



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

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## ABSTRACT

Anaerobic co-digestion of concentrated pig manure (PM) with grass silage (GS) at five different PM to GS volatile solid (VS) ratios of 1:0, 3:1, 1:1, 1:3 and 0:1 was evaluated by examining operation stability and methane (CH<sub>4</sub>) production potentials. The highest specific CH<sub>4</sub> yields were 304.2 and 302.8 ml CH<sub>4</sub>/g VS at PM to GS ratios of 3:1 and 1:1, respectively. The digestion systems failed at the ratio of 0:1. The lag phase lasted 29.5, 28.1, 24.6 and 21.3 days at the ratios of 1:0, 3:1, 1:1 and 1:3, respectively. The daily methane yield was linearly correlated with the acetic acid concentration, indicating methane production was probably associated with acetoclastic methanogenesis. The hydrolysis constant linearly decreased with increasing the fraction of GS in the feedstock. This study recommends applying the PM to GS ratio of 1:1 in practice due to a high specific methane yield and a short lag phase.

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## 1. Introduction

There is growing worldwide interest in renewable energy sources as a result of issues such as global warming, the increasing cost of fossil fuels and the projected decrease in fossil fuel reserves. Biogas, a methane rich biofuel, produced from renewable biomass by means of anaerobic digestion has received intense attention. Animal manure is a source of biomass for biogas production.

In the European Union (EU), pig farming is a major agricultural industry and large pig farms are now the norm (Molinieuo et al., 2009). The pig production sector in Ireland contributes 6% to the gross agricultural output and is the third most important agricultural sector (Martin, 2007). It is estimated that 3.2 million m<sup>3</sup> of pig manure (PM) is produced in Ireland annually, containing 13 kilotonnes of nitrogen (N) and 2.5 kilotonnes of phosphorus (P). PM is therefore an excellent fertiliser for grass and other crops 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 im-

proved flow characteristics (Ward et al., 2008); and (iii) reduction of pathogens and unpleasant odour.

Ireland has a suitable climate for grass production and has 4.3 million ha of grassland in comparison with only 280,000 ha of arable land. Grass is often conserved as winter forage for ruminant livestock as grass silage (GS). Grass silage has a high digestible organic matter and volatile solid (VS) content and is an excellent feedstock for AD, either as a single feedstock or co-digested with PM. A few studies have shown that energy crops/crop residues can be co-digested with PM (Kaparaju and Rintala, 2005; Gelegenis et al., 2007; Lehtomäki et al., 2007a, b; Alvarez and Lidén, 2008). 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. At a given VS loading rate, GS has a higher specific methane yield (330 ml methane/g VS added) than PM (226 ml methane/g VS added) (Xie et al., 2009). Therefore, when co-digesting PM and GS, increasing the fraction of GS in the feedstock should increase the methane yield. However, there is not much information on the effects of the PM to GS ratio on the methane production potential and the operation stability. This information would be beneficial to determine the maximum amount of GS to co-digest with PM.

The rate-limiting step in AD of grass silage is hydrolysis of complex polymeric substances, such as cellulose, hemicellulose, and

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lignin which comprise up to 75% of the dry matter of GS (Lynd et al., 2002). The rate and extent of hydrolysis of lignocellulosic components is limited due to intense cross-linking of cellulose with hemicellulose and lignin. The crystalline structure of cellulose prevents penetration of microorganisms or extracellular enzymes. The hydrolysis process can be studied by analysing the parameter of soluble chemical oxygen demand (COD), which can be used to describe the hydrolysis kinetics (Batstone et al., 2002).

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.

## 2. Methods

### 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 lab, PM was sieved through 2-mm sieve to remove coarse materials thus ensuring that laboratory tubing was not blocked. The PM was dilute due to rain water and settlement in the storage pond, with the total solid (TS) content of 3.7%, volatile solid (VS) content of 2.5% and soluble COD concentration of  $33200 \pm 640$  mg/l. The PM was then concentrated by sieving through 0.5-mm sieve. The PM fraction passing the sieve was settled down in a container for 2 h 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 concentrations and PM concentrated with 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 Lehtomäki et al. (2008). Prior to commencement of the experiment, the frozen PM and cut GS were transferred to a refrigerator at 4 °C for 1 day. The characteristics of PM and GS are given in Table 1.

### 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 digesters was inoculated with 500 ml of mixed liquor (inoculum) taken from lab-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 solids (TSS) and 15.6 g/l of volatile 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 800 ml working volume. The initial pH of the mixed liquor in each digester was adjusted to  $7.5 \pm 0.1$  by using 1 M HCl or 1 M NaOH. Finally, the digesters were flushed with N<sub>2</sub>, 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.

### 2.3. Analytical methods

The liquid samples were taken from digesters once every 3 days using 5-ml syringe. After immediate measurement of pH, the samples were then centrifuged at 3900 rpm for 10 min and then at 18,000 rpm 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% H<sub>2</sub>SO<sub>4</sub> 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 (TS), VS, soluble COD and alkalinity were analysed according to standard methods (APHA, 1995). The NH<sub>4</sub><sup>+</sup>–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 atm. The CH<sub>4</sub> 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, 60 and 105 °C, respectively. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

## 3. Results and discussions

### 3.1. Process stability

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

**Table 1**  
Characteristics of raw pig manure, grass silage and inoculum.

Characteristics	Grass silage	Pig manure	Inoculum
pH	4.5	7.4	7.9
TS (% fresh weight)	21.4	12.6	2.5
VS (% fresh weight)	20.2	9.3	1.6
NDF <sup>a</sup> (% DM)	68.0	–	–
Protein (% DM)	14.7	–	–
Soluble sugars (% DM)	0.9	–	–
Soluble COD (mg/l)	–	31200	5570
Total COD (mg/l)	–	126000	22420
Total COD (mg/mg VS)	1.4	–	–
TKN <sup>b</sup> (% DM)	1.6	4.3	–
NH <sub>4</sub> –N (mg/l)	–	1650	1930
Lactic acid (% DM)	1.7	–	–
VFA <sup>c</sup> (% DM)	4.9	3.1	–

<sup>a</sup> NDF: neutral detergent fiber.

<sup>b</sup> TKN: total kjeldahl nitrogen.

<sup>c</sup> VFA: volatile fatty acids.

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