1 (0.7)</td>
<td>0.303</td>
<td>23.2 (1.1)</td>
<td>60.3 (2.5)</td>
<td>8.1 (0.9)</td>
<td>61.0</td>
</tr>
<tr>
<td>4</td>
<td>LBR1</td>
<td>58.2 (1.3)</td>
<td>58.2 (1.3)</td>
<td>60.3 (1.3)</td>
<td>0.351</td>
<td>30.1 (1.7)</td>
<td>74.38 (2.6)</td>
<td>9.3 (1.0)</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>LBR2</td>
<td>54.6 (1.2)</td>
<td>55.6 (1.2)</td>
<td>59.8 (0.9)</td>
<td>0.332</td>
<td>26.8 (1.7)</td>
<td>71.97 (2.0)</td>
<td>8.8 (0.8)</td>
<td>64.8</td>
</tr>
<tr>
<td></td>
<td>LBR3</td>
<td>44.4 (1.3)</td>
<td>51.5 (1.6)</td>
<td>57.1 (1.0)</td>
<td>0.294</td>
<td>23.4 (1.6)</td>
<td>64.35 (1.3)</td>
<td>8.2 (0.9)</td>
<td>62.1</td>
</tr>
<tr>
<td>5</td>
<td>LBR1</td>
<td>55.4 (1.3)</td>
<td>55.4 (1.3)</td>
<td>62.8 (1.7)</td>
<td>0.348</td>
<td>28.3 (1.2)</td>
<td>68.0 (2.2)</td>
<td>9.0 (0.7)</td>
<td>61.3</td>
</tr>
<tr>
<td></td>
<td>LBR2</td>
<td>49.0 (1.0)</td>
<td>49.3 (1.1)</td>
<td>62.3 (1.1)</td>
<td>0.307</td>
<td>21.6 (0.9)</td>
<td>60.2 (1.8)</td>
<td>8.2 (0.6)</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>LBR3</td>
<td>43.4 (0.5)</td>
<td>45.7 (0.4)</td>
<td>59.6 (1.9)</td>
<td>0.253</td>
<td>18.6 (0.8)</td>
<td>54.3 (1.5)</td>
<td>7.8 (0.7)</td>
<td>55.1</td>
</tr>
</tbody>
</table>

a hydrolysis yield (%) achieved on Day 24 of each batch test for comparison.

b achieved at the end of batch tests.
As shown in Table 6.3, VFA yields on a tCOD basis for LBRs ranged 0.29 - 0.35 gVFA/COD/g tCOD. In all LBRs, the VFA components were acetic acid (HAc; 38.3-41.2% w/w of tVFA/COD), propionic acid (HPr; 26.9-30.6% w/w of tVFA/COD) and butyric/isobutyric acids (HBu; 25.4-28.2% of w/w tVFA/COD). Valeric/isovaleric acids were produced in very low levels (2.8-5.1%).

The high percentages of HAc, HPr and HBu were associated with carbohydrate degradation, since GS contained high contents of cellulose and hemicellulose. This is in keeping with Hwang et al. (2009) who reported that short-chain fatty acids were dominant in acidogenic reactors treating a glucose substrate; while, Parawira et al. (2004) have reported that higher molecular weight VFAs are commonly found in protein fermentation.

6.3.3 Degradation of lignocellulosic materials

The TS content of grass comprised of 72.5% cellulose, hemicelluloses and lignin (Table 6.1). The reductions of cellulose, hemicellulose and lignin were low in Batch Tests 1 and 2 (Table 6.3); while they were much higher in Batch Tests 3, 4 and 5. For instance, in Batch Test 4, up to 74.4% of hemicellulose, 30.1% of cellulose and 9.3% of lignin were removed in 32 days (Table 6.3). The reduction of lignin in LBRs in all batch tests was much lower than that (up to 69.5%) reported by Xie et al. (2011b) in thermo-chemical pretreatment of GS (Chapter 5). The reduction of lignin in LBRs was probably due to solubilisation rather than degradation (Lehtomäki et al., 2008), as lignin is known to be refractory under anaerobic conditions (Fan et al., 1981). The highest VS removals obtained in Batch Test 4 were up to 66.3%.

6.3.4 Cellulase activities

Cellulase activities were examined in Batch Test 4 (Fig. 6.3). During the entire test period, the cellulase activity ranged from 0 to 0.20 IU/ml, 0.02 to 0.22 IU/ml and 0.02
to 0.23 IU/ml for LBR1, LBR2 and LBR3, respectively. The cellulase activity was the highest on the first day and then decreased steadily from an average of 0.21 to 0.05 IU/ml from Day 1 to Day 6 in the LBRs. Leachate dilution with tap water and pH adjustment occurred on Day 6. Cellulose activities recovered after leachate dilution in the first two days but decreased thereafter. Leachate dilution and pH adjustment were also carried out on Day 12 and Day 18 for LBR 1, LBR2 and LBR 3, and on Day 24 for LBR3 due to its higher OLR. A similar trend of the cellulase activity was observed after each leachate dilution and pH adjustment. The cellulase activity was not detectable on Day 24 in LBR1 and on Day 26 in LBR2.

![Cellulase activity in the leachate measured in Batch test 4](image)

6.3.5 Methane production

In Batch Test 4, the methane production in LBRs was measured (Fig. 6.4). The cumulative methane yields when the batch test ended were 1121 ml, 1358 ml and 1624 ml in LBR 1, LBR 2 and LBR 3, respectively, which accounted for 13.8%, 11.1% and 10% of biological methane potentials (BMP) of GS, respectively, in consideration that the tested GS had a BMP of 325.8 ml CH₄/g VS added (Xie et al., 2011a), respectively. The methane content in biogas was below 9.2% for all LBRs throughout the batch test.
6.4. DISCUSSION

6.4.1 Overall performance

Hydrolysis is the first step in anaerobic digestion of grass silage, which converts particulate biomass, such as starch, lignocellulosic materials (cellulose, hemicellulose and lignin), fats and proteins, into small molecules like sugars, long chain fatty acids and amino acids. These compounds are then further broken down, with VFAs as the major final products; this process is called acidification. In the five batch tests, Batch Tests 3, 4 and 5 achieved much higher hydrolysis yields and VS removals than Batch Tests 1 and 2. Jagadabhi et al. (2010) investigated hydrolysis and acidification during mono-digestion of GS in one-stage LBRs for 57 days and obtained up to 74% and 63% of VS removals with the operational condition of leachate replacement with and without initial pH adjustment at 7. Lehtomäki et al. (2008) evaluated VS removals in LBRs and obtained: 1) 34% VS removal with pH adjustment to 7; and 2) 55% VS removal with recirculation of the effluent from the successive UASB reactor treating the leachate produced in the LBR without pH adjustment. In Batch Test 4, taking biogas production into account, the hydrolysis yield would be up to 68.1% which was slightly higher than that reported by Nizami et al. (2010) who examined efficiencies of LBRs in hydrolyzing
different sources of GS and obtained up to 67% of the hydrolysis yield and 32-64% of VS removals in 30 days using 40 °C hot water.

The acidification process occurred in all batch tests, with the acidification yields in the range of 54.1% - 67.1%. In Batch Tests 3-5, the VFA yields were 45-75% higher than that (0.2 g VFA-COD/g tCOD) obtained by Morgan-Sagastume et al. (2011) during fermentation of waste activated sludge pre-treated in thermal hydrolysis plants. Alkaya and Demirer (2011) reported that the highest acidification yield was up to 64.2% treating sugar-beet processing wastewater and beet pulp in continuously mixed anaerobic reactors. The high tVFA levels observed in all LBRs in Batch Tests 3 and 4 indicate the accumulation of VFAs which would result in reduction or inhibition of hydrolysis. For instance, TVFA concentrations reached 4.4 g/l (pH=6.2) by Day 6 indicating the onset of inhibition of hydrolysis as tVFA concentration of 2 g/l was found to inhibit the process (Siegert and Banks, 2005).

The longer operation duration in LBR 2 and LBR3 (Batch Tests 3, 4 and 5) helped to achieve higher hydrolysis yields (P<0.05) as shown in Table 6.3. For example, the hydrolysis yield in LBR3 (Batch Test 4) increased from 44.4% on Day 24 to 51.5 % on Day 32.

**6.4.2 Effect of the OLR on the LBR performance**

By increasing the OLR from LBR1 to LBR3, the overall pH value dropped as more substrate was available for hydrolysis and acidification, and consequently, more VFAs were produced.

As shown in Fig. 6.5, increasing the GS OLR resulted in a linear decrease in hydrolysis yields (P<0.05), as explained by Vavilin et al. (2008) who described hydrolysis as the first-order reaction kinetics and higher OLRs resulted in lower hydrolysis yields. The increase in GS OLRs caused a linear decrease in the acidification yield in all batch tests.
(P<0.05). Similarly, the acidification yield was found to decrease with increasing OLRs in a complex wastewater acidification study (Dinopoulou et al., 1988). However, Jagadabhi et al. (2010) investigated hydrolysis/acidification of GS in LBRs at an OLR of 3.78 g fresh GS/l/d at 55 days solid retention time and reported a higher VS removal rate up to 74% by initial pH adjustment to 7.5 at 35 °C, probably due to the higher operational temperature and higher initial pH.

Fig. 6.5. Relationship between the OLR and the cumulative hydrolysis yield (a) and the cumulative acidification yield (b) on Day 24 in batch tests.
6.4.3 Effect of addition of inoculum on the LBR performance

In comparison with Batch Test 2, low hydrolysis yields were obtained in Batch Test 1, probably due to the lack of inoculum. The initial pH adjustment in Batch Test 2 was not as important as the addition of inoculum since the initial pH (6.32-6.63) in Batch Test 1 was within the optimum hydrolysis pH range. The only difference between Batch Test 4 and Batch Test 5 was the addition of inoculum in Batch Test 4. The slightly lower hydrolysis yields (P<0.05) and lower VS removals (P<0.05) in Batch Test 5 indicate that provision of the inoculum at the start of the LBR would enhance the hydrolysis and acidification of GS. This has been confirmed by Jagadabhi et al. (2010), who thought a continuous supply of anaerobic seed (inoculum) by recycling the leachate back to the LBRs was vital for successful operation of LBRs, as it would overcome the continuous loss of inoculum from LBR.

However, efficient hydrolysis was obtained in Batch Test 5 without the addition of inoculum, in comparison with the results obtained in Batch Test 2. This indicates that the effect of inoculum on the performance of LBRs may not be as significant as leachate dilution and pH adjustment.

6.4.4 Effects of pH adjustment and leachate dilution on the LBR performance

The optimum pH for hydrolysis is 6.0–6.5 and inhibition results from pH below 5 (Arntz et al., 1985; Veeken et al., 2000). Therefore, maintaining pH in this optimal range should improve the hydrolysis of GS. In addition, the presence of high concentrations of VFAs in the reactors would also inhibit the hydrolysis process. One of the reasons is that higher concentrations of VFAs lower pH. Furthermore, even when the process pH is optimal, the accumulation of VFAs may lead to a reduced hydrolysis rate of the solid organic substrate (Banks and Wang, 1999). Bhattacharya et al. (2008) suggest that in one-stage LBR systems, high concentrations of hydrolysis products (sCOD) and acidogenesis products (VFAs) in the leachate should result in inhibition to
the hydrolysis process. Further solubilisation and acidification of sCOD can be achieved either by diluting or consumption of the produced sCOD and VFAs from the leachate through methanogenesis. Thirdly, the dilution would benefit the hydrolysis kinetics.

In Batch Tests 3 - 5, pH of the leachate was adjusted to 6.5 and the leachate was diluted on Days 6, 12 and 18 with tap water or inoculum (v:v = 1:1). This improved the hydrolysis yields in the three batch tests. For instance, the hydrolysis yields in Batch Test 2 were much lower than in Batch Test 3, due to the combined influence of a lack of dilution of leachate and inadequate pH. Similarly, the benefit of pH adjustment was proven by Zhang et al. (2005) during investigation of hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion. They found that pH of 7 provided an optimum working condition. Jagadabhi et al. (2010) reported that leachate dilution with tap water increased the generation of sCOD from GS in LBRs by 2.7 times compared to that without dilution. The dilution agent was inoculum in Batch Test 3, but it was tap water in Batch Test 4. There was no significant difference in the performance of Batch Tests 3 and 4 in terms of the hydrolysis yield, acidification yield and VFAs yield (for example, VFAs yields in Batch Tests 3 and 4 were similar (P>0.10). This clearly indicates that addition of inoculum did not affect the hydrolysis and acidification process as significantly as leachate dilution.

6.4.5. Cellulase activity

The cellulase activity is affected by several factors, such as pH, temperature, VFAs concentration, microbial enrichment, production and stability of enzymes, and substrate availability (Parawira et al., 2005). In Batch Test 4, during the overall test period, the cellulase activity ranged from 0 to 0.20 IU/ml, 0.02 to 0.22 IU/ml and 0.02 to 0.23 IU/ml for LBR1, LBR2 and LBR3, respectively. Parawira et al. (2005) found that the total FPase (filter paper cellulase) activity varied between 0.3 and 0.5 IU/ml in acidogenic reactors treating solid potato waste. Zhang et al. (2007) obtained the FPase activity of up to 0.12 IU/ml treating vegetable and flower wastes, similar to the results obtained in this study. High VFAs concentrations in LBRs might have inhibited the
hydrolysis process to some extent, which was reflected by the predominantly low cellulase activities (< 0.10 IU/ml). Lü et al. (2006) investigated how pH (5-9) and acetate concentrations (0-20 g/l) affected the hydrolysis of potato samples and concluded that the hydrolysis of carbohydrates was both affected by pH and acetate. They further concluded that at lower pH values, the VFA concentrations affected the hydrolysis yield significantly. In the present study, since pH value was around 6.5, and did not fluctuate significantly, the cellulase activity was more affected by the VFAs concentrations. After the dilution of leachate and pH adjustment, the cellulase activity recovered due to: (1) lower VFAs concentrations; and (2) more suitable pH. The cellulase activity gradually decreased due to higher VFAs levels and lower pH in the leachate. The highest cellulase activity occurred in LBR3, following LBR2 and LBR1. With increasing OLR, a slight increase in the cellulase activity was observed due to the availability of more substrate.

The higher the cellulase activity, the higher the soluble COD production rates were. A linear relationship between the daily sCOD yield production and the cellulase activity was observed (Fig 6.6):

\[ Y_{COD} = 12.8 A_{cellulase}^{0.34} \quad (R^2=0.72; \ p=0.00) \]

where, \( Y_{COD} \) is the daily sCOD yield and \( A_{cellulase} \) is the cellulase activity.

This indicates that cellulase activity can be used as a key indicator to monitor the hydrolysis process. More research should be conducted on the cellulase activity in LBRs under various operation conditions. The relatively high cellulase activity during the first two days after dilution indicated that dilution was beneficial for the hydrolysis of GS.
6.4.6. Biomethane production from LBRs

The methane production in LBRs was due to the methanogenic activity. In LBRs, the accumulation of VFAs led to low pH, which helped to inhibit the activity of methanogens, and consequently to reduce the loss of soluble organic matter products (like VFAs) from LBRs. Xie et al. (2011a) found that methane production during mono-digestion of GS was severely inhibited when pH dropped to below 6.5 (Chapter 3). In Batch Test 4, pH values in leachate were relatively low, ranging 6.18 - 6.50. As a result, methane production was suppressed to some extent with a daily methane production of 28 - 65 ml/d. In addition, the methane contents in biogas were low, 5.8% - 9.0%, compared with the methane content up to 60 - 70% in Chapter 3 and Chapter 4 when GS was co-digested with pig manure. Alkaya et al. (2011) also reported low methane contents in produced biogas (5.6-16.3%) during the hydrolysis and acidification of sugar-beet processing wastewater and beet pulp under a relatively low pH range (5.7 - 6.8). Lehtomäki et al. (2008) obtained a SMY of 60 ml/g VS of GS added in LBRs, which was equal to 20% of its BMP (300 ml/g VS added) when GS was treated in LBRs at an OLR of 0.91 kg VS/m³/d with pH automatically adjusted and maintained at 7. The lower methane production from LBRs in the current study (13.8%,
11.1% and 10% of BMP of GS in LBRs 1-3, respectively) was likely due to the lower pH values.

6.5 SUMMARY

Hydrolysis and acidification of GS was examined in LBRs under three OLRs: 2.08, 3.13 and 4.17 g grass silage/l/d. The LBRs were run in duplicate over five consecutive batch tests (Batch Tests 1 - 5) to examine the effects of pH, leachate dilution and addition of inoculum on the process of hydrolysis and acidification. The highest GS hydrolysis yields of 51.5 - 58.2%, acidification yields of 57.2 - 60.3% and volatile solids removals of 62.1 - 66.3% were obtained in Batch Test 4. Increasing OLRs affected the hydrolysis yield negatively. In Batch Test 4, the reduction of lignocellulosic materials was up to 74.4% of hemicellulose, 30.1% of cellulose and 9.3% of lignin in 32 days. The cellulase activity can be used as an indicator for the hydrolysis process. Methane production from the LBRs only accounted for 10.0 - 13.8 % of the BMP of GS.
CHAPTER SEVEN

Pilot-scale study on anaerobic co-digestion of pig manure and grass silage: an approach to mitigate greenhouse gas emissions

7.1. INTRODUCTION

In Chapter 3, it has been found that in the feedstock of GS and PM mixture for co-digestion, the optimum ratio of GS and PM to be co-digested is 1:1 at a volatile solids (VS) basis. Therefore, in this study, anaerobic co-digestion of PM with GS was investigated in a pilot-scale anaerobic digester to examine the effect of anaerobic co-digestion of PM and GS on biogas productivity. Furthermore, mitigation of GHG with the adoption of the AD technology was evaluated.

7.2. MATERIALS AND METHODS

7.2.1 Materials

Pig manure was obtained from the Pig Unit in Pig Development Department, Teagasc Moorepark Research Centre, Ireland. In order to maintain relatively constant solids content in PM during the study period, it was stored at two 1 m³ intermediate bulk containers (IBCs). Grass silage was collected from a silage pit in Teagasc Moorepark Research Centre, Ireland and was chopped on site. The precision-chop GS had an average size of 5 cm and was stored in a freezer (-17 °C) until further use. The characteristics of fresh PM and fresh GS are given in Table 7.1.
Table 7.1. Characteristics of grass silage raw pig manure and inoculum.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Grass silage</th>
<th>Pig manure</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (% of FW)</td>
<td>34.5</td>
<td>3.7</td>
<td>1.6</td>
</tr>
<tr>
<td>VS (% of FW)</td>
<td>31.6</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Ash (% of FW)</td>
<td>2.9</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>NDF (% of DM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADF (% of DM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>4.5</td>
<td>7.9</td>
<td>8.0</td>
</tr>
<tr>
<td>Lactic acid (% of DM)</td>
<td>10.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VFAs (% of DM)</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CP (% of DM)</td>
<td>14.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WSC (% of DM)</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMD (% of DM)</td>
<td>68.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sCOD (g/l)</td>
<td>24.4</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>tCOD (g/l)</td>
<td></td>
<td>128.9</td>
<td>36.6</td>
</tr>
<tr>
<td>sCOD (% of DM)</td>
<td>24.6</td>
<td>65.9</td>
<td>41.9</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/l)</td>
<td>1640</td>
<td>2387</td>
<td></td>
</tr>
</tbody>
</table>

Note: DM: dry matter; VS: volatile solids; NDF: neutral detergent fiber; ADF: acid detergent fiber; VFAs: volatile fatty acids; CP: crude protein; WSC: water soluble carbohydrate; DMD: dry matter digestibility; sCOD: soluble COD

7.2.2. Pilot-scale anaerobic digester

The pilot-scale anaerobic digester (Fig. 7.1) was designed with purposes of ease of transportation of the digester to the site and automatic operation and control. Its footprint is 0.52 m² with a height of 2.57 m. The system consists of four parts: (a) a control panel, (b) a feeding system, (c) a digester tank body, and (d) a biogas storage system.

(a). The control panel, located in a protective and closed electrical box, contains all the electronic controls required for the functioning of the digester, which is controlled by Allen Bradley MicroLogix 1200 programmable logic controllers (PLC; Rockwell Automation Inc., USA). In the upper part of the control panel, two LCD monitors are mounted (Thermo Scientific Alpha transmitter, USA); one is connected to a pH probe (Hamilton electro-chemical sensors, Esslab, UK) and the other is connected to an ORP (oxidation-reduction potential) probe (Hamilton electro-chemical sensors, Esslab, UK).
The mechanical stirrer speed regulator (Hitachi SJ200, Japan) is located in the lower part of the panel.

(b). The feeding system is at the top of the reactor. In order to feed GS to the digester, two 15.2 cm (6 inches) diameter chambers are installed (Fig. 7.1). The GS feeding system comprises a pipe and the two chambers controlled using two compressed-air operated valves. These valves allow the feeding of GS into the reactor tank, while avoid air entering the digester. The valves are operated as follows: firstly, GS is filled into the upper chamber by hand; Secondly, the upper compressed-air operated valve is opened, allowing GS to fall into the lower chamber; and finally, the upper compressed-air operated valve is closed and the lower compressed-air operated valve is opened, allowing GS to fall into the digester tank. Pig manure is fed into the digester with a 1 litre chamber whose both ends are connected with 3.8 cm (1.5 inches) diameter pipes; one pipe is connected to the inlet of a water submersible pump (FTS 1100A1, Florabest) placed in the PM storage IBCs, and the other is submerged in the IBCs. This helps to form a uniform feedstock before feeding. The chamber is controlled with a compressed-air operated valve, avoiding air entering the digester.

(c). The digester tank is cylindrical in shape and is made of 316-stainless steel, with a height of 94 cm and a diameter of 40.3 cm. Its capacity is 480 l, with the working volume of 360 l. The mixing system consists of a mechanical stirrer and a submersible pump, both installed inside the reactor. The mechanical stirrer has two stainless steel propellers, whose rotation (30-60 rpm) is provided by an electric three-phase motor (380 V) operated by an inverter (Hitachi SJ200, Japan) through the control panel. It is installed for continuously homogenizing the digesting material. A Tiger 80 submersible vortex chopper pump (Arven S.R.L., Italy) placed inside the digester with the capacity of about 250 l/ min is used to circulate the digestate for 100 seconds after each feeding and before each digestate discharge and to avoid a build up of GS on the surface of the bulk liquid.

The digester tank is equipped with pH and ORP probes with individual transmitters (Thermo Scientific Alpha, USA). The pH sensor and ORP sensor are manually inserted
into the reactor, through a hole equipped with a series of scaling valves on the top of the reactor. pH and ORP are measured by the sensors in situ. The lower part of the external surface of the digester is wrapped with a water jacket to maintain the bulk fluid at a constant temperature of 37 °C, and the external surface is fully covered with insulating material to reduce heat loss. Two compressed-air operated valves of 10.16 cm in diameter (4 inches) installed at the bottom of the digester tank allows the withdrawal of the digestate and collection of the digestate samples for analysis. The digestion temperature is set manually with a switch located at the bottom of the digester tank. A pressure release valve is designed to be located at the top of the digester.

(d) The biogas measurement and storage system consists of biogas piping, a biogas flow meter and a biogas bag. The volume of biogas produced was measured by a volumetric flow meter (FMA-1620A, Omega, UK) with 0.48 cm (3/16 inch) tubing (Tygon, USA). An in-line water trap element was installed at the upper part of the tubing to avoid the water vapour entering the biogas flow meter and biogas bag.

7.2.3. Operation of the pilot-scale anaerobic digester

The digester was loaded with PM 12 times per day and GS was fed once every day. One day before the commencement of the operation, the pilot-scale anaerobic digester was filled with 360 litre of seed sludge (inoculum) taken from an anaerobic digester treating PM in Kilgavan, Co. Kerry, Ireland.

From Day 1 to Day 61 (Phase I), only PM was fed into the digester with a loading rate of 12 litre/day (1 litre each time of feeding). The OLR was 0.87 kg VS/m$^3$/d. From Day 62 to Day 109, besides PM, GS was added into the digester manually once a day at a PM to GS VS ratio of 1:1 (Phase II). In this phase, the OLR was 1.74 kg VS/m$^3$/d. The ratio was chosen based on a previous study on co-digestion of GS and PM, which shows that the PM to GS ratio should be no lower than 1:1 in order to achieve stable digestion.
(Xie et al., 2011). The digestate was discharged 6 times a day (12 litres/day) controlled by the PLC programme to maintain a constant HRT of 30 days.

Fig. 7.1 Pilot-scale anaerobic digester (a) and schematic of the AD unit (b)
7.2.4. Measurement of GHG emissions from storage of PM and digestate

Small scale experiments were conducted on site in order to quantify GHG emissions during storage of PM and digestate (Fig. 7.2). For this purpose, PM was stored in three air-tight \(1 \text{ m}^3\) IBCs. A 10 litre Tedlar gas sampling bag was attached to the top of each IBC for the collection and analyse of biogas. The characteristics of PM were the same as the PM digested in the reactor (Table 7.1).

![Fig 7.2. Measurement of GHG emissions in pilot-scale IBCs (1 m³)](image)

The post-methane production potential of the digestate was measured in batch experiments using 25 litre containers placed on site. Digestate (20 litre) was drawn from the pilot-scale AD unit on Day 34 – Day 38 and was thoroughly mixed and added to the containers, 20 litres each. A 10 litre Tedlar gas sampling bag was attached to the top of each container for the collection and analyse of biogas. The experiment lasted for 74 days and was conducted in triplicate.

7.2.5. Field application
GHG emissions from the field application of pig manure are mainly due to N$_2$O (Fangueiro et al., 2008, 2010). Direct N$_2$O emissions (Gg) from manure management (including storage, treatment and field application) can be estimated according to Eq. 10.25 of IPCC guidelines (Eq. 7.1).

$$N_2O = (N_T \times N_{ex} \times MS_{(T,S)}) \times EF_{3(S)} \times 44/28 \quad \text{(Eq. 7.1)}$$

where, N$_2$O, direct N$_2$O emissions from manure management in the country studied, kg N$_2$O / yr; N$_T$, number of head of livestock species/category T in the country; N$_{ex}(T)$, annual average N excretion per head of species/category T in the country, kg N / animal / yr, and N$_{ex}$ per manure management system (kg N / yr) is given as the number of animals of each category multiplied by the fraction of manure N per manure management system (%/100; fraction); MS$_{(T,S)}$, fraction of total annual nitrogen excretion for each livestock species/category T that is managed in manure management system S in the country, dimensionless (0.6 for swine in IPCC); EF$_{3(S)}$, emission factor for direct N$_2$O emissions from manure management system S in the country, kg N$_2$O-N/kg N in manure management system; S, manure management system; T, species/category of livestock; and 44/28, conversion of (N$_2$O-N) emissions to N$_2$O emissions. In this study, the same animal category was used to calculate CH$_4$ emissions from manure management. IPCC default emission factor EF3 value for Liquid/Slurry manure management system is 0.005 (IPCC, 2006).

CH$_4$ emissions from manure management can be calculated according to Eqs. 10.22 and 10.23 of IPCC (Eq. 7.2 and Eq. 7.3, respectively) as follows.

$$CH_4_{\text{manure}} = \left( EF_{(T)} \times N_{(T)} \right) / 10^6 \quad \text{(Eq. 7.2)}$$

where, CH$_4_{\text{manure}}$, CH$_4$ emissions from manure management, for a defined population, Gg CH$_4$/year; EF$_{(T)}$, emission factor for the defined livestock population, kg
CH₄/head/year; \(N_{(T)}\), the number of head of livestock species/category T in the country studied; and T, species/category of livestock.

\[
EF_{(T)} = (VS_{(T)} \times 365) \times \left( B_{0(T)} \times 0.67 \text{kg/m}^3 \times \left( \text{MCF}_{(S,k)/100} \right) \right) \times MS_{(T,S,k)} \quad \text{(Eq. 7.3)}
\]

where, \(VS_{(T)}\), daily volatile solids excreted for livestock category T, kg dry matter manure/animal per year; 365, basis for calculating annual VS production, days/year; \(B_{0(T)}\), maximum methane producing capacity for manure produced by livestock category T, m\(^3\) CH\(_4\)/kg of VS excreted (0.26 for PM was obtained in the present study); 0.67, conversion factor of m\(^3\) CH\(_4\) to kilograms CH\(_4\); \(\text{MCF}_{(S,k)}\), methane conversion factors for each manure management system S by climate region k, % (IPCC default value of 10% for liquid/slurry type manure management systems for cool climate of a mean annual temperature of <10 °C); and \(MS_{(T,S,k)}\), fraction of livestock category T’s manure handled using manure management system S in climate region k, dimensionless (0.6 for swine, IPCC, 2006).

In order to compare the total GHG emissions, CH\(_4\) and N\(_2\)O emissions were converted into CO\(_2\) equivalent by using the 2001 GWP (over a 100 year period) of 23 for CH\(_4\) and 310 for N\(_2\)O (IPCC, 2001).

In calculating the reduction in GHG emissions from the replacement of fossil fuels, 1 MWh of electricity from coal would produce 0.8684 t of CO\(_2\) and 0.4342 t of CO\(_2\) is emitted when producing 1 MWh of heat (IPCC, 1996). The conversion factor for N\(_2\)O emission from the applied N fertiliser (t) was estimated to be 1.25% of the quantity of N fertiliser (Kaparaju and Rintala, 2011). The amount of fossil energy required to produce 1 kg of inorganic N fertiliser is about 2 kg of mineral oil (Klinger, 1999), with a calorific value of 12 kWh/t for oil.
In addition, the following energy equivalents and conversion factors were used: in a CHP unit, 1 m³ of biogas with the CH₄ content of 60% would produce 1.7 kWh of electricity (35-40%) and 2 kWh of heat (45-50%) at 85-90% conversion efficiency (ETSU, 1997). The energy content of 1 m³ of CH₄ is 35.7 MJ. 3.6 MJ of energy is equal to 1 kWh of electricity.

### 7.2.6 Calculations

#### 7.2.6.1 VS removal

There are two calculation methods used to determine VS removals: the Van Kleeck method and the mass balance method. The Van Kleeck method (Eq. 7.4) assumes the amount of mineral solids is conserved during digestion (Switzenbaum et al., 2003), and uses the volatile fraction, VSfrac (VS/TS), in the feedstock and the digestate as references.

\[
VS_{\text{removals}}(\%) = \frac{VS_{\text{frac,in}} - VS_{\text{frac,out}}}{VS_{\text{frac,in}} - (VS_{\text{frac,in}} * VS_{\text{frac,out}})}
\]  
(Eq. 7.4)

where, \(VS_{\text{frac,in}}\), volatile fraction in the feedstock solids; and \(VS_{\text{frac,out}}\), volatile fraction in the digestate solids.

The mass balance (Equ. 7.5) uses VS concentrations (\(VS_{\text{conc}}\)) in the feedstock and the digestate to calculate the VS removal rate (Ge et al., 2011), expressed as

\[
VS_{\text{removals}}(\%) = \frac{VS_{\text{conc,in}} - VS_{\text{conc,out}}}{VS_{\text{conc,in}}}
\]  
(Eq. 7.5)
where, \( VS_{\text{conc,in}} \), VS concentration in the feedstock; \( VS_{\text{conc,out}} \), VS concentration in the digestate.

Results calculated using Eq. 7.4 are sensitive to systematic sampling issues, which may cause dilution, while the results calculated using Eq. 7.5 are influenced by accumulation of mineral inerts within the reactor. In this study, Eq. 7.5 was used to calculate the VS removal efficiency.

### 7.2.6.2 Soluble COD removal

The soluble COD removal was determined using Eq. 7.6 as follows:

\[
\text{SolubleCOD removals(\%) = } \frac{sCOD_{\text{conc,in}} - sCOD_{\text{conc,out}}}{sCOD_{\text{conc,in}}} \quad \text{(Eq.7.6)}
\]

where, \( sCOD_{\text{conc,in}} \), soluble COD concentration in the feedstock; \( sCOD_{\text{conc,out}} \), soluble COD concentration in the digestate.

### 7.2.7 Analytical methods

In the pilot-scale digestion experiment, the liquid digestate samples were taken from the digester once every day using a 100-ml container. After immediate measurement of pH, the samples were stored in a freezer (-17 °C) until delivery to the laboratory in NUI Galway for measurement of required parameters.

Methods used to measure TS, VS, soluble COD and \( \text{NH}_4^+ \)-N concentration in the liquid samples were described in Section 3.2, Chapter 3. The volume of biogas was measured by a volumetric flow meter (FMA-1620A, Omega, UK), and the value was shown in
standard temperature and pressure (STP) conditions of 0 °C and 1 atmosphere. Free ammonia (NH₃) in the liquid phase was calculated after Anthonisen et al. (1976):

\[
\text{NH}_3 = \frac{\text{NH}_4^+ \times 10^{\text{pH}}}{10^{\text{pH}+0.0344/(273+t)}} \quad \text{(Eq. 7.7)}
\]

where, \(t\) is the temperature, °C.

For GHG emission from storage of PM and the digestate, the gas volume was measured by a volumetric flow meter (FMA-1620A, Omega, UK) or by displacement of water. Methane, CO₂ and hydrogen sulphide (H₂S) were measured by a GA3000 biogas analyser (Geotechnical Instruments Ltd, UK). Nitrous oxide (N₂O) was measured by Varian 450-gas chromatograph with an electron capture detector. The ammonia (NH₃) content in biogas was measured with Dräger tubes (Dräger, Germany). The data of ambient temperature was obtained from the local weather station (1 km distance from the experimental site). Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc; Chicago, IL, USA).

7.3. RESULTS AND DISCUSSION

7.3.1 Process stability

Key factors measured to assess the pilot-scale system stability were pH, ORP, and \(\text{NH}_4^+\) /free ammonia (NH₃) concentrations.
7.3.1.1 pH and ORP

In Phase I, pH of the bulk fluid fell immediately after the commencement of the experiment from 8.0 to 7.8 on Day 12 (Fig. 7.2). The decrease of the pH value indicates the onset of hydrolysis and acidification of PM and a sufficient population of methanogens had not yet been established for the biogas production by consuming VFAs. From Day 12, the pH gradually increased. After 33 days of operation, pH reached 8.0 and levelled off with steady biogas production. In Phase II, the increase of the loading rate from 0.87 to 1.74 kg VS/m$^3$/d resulted in the decrease of pH to an average value of 7.77 (Day 80 - Day 109).

ORP can be used as a control and monitoring parameter in anaerobic treatment systems because organic material is subjected to degradation by redox reactions catalyzed by enzymes under anaerobic conditions (Khanal and Huang, 2003). It measures the net value of all complex oxidation-reduction reactions within an aqueous environment. Therefore, it can be used to control and monitor soluble COD production during the hydrolysis process. Chang et al. (2002) observed a linear relationship between the change in OPR value and the increase in soluble COD concentration during biochemical hydrolysis of waste activated sludge by dosing different concentration of NaOH. The decrease in the ORP value from -361 mV at the steady state in Phase I to -389 mV in Phase II indicates a more stable digestate, which has been confirmed by a lower level of soluble COD concentrations present in the digestate (Section 7.3.2.2).
7.3.1.2. NH$_4^+$ / NH$_3$

The NH$_4^+$-N concentrations were increased from an average of 2323 mg/l (Day 34 - Day 61) in Phase I to 2541 mg/l (Day 80 - Day 109) in Phase II (Fig. 7.3). Free ammonia is dependent on pH and the average NH$_3$ concentrations calculated using Eq. 7.7 decreased from 233.0 mg/l in Phase I (Day 34 - Day 61) to 158.3 mg/l in Phase II (Day 80 - Day 109) due to the lower pH value in the digestate in Phase II. It is reasonable to speculate that less inhibition would be caused by lower NH$_3$ concentrations. The concentrations of NH$_4^+$-N and NH$_3$ that cause inhibition of AD vary in different AD systems. For example, 50% reductions in methane production have been found for NH$_4^+$-N concentrations from 1.7 to 14 g/l (Chamy et al., 1998; Hansen et al., 1998; Sung and Liu, 2003). The result clearly shows that co-digestion of GS and PM is advantageous over mono-digestion of PM.

![NH$_4^+$ and NH$_3$ concentrations over time](image)

Fig. 7.3 NH$_4^+$ - N and free NH$_3$ concentrations

7.3.2 Performance of the pilot-scale anaerobic digester
7.3.2.1 Methane yields

Fig. 7.4 Daily biogas yield, and CH$_4$ and CO$_2$ contents in biogas

Daily biogas production, and the CH$_4$ and CO$_2$ contents in biogas during the operation period is shown in Fig. 7.4. The daily biogas production increased from the average of 84 litre/d (Phase I) to 254 litre/d (Phase II) at steady state. The average methane content in biogas in Phase I and Phase II were 58% and 62% at steady state from Day 34 to Day 61 and from Day 80 to Day 109, respectively. The SMYs of Phase I and Phase II calculated were 154 and 251 ml CH$_4$/g VS added, respectively. Koch *et al.* (2009) obtained a SMY of 260 ml CH$_4$/g VS added and a methane content of 52% in a loop reactor where GS was fermented at 38 °C. Dubrovskis *et al.* (2009) obtained a SMY of 126, 294 and 295 ml CH$_4$/g VS added from AD of reed canary grass, oats-barley silage and maize silage, with corresponding methane contents in biogas of 48%, 56% and 53%, respectively. The co-digestion SMYs obtained in this research are in the range of literature review results. The advantage of co-digestion was examined by Kaparaju and Rintala (2005). In their study, at a loading rate of 2 g VS/(l·d) in a CSTR at the HRT of 15 – 20 days and at 35 °C, the SMYs were 130 – 150 ml CH$_4$/g VS added at a PM to potato ratio (VS:VS) of 100:0, 210 – 240 ml CH$_4$/g VS added at a ratio of 85:15 and 300 – 330 ml CH$_4$/g VS added at a ratio of 80:20. Panichnumsin *et al.* (2010) reported 41% higher SMYs when PM was co-digested with 60% VS of cassava pulp compared to the digestion of PM alone.
Co-digesting animal manure that has a low C/N ratio along with feedstock containing low levels of nitrogen (high C/N ratio) gives more stable operation performance and a higher methane yield than digesting manure only (Callaghan et al., 2002). In the batch experiment, the biomethane production potential (BMP) of GS was 330 ml CH$_4$/g VS (Xie et al., 2011). It can be observed from Section 3.1 and 3.2 that co-digestion of PM with GS improved both the stability of the digestate and the methane production. Assuming that the SMY of PM in Phase I of 154 ml CH$_4$/g VS would not change in Phase II with respect to PM, the SMY of GS calculated using the SMY in Phase II of 251 ml CH$_4$/g VS was 348 ml CH$_4$/g VS, which exceeded the BMP of GS of 330 ml CH$_4$/g VS. Alternatively, assuming that the SMY of GS was equal to the BMP, meaning that 100% of degradable organic matter in GS would be used for methane production, the minimum amount of CH$_4$ production contributed by PM in Phase II was 172 ml CH$_4$/g VS, respectively, which was greater than that in Phase I (154 ml CH$_4$/g VS). Hence, co-digestion of PM and GS had synergic effects, and consequently improved the digester performance. Similarly, Callaghan et al. (2002) reported that increasing the proportion of fruit and vegetable wastes from 36% to 69% at a VS basis when co-digested with cattle slurry improved the SMY from 230 to 450 ml CH$_4$/g VS added. One possible reason was the critical C/N ratio. The optimum C/N in feedstock for AD is 20/1–30/1 (Parkin and Owen, 1986). The C/N ratio of PM (< 12/1) is less than that of GS, which is more than 20/1 (Huang et al., 2004; Koch et al., 2009). Inappropriate (too high or too low) C/N ratios in the feedstock could result in a release of high ammonia or accumulation of VFAs in the digester, which are potential inhibitors in the AD process and would decrease the activity of methanogens and eventually terminate the AD process. The operation performance is summarised in Table 7.2.

7.3.2.2 VS and sCOD removals

Soluble COD and VS removal efficiencies increased from 81.4% and 41.4% in Phase I to 87.8% and 53.9% in Phase II, respectively (Table 7.2). AD of GS only can remove 37% - 67% of VS (Yu et al., 2002; Lehtomaki and Bjornsson, 2006; Cirne et al., 2007; Lehtomaki et al., 2008), depending on the reactor configuration, temperature, GS type,
pre-treatment methods, etc. Linke (2006) reported an increase in VS removals and biogas yields with increasing HRT on the basis of a mass balance in a CSTR treating solid potato processing wastes. VS removals during AD of PM alone or with various agro-industrial wastes range from 42% to 82% (Monou et al., 2009; Panichnumsin et al., 2010).

Table 7.2 Performance of the pilot-scale anaerobic digester at steady state (Day 34 - Day 61 in Phase I and Day 80 - Day 109 in Phase II)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phase I</th>
<th>Phase II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (day)</td>
<td>0 – 61</td>
<td>62 - 109</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.99</td>
<td>7.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>-361</td>
<td>-389</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/l)</td>
<td>2323</td>
<td>2541</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Free NH$_3$ (mg/l)</td>
<td>233.0</td>
<td>158.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SMY (ml CH$_4$/g VS added)</td>
<td>154</td>
<td>251</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Volumetric methane yield (l/l/d)</td>
<td>0.134</td>
<td>0.437</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VS removals (%)</td>
<td>41.4</td>
<td>53.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VS$_{conc,in}$ (%)</td>
<td>2.6</td>
<td>5.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VS$_{conc,out}$ (%)</td>
<td>1.5</td>
<td>2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Soluble COD removals (%)</td>
<td>81.4</td>
<td>87.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>sCOD$_{conc,in}$ (g/l)</td>
<td>24.4</td>
<td>31.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>sCOD$_{conc,out}$ (g/l)</td>
<td>4.5</td>
<td>3.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

7.3.3 Mitigation of GHG emissions
7.3.3.1. GHG emissions from storage of raw PM

The main contributor of GHG emissions was CH₄ whose contribution was in the range of 92.6% - 95.6%, followed by CO₂ (4.4% - 7.4%). N₂O contributed the least to GHG emissions (nearly equal to 0), and can be neglected. The daily GHG emissions ranged 447.9 - 577.4 g CO₂ equivalent/m³ PM /day when the daily maximum temperature ranged 20.5 - 24.6 °C and reduced to less than 4 g CO₂ equivalent/m³ PM /day from Day 74 to Day 81. Since the storage of PM was carried out in covered IBCs under anaerobic conditions, it is speculated that the low GHG production from Day 74 to Day 81 was probably due to low pH in the IBCs. However, long-term evaluation of GHG emission from storage of PM needs to be carried out. As reported by Masse et al. (2003), over a period of 180 days, methane production from PM storage in closed barrels increased from 26.2 ml/g VS at 10 °C to 127.8 ml/g VS at 15 °C. The total GHG emissions from 1 m³ of PM obtained in this study was 9525.8 g CO₂ equivalent with 546.3 litres of CH₄ emissions at STP. GHG emissions were greatly associated with ambient temperature, especially at the maximum temperature (Fig. 7.5) in the first 55 days:

\[
\text{Temperature range (12.1 – 24.6 °C): } \ E_{GHG} = 39.54T_m - 519 \ (R^2 = 0.68) \\
\text{(Eq.7.8)}
\]

where, \( E_{GHG} \) is the GHG emissions (g CO₂ equivalent) and \( T_m \) is the maximum temperature (°C). Other researchers have also observed a positive correlation between CH₄ emissions during animal manure storage and the manure temperature (Massé et al., 2003; Møller et al., 2004; Pattey et al., 2005; VanderZaag et al., 2010). Sommer et al. (2007) observed that methane production was low at temperatures <15 °C but increased exponentially as temperature rose to above 15 °C.

The results from this research show that the storage strategy used resulted in 14.5% of the SMY obtained in the pilot-scale AD unit digesting PM in Phase I, while the N₂O
emissions were significantly lower in the covered storage.

The SMY of mono-digestion of PM obtained from the pilot-scale anaerobic digester was 154 ml/g VS added in steady state operation. Therefore, the mitigation of GHG from 1 m$^3$ of PM is equal to 15.6 kg of CO$_2$ equivalent at mesophilic conditions by replacement of fossil fuel via CHP. The methane gas produced from covered pig manure storage at ambient temperature (SMY = 21 ml/g VS added), which can be collected and used as a renewable energy, can potentially mitigate GHG emissions of 2.1 kg of CO$_2$ equivalent by replacement of fossil fuel via CHP (Referred to Table 7.3).

![Graph (a)](image1)

![Graph (b)](image2)

**Fig. 7.5** Daily GHG emissions and maximum temperature in pig manure storage (a) and linear relationship of GHG emissions and maximum temperature (Day 0- Day 55) (b)
7.3.3.2. GHG emissions from post-storage of pig manure digestate

The post-storage of PM digestate produced GHG of 657.7 g CO\textsubscript{2} equivalent per 1 m\textsuperscript{3} of digestate, which is 93.1% less compared with GHG emissions of storage of raw PM under the same temperature range, due to a much lower organic matter content in the digestate after AD.

7.3.3.3. GHG emission from field application of raw pig manure and digestate

The use of digestate to replace inorganic fertiliser is an effective option to reduce GHG emissions (Kaparaju and Rintala, 2011). Kaparaju and Rintala (2011) have presented that the quantity of inorganic N fertiliser potentially saved can be up to 1462 kg/yr based on a typical pig farm in Finland by using digestate as a source of N for crop production, due to more efficient use of N and reduced need for inorganic N fertilisers compared to field application of raw PM. As reported by Klinger (1999), the nitrogen use efficiency (amount of N available for plants in 100 kg applied N) is 35-43% and 70-100% for undigested and digested manure, respectively. In this study, the amount of inorganic fertiliser that could be saved due to the increased nitrogen use efficiency of the digestate was determined by multiplying the difference in efficiencies (35%/70%=50%) with the total inorganic N content in the digestate.

Anaerobic digestion reduces organic matter in the manure and affects infiltration of manure slurry and the VS contents in the soil slurry mixture, which can lead to the reduction of N\textsubscript{2}O emissions. As reported by Petersen \textit{et al.} (1996), a lower VS content decreases the microbial demand for O\textsubscript{2} and consequently heterotrophic denitrification. Amon \textit{et al.} (2006) reported lower N\textsubscript{2}O emissions (2.7 g N\textsubscript{2}O per m\textsuperscript{3} slurry) from soils amended with digested slurries than from untreated slurries (3.8 g N\textsubscript{2}O per m\textsuperscript{3} slurry). The reduction of N\textsubscript{2}O from land application due to the adoption of the AD technology is quantified by using literature data.
By using data obtained from Sections 7.3.1 and 7.3.2, the mitigation of GHG emissions through adoption of the AD technology is summarised in Table 7.3. The estimation was based on: (1) a pig farm with an average herd size of 654 sows in Ireland (Teagasc, 2011); (2) comparison of GHG emissions in the pig farm with and without AD; (3) the use of raw manure and digestate for land application; (4) CH$_4$ emissions from livestock enteric fermentation is not considered in this study; (5) CH$_4$ emissions from manure management were calculated using IPCC tier 2 method (Section 10.44 of IPCC, 2006); (6) the animal category of swine was used and the emission factor was calculated by using Eu. 7.2 (Eq. 10.22, IPCC, 2006); and (7) CH$_4$ emission factor from manure management (storage and treatment of manure, land application of manure on pasture) is derived from IPCC Tier 2.

The results show that the total GHG mitigation based on an average Irish pig farm (Teagasc, 2011) is 751.3 Mg CO$_2$ eq. The impact of AD on mitigating GHG emissions was mainly through reduced emissions due to reduced fertiliser use, reduced emissions during manure management, and replacing of fossil fuels with the methane-rich biogas.
Table 7.3. GHG emissions from pig manure with and without AD technology

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Pig farm (654 sows)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of pig manure</td>
<td>m³</td>
<td>13538</td>
<td>-</td>
</tr>
</tbody>
</table>

A. GHG emissions without AD

| CH₄ emissions from manure management*                 | kg CH₄ / year   | 2406                | Eq.(7.2) and Eq.(7.3) |
| Direct N₂O emissions from manure management*        | kg N₂O / year   | 268                 | Eq.(7.1)              |
| Total CH₄ and N₂O emissions from livestock management in CO₂ eq | Mg CO₂ eq / year | 138.4               | Refer to Section 2.5  |
| CH₄ emissions from manure management                | %               | 40%                 | -                     |

B. GHG emissions with AD

(i) Avoided GHG emissions by replacement of fossil fuels in CHP with biogas

| Biogas production                                   | m³              | 90342.3             | Assuming 60% of the methane content |
| For heat produced                                   | Mg CO₂ / year   | 78.8                | Refer to Section 2.5               |
| For electricity produced                            | Mg CO₂ / year   | 133.9               | Refer to Section 2.5               |

(ii) Reduction of N₂O emission due to reduced fertiliser use by using the digestate

| Inorganic N equivalent of digestate                  | g / m³          | 2323                | Table 2                          |
| Inorganic N equivalent of untreated                  | g / m³          | 1162                | Refer to section 3.3.4            |
| Saved amount of inorganic N fertiliser              | kg / yr         | 15731               | -                                 |
| Avoided N₂O emissions                                | kg N₂O / year   | 236                 | Refer to Section 2.5               |
| CO₂ equivalent                                      | Mg CO₂ eq / year| 73                  | -                                 |

(iii) Reduction in CO₂ emissions due to reduced fertiliser production

| Saved application of Inorganic N                    | kg / year       | 15731               | -                                 |
Electricity saved  MWh/ year  378  Refer to Section 2.5
Avoided CO₂ emissions  Mg/ year  328  Refer to Section 2.5

C. Total GHG mitigated  Mg / year  751.3

* In Eq. 7.1, 𝑁_{(T)} = 654; there is 4.2 kg N/ m³ of pig manure, so 𝑁_{ex} = 20.7*4.2 kg N/ pig / year; MS=0.6; EF₃=0.005 (liquid/slurry type with natural crust cover)

Note: (1) the calculation was based on the comparison between the two PM management methods: land application of PM and land application of digestate after anaerobic digestion of PM; and (2) it was assumed that after using AD, PM storage can be omitted so GHG emission during PM storage was ignored.
The anaerobic digestion of PM and co-digestion of GS and PM at the VS basis of 1:1 was successful in the pilot-scale study. Co-digestion of PM with GS offered several advantages, such as higher SMYs, higher methane contents in biogas, lower free ammonia concentrations in the bulk fluid, and higher VS and soluble COD removals. The results show that AD of PM is a very effective way to reduce GHG emissions in terms of the production of renewable energy, reduced inorganic N fertiliser use, and avoidance of uncontrolled GHG emissions during the manure management.
CHAPTER EIGHT

Conclusions

8.1 OVERVIEW

The thesis consists of laboratory-scale and pilot-scale studies evaluating biogas production from anaerobic digestion of pig manure and energy crops. Thermo-chemical pretreatment of grass silage was carried out to enhance biogas production. Leaching bed reactors were tested for the hydrolysis and acidification of grass silage.

8.2 MAIN CONCLUSIONS

Effect of pig manure to grass silage ratio on methane production from batch anaerobic co-digestion of pig manure and grass silage

Anaerobic co-digestion of concentrated PM with GS at five different PM to GS VS ratios of 1:0, 3:1, 1:1, 1:3 and 0:1 was evaluated by examining operation stability and methane production potentials in 1-litre digesters made from glass bottles. The following conclusions were drawn:

(i) The co-digestion systems were stable in operation in terms of pH, VFA/alkalinity ratios and concentrations of ammonium/free ammonia at PM to GS ratios of 1:0, 3:1, 1:1 and 1:3, while the digestion systems digesting pure GS failed.
(ii) The highest SMYs were achieved at PM to GS ratios of 1:3 and 1:1 (302.8 – 304.2 ml CH₄/g VS added). The efficient methane production period lasted 23.0, 18.0, 16.4 and 15.8 days at PM to GS ratios of 1:0, 3:1, 1:1 and 1:3, respectively. (iii) The PG/GS VS ratio of 1:1 was recommended for commercial application because of its high methane production potential, short effective biogas production period, and as a result, utilisation of a high amount of GS in co-digestion with PM.

Methane production from anaerobic co-digestion of the separated solid fraction of pig manure with dried grass silage

Anaerobic co-digestion of the solid fraction of separated PM with dried GS (DGS) was evaluated in three identical CSTRs at 35 ± 1 °C with a HRT of 30 days at three DGS VS ratios in the feedstock (20%, 30% and 40% on a VS basis) and four OLRs. The following conclusions were drawn:

(i) Digestion was successful at all OLRs examined. Digestion was successful at all DGS ratios examined.

(ii) Tripling the OLR (from 1.0 to 3.0 kg VS/m³/d) increased the volumetric methane yield by an average of 88% and decreased the specific methane yield by an average of 38%. The methane yield at each OLR was little affected by the proportion of DGS in the feedstock.

Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion

Dried GS was pre-treated at different NaOH loading rates (1%, 2.5%, 5% and 7.5% by VS mass in GS) and temperatures (20 °C, 60 °C, 100 °C and 150 °C) and the
bio-degradability of pretreated DGS was examined, in terms of the hydrolysis yield and degradation of ligno-cellulosic materials. The following conclusions were drawn:

(i) Increasing pre-treatment temperature and NaOH loading rates enhanced the solubilisation and biodegradability of GS biomass. At 100 °C, 45% of the total biomass COD was solubilised, and the reduction of lignin, hemi-cellulose and cellulose was up to 65.6%, 36.1% and 21.2% at the NaOH loading rates of 1%, 2.5%, 5% and 7.5%, respectively.

(ii) The SMYs of GS treated at 100 °C and NaOH loading rates of 1%, 2.5%, 5% and 7.5% were 359.5, 401.8, 449.5 and 452.5 ml CH₄/g VS added, respectively, which were increased by 10% - 38.9% compared to that of untreated GS (325.8 ml CH₄/g VS added). The VS removals achieved were in the range of 76.9 - 96.7%.

(iii) Pre-treatment at the NaOH loading rate of 7.5% resulted in the longest lag phase, probably due to the production of inhibitory or refractory compounds.

(iv) It is recommended that the pre-treatment condition of 100 °C and the NaOH loading rate of 5% be used because of the highest methane production potential and the highest utilisation of VS.

Study on hydrolysis and acidification of grass silage in leaching bed reactors

Hydrolysis and acidification of GS was examined in LBRs under three OLRs: 2.08, 3.13 and 4.17 g grass silage/l/d. The LBRs were run in duplicate over five consecutive batch tests (Batch Tests 1-5) to examine the effects of pH, leachate dilution and addition of inoculum on the process of hydrolysis and acidification. The following conclusions were drawn:

(i) The hydrolysis yields, acidification yields and the degradation of lignocellulosic materials can be increased by: (1) addition of inoculum before the
commencement of experiment; (2) leachate dilution; and (3) adjustment of pH to 6.5. The highest GS hydrolysis yields of 51.5 - 58.2%, acidification yields of 57.2 - 60.3% and VS removals of 62.1 - 66.3% were obtained in Batch Test 4. The reduction of lignocellulosic materials was up to 74.4% of hemicellulose, 30.1% of cellulose and 9.3% of lignin in 32 days.

(ii) Increasing the OLR reduced the hydrolysis and acidification yields.

(iii) The cellulase activity can be used as a key indicator to monitor the hydrolysis process.

(iv) Methane production was low, and accounted for 10.0 - 13.8 % of BMP of GS.

Pilot-scale study on anaerobic co-digestion of pig manure and grass silage

A pilot-scale CSTR (480 litre) was fabricated to further examine biogas production from PM and GS in Teagasc pig research unit. The experiments consisted of two phases: Phase I (day 0 - day 61) at an OLR of 0.87 kg VS/m$^3$/d with the feedstock of PM only and Phase II (day 62 - day 109) at an OLR of 1.74 kg VS/m$^3$/d with the feedstock of PM and GS at 1:1 of VS basis. Three 1 m$^3$ IBCs were set up for the measurement of GHG emissions from raw PM storage. The post-methane production potentials of the digestate produced in Phase I was also measured. The following conclusions were drawn:

(i) The mono-digestion of PM and co-digestion of GS and PM at the VS basis of 1:1 were stable and successful with the pilot-scale anaerobic digester. The specific methane yields were 154 ml CH$_4$/g VS added with mono-digestion of PM and 251 ml CH$_4$/g VS added with co-digestion of PM and GS. Soluble COD and VS removal rates increased from 81.4% and 41.4% in Phase I to 87.8% and 53.9% in Phase II, respectively. Co-digestion of PM with GS offered several advantages, such as a higher specific methane yield, higher methane contents in
biogas, lower free ammonia concentrations, and higher VS and soluble COD removals.

(ii) The results show that AD of pig manure is a very effective way to reduce GHG emissions in terms of the production of renewable energy, reduced inorganic fertiliser use by using digestate and avoidance of uncontrolled GHG emissions during manure management. By using IPCC guidelines, it was estimated that AD of PM can reduce GHG emissions by 781.4 Mg CO$_2$ equivalent compared to not utilizing AD for PM managemenet based on a pig farm with an average herd size of 654 sows in Ireland.

Summary

Co-digestion of pig manure and grass silage has been demonstrated through both laboratory-scale and pilot-scale studies. The amount of energy generated from an Irish farm with 654 sows via co-digestion of PM and GS is estimated; it is up to 530 MWh/a of heat and 371 MWh/a of electricity from CHP. The operation data obtained indicates the benefits of adopting the AD technology for sustainable animal manure management in terms of renewable energy production and GHG emission reduction. The co-digestion technology should be applied by Irish farmers for building “green” farms, given that the government provides due economic and policy incentives. Improvements in biodegradation of grass silage through thermal-chemical and leaching bed reactor technologies are further examined, and the results demonstrates that grass silage can be an excellent feedstock for subsequent biogas production. It is practical for Irish pig farms to co-digest grass silage with pig manure at existing on-site biogas plants.
8.3 RECOMMENDATIONS FOR FUTURE RESEARCH

It is recommended that further studies on biogas production from AD of animal manure and grass silage should be conducted. The recommended research topics include:

(i). A dynamic mathematical modeling of the co-digestion process should be developed to aid practical operation of AD.

(ii). The quality and biosafety of digestate with a view to use as a fertiliser need to be evaluated. The physical, chemical and microbiological parameters including concentrations of heavy metals, organic pollutants, biological stability, pH, salinity, conductivity, and phyto-toxicity, etc., need to be monitored.

(iii). GHG emissions from land application of digestate and raw animal manure need to be quantified in field-scale experiment. This will help to evaluate the mitigation of GHG emissions by means of anaerobic digestion of animal manure and grass silage from a life cycle assessment prospective.

(iv). A cost-benefit analysis of anaerobic digestion in a typical Irish pig farm via life cycle assessment should be carried out in order to present a comprehensive picture of the adoption of the AD technology.

(v). The structure of the bacterial community, especially the cellulose degrading bacterial community, in the anaerobic digesters should be analyzed using molecular techniques. The application of isolated and enriched cellulose degrading microorganisms to degrade lignocellulosic biomass for the biogas production should be examined so as to improve the biogas yields from grass silage.
BIBLIOGRAPHY


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124. Jakobsson C., Sommer E. B., De Clercq P., Giuseppe Bonazzi, Jaap Schröder, 2002. The policy implementation of nutrient management legislation and effects in some European Countries. A presentation held on 18th April 2002 in Gent, Belgium at the final Workshop of the EU concerted action Nutrient Management Legislation in European Countries NUMALEC.


thermal hydrolysis plants. Bioresource Technology 102, 3089-3097.


in a Two-Phase Grass Digestion System. Energy Fuels 24 (8), 4549–4559.
solubilization. Journal of the Water Pollution Control Federation 61, 1678–1683.


258. Wang Y.W., Xu W.Y., 1987. The method for measurement the content of cellulose,
hemicellulose and lignin in solid materials (Chinese). Microbiology 2, 81–85.
two-phase AD. Waste Management 22, 1-5.


APPENDIX A

Publication and Presentation
Journal publication


Conference presentations

Xie S., Lawlor P.G., Frost P., T. Nolan, Zhan X. *Biogas production from anaerobic co-digestion of pig manure and grass silage: a pilot-scale study*. International
Symposium on Anaerobic Digestion of Solid Biomass. 23-24 February 2012, Berlin, Germany


APPENDIX B

Glossary of Term
\( \eta \): fraction of the post methane production potential of the digestate to the total methane yield (in consideration with the digestate) of the feedstock

\( C_{EVS} \): concentration of VS in the digestate, g/l

\( C_{IVS} \): concentration of VS in the feedstock, g/l

CHP: Combined Heat and Power

COD: chemical oxygen demand

CSTR: continuously stirred tank reactor

DGS: dried grass silage

HRT: hydraulic retention time

\( \text{NH}_4^+ \): ammonium-nitrogen

OLR: organic loading rate, kg VS/m\(^3\)/d

PP: specific post-methane production potential, ml CH\(_4\)/g VS

\( r_{DGS} \), DGS VS ratio in the feedstock

SMY: specific methane yield, ml CH\(_4\)/g VS

TKN: total kjeldahl nitrogen

TS: total solid

\( V_0 \): total mass of VS contained in the digestate used, g

\( V_{cm} \): volume of cumulative methane production from the digestate after the post methane production potential test was complete, ml

\( V_d \): volume of digestate used, ml.

\( V_D \): digester volume, l

\( V_E \): volume of the digestate, l/d
\( V_I \): volume of the feedstock, l/d

\( V_m \): volume of methane produced per day, ml/d

VFA: volatile fatty acids

VS: volatile solid

VP: volumetric post-methane production potential, ml CH\(_4\)/ml digestate

\( W_{VS} \): VS mass added to the reactor per day, g/d

\( Y_v \): daily volumetric methane production, ml/l/d