Effect of co-digestion ratio and enzyme treatment on biogas production from grass silage and chicken litter

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Abstract Biomethane production potential was evaluated by anaerobic co-digestion of grass silage and chicken litter in batch experiments. The aim of this study was to identify optimum enzyme treatment and co-digestion ratio for these substrates. Preliminary batch assays for biomethane potential determination were performed using a co-digestion ratio of 2:1 (grass silage:chicken litter) treated with various concentrations of enzyme. The highest specific methane yield of 59.28 ml CH_4/ g was observed for 0.1% (w/v) enzyme treatment.

Keywords: anaerobic co-digestion, biomethane potential, grass silage, chicken litter, enzyme treatment

Introduction

As a result of high fossil fuel prices and the threat of climate change, the demand for sustainable fuel has led to increased research into sustainable fuel sources. Biogas generated from the anaerobic digestion (AD) of organic waste matter represents a CO_2 neutral and renewable option for this sector. AD of waste with high organic content is a natural process carried out by microorganisms with biogas (70% CH_4, 25-30% CO_2, 0-10% N_2, and trace amounts of H_2, NH_3, H_2O and H_2S) as an end product (Ray *et al.*, 2013).

Methane, which burns upon combustion to produce carbon dioxide and water, is used for lighting, cooking and electricity in developing countries. Currently, it is gaining importance as a fuel in developed countries as also a cleaner fuel available, besides compressed natural gas (CNG), when compared to coal and petroleum based fuels. Sweden tops the list of countries using biogas as a transportation fuel with a large percentage of public transport vehicles now running on biogas (IGU Biogas Report, 2015).

Anaerobic co-digestion has gained much attention recently as it was realized that it can increase stability of the process of AD. Co-digestion refers to the process of simultaneous digestion of a mixture of two or more substrates with different characteristics that provide a more balanced nutrient composition and positive synergism thus helping in improving biogas yield Mata-Alvarez *et al*., 2000; Esposito *et al*., 2012; Søndergaard *et al*., 2015).

Poultry litter (PL) is a mixture of excreta and bedding material. PL contains high levels of nitrogen and phosphorous which can be used as a fertilizer. However, due to these rich nutrient levels, significant run off from land can occur resulting in eutrophication of adjacent water bodies (Gerber *et al.*, 2007). In 2003, a report released by the sustainable energy authority of Ireland (SEI) noted the resource potential of poultry litter as a fuel. According to this report, each turkey produces 0.014 tons, each broiler produces 0.0018 tons and about 140,000 tons of litter altogether is produced per annum. Out of the total amount of litter produced, approximately 80% is produced by chicken (SEI, 2003).

Grass silage (GS) is obtained after the process of ensilage that includes fermenting and storing harvested grass to enhance its shelf life for use as an animal feed. Anaerobic digestion of grass silage in batch leach bed reactors resulted in methane yields up to 0.204 m^3 CH_4/ kg VS added (Lehtomäki *et al*., 2008). Pakarinen *et al.*, 2008 confirmed that storing energy crops like grass and ryegrass helps to preserve methane yield thus making grass silage a preferable substrate for AD. Nonetheless, it is an expensive substrate and thus co-digestion with waste makes the process more economically viable, provides a better nutrient balance, reduces odor and helps to reduce gas emissions (Babaee *et al*., 2013).

Hydrolysis is regarded as the rate limiting step during AD, improvements to which can make the overall process more economically favorable and result in reduced hydraulic retention time (HRT) (Gerardi, 2003). Studies testing various pretreatment methods that can be applied to disintegrate feedstock, making them more readily bioavailable have been carried out (Taherzadeh and Karimi, 2008). Enzymatic hydrolysis is more competitive than other methods as it consumes low water and energy, offers lower costs of waste utilization and also avoids the problems associated with equipment corrosion (Kumar *et al*., 2009).

There is little information available on studies concerning co-digestion of chicken litter (CL) with GS and enzyme treatment of the substrates. In light of this the current study aims to determine if enzyme addition and co-digestion helps in enhancing methane production from a given feedstock. Batch experiments were performed to determine

percentage of enzyme treatment that gave maximum methane yield for the co-digestion ratio of 2:1 (GS: CL).

1. Materials and methods

1.1 Source and preparation of co-digestion materials and inoculum

GS and CL were obtained directly from local farms in Dunboyne, Co Meath. After delivery to the laboratory, the samples were stored at -20^{o}C and defrosted at 4^{o}C for 24 hours prior to experimentation. The anaerobic digestate used as an inoculum was obtained from an anaerobic reactor treating dairy slurry at Alltech and was stored at 4^{o}C. GS was prepared for assays by manual cutting into approximately 2 cm lengths. Inoculum was prepared for BMP assays by diluting the digestate 1:1 with deionized water and passing it through a 2 mm sieve.

1.2 Substrate characterization

All of the above materials were weighed and dried at 105°C for 24 hours for measuring total solids. Dried samples were heated in a muffle furnace at 550°C for 6 hours (APHA 2012, Allen *et al*., 2016) to determine volatile solids.

$$TS\ \% = \left(\frac{B-w}{A-w}\right) * 100$$

$$VS\ \% = \left(\frac{B-C}{A-w}\right) * 100$$

w: weight of the dish

A: weight of fresh sample+ dish (g)

B: weight of dry sample +dish (g)

C: weight of baked sample +dish (g)

TS: total solids percentage

VS: volatile solids percentage

Total kjeldahl nitrogen, phosphate phosphorous and chemical oxygen demand (COD) were determined with Hach Lange test kits LCK 338, LCK 350 and LCK 014 respectively as per kit protocol (Dr. Bruno Lange GmbH, Düsseldorf, Germany).

1.3 Bio-methane production potential (BMP) tests

The effect of different enzyme concentrations on the production of biomethane was determined using biomethane potential (BMP) assays (Moody *et al*., 2009).

GS and CL were used as mixed feed (MF), in a co-digestion ratio of 2:1 and were employed as substrate (2 g VS per assay). Each 125 ml serum bottle received 50 ml of prepared inoculum (digestate) as described above. Inoculum without substrate or enzyme addition was employed as the blank and inoculum with MF as the positive control. Test samples contained MF and were supplemented with enzyme for the concentrations (% w/v) - 0.05, 0.1, 0.25, 0.5 and 1 respectively. Serum bottles were capped with butyl septa and sealed with aluminum crimp seals (fig. 1).

Pressure readings were recorded regularly over a 30 day period using the pressure transducer method (Coates *et al*., 1996) and pressure values were converted to gas volumes (V) using the formula below. All assays were performed in triplicate at 37°C.

$$V = \frac{P1 * V1}{P2}$$

P1: Pressure reading on the transducer (in psi)

P2: atmospheric pressure (14.7 psi)

V1: headspace volume (70ml)

Figure 1 BMP assay experimental set up

1.4 Methane percentage

Biogas samples were pooled from triplicates for each test sample into gas bags (Supelinert foil, sigma Aldrich). Percentage methane of biogas was determined using gas chromatograph at the conclusion of the experiment. Gas was drawn from the bags using a gas tight syringe and fed into the injector of GC Varian CP 3800 (Varian Inc., Walnut Creek, CA) installed with an FID detector. The GC was equipped with a glass column (1.8m x 6mm od, 4mm id) packed with Poropak Q 100-120 mesh in a Philips PYE-Unicam Series 304 chromatograph. The injector volume was 2 ml and the injector temperature was 100°C. The oven was maintained at 35°C and the detector temperature was 105 °C. Nitrogen was used as the carrier gas at a flow rate 25 ml/min.

Table 1 Characteristics of grass silage, chicken litter, digestate and prepared inoculum

Characteristics	Digestate	Grass silage	Chicken litter	Inoculum
pH	8.57	3.70	7.90	8.3
Total solids (%)	7.7	54	58	1.4
Volatile solids (%)	5.4	50	52	1.2
Total COD (mg/l)	74000	-	-	18573
Total COD (g/g VS)	-	0.9	0.89	-
TKN (mg/l)	12200	-	-	1881
TKN (g/kg VS)	-	38.6	55.2	-
Orthophosphate(mg/l)	2000	-	-	176.3
Orthophosphate (g/kg VS)	-	3.9	15.3	-
VS/TS	0.7	0.92	0.89	0.85

Results

2.1 Substrate and inoculum characterization

Physiochemical characteristics of the substrates and inoculum are shown in Table 1. Both CL and GS recorded high COD values of 0.9 g/g VS, indicating that 90% of the organic matter in the substrates are biodegradable making them an ideal AD feedstock.

2.2 Biogas (CH_4) volume

Cumulative daily biogas and methane volumes (ml), and % CH_4 were recorded for all assays, minus blank assay values (fig. 2). Table 2 shows the biogas and corresponding methane values after subtracting blank from the samples.

The highest biogas volume was obtained for the 0.1% enzyme treatment, 363 ml, corresponding to 131 ml of CH_4. Interestingly, in comparison to the positive control (229 ml biogas, 64 ml CH_4), all other enzyme concentration treatments recorded significantly lower results of c.170 ml biogas, corresponding to between 12-20 ml CH_4.

2.3 Biomethane potential

Biomethane potential (BMP), is the volume of methane produced (l) per unit weight of volatile solids (kg) of the substrate and it is calculated as per the following formula:

$$BMP = \frac{Volume\ of\ methane\ (V - V')}{Mass\ of\ VS\ added}$$

BMP: biomethane potential or SMY (ml/g or l/ kg)

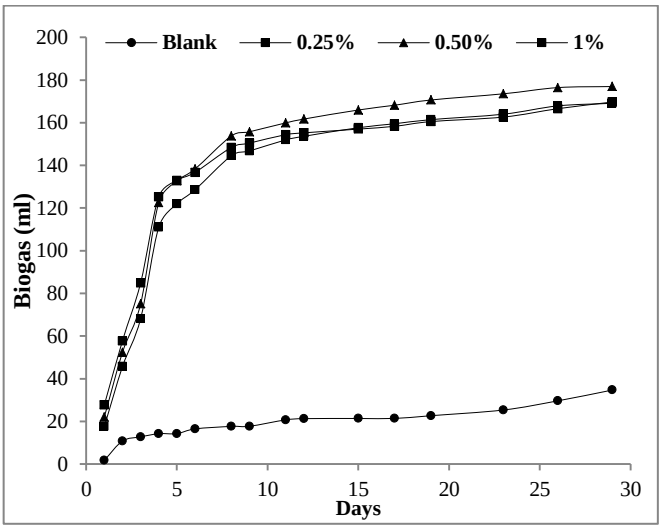
V: Volume of methane produced by test sample

V': Volume of methane produced by blank

0.1% enzyme treatment produced 134 ml higher biogas and 22 l/ kg VS higher methane than the untreated positive control. Figure 3 shows the comparison between biogas and methane yield among different test samples.

Table 2 Cumulative biogas and methane volume

Assay	Biogas (ml)	CH_4 %	CH_4 (ml)
+ control	229	28	64
0.05%	171	8.4	14
0.1%	363	36	131
0.25%	169	12	20
0.5%	177	10	18
1%	170	7.2	12



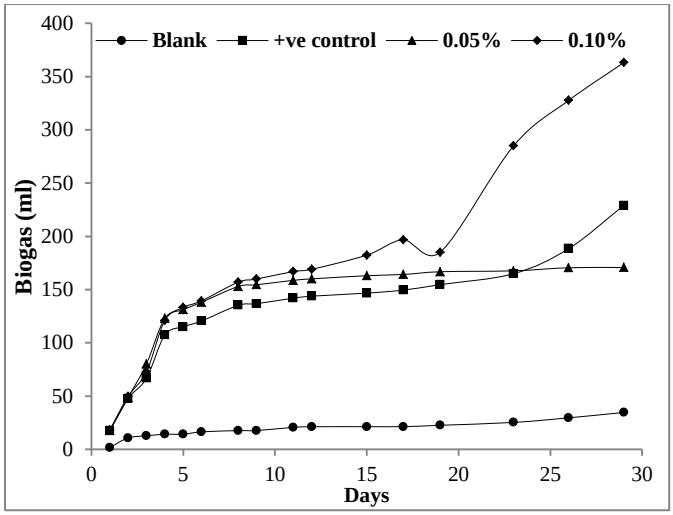


Figure 2 Daily cumulative biogas production curve

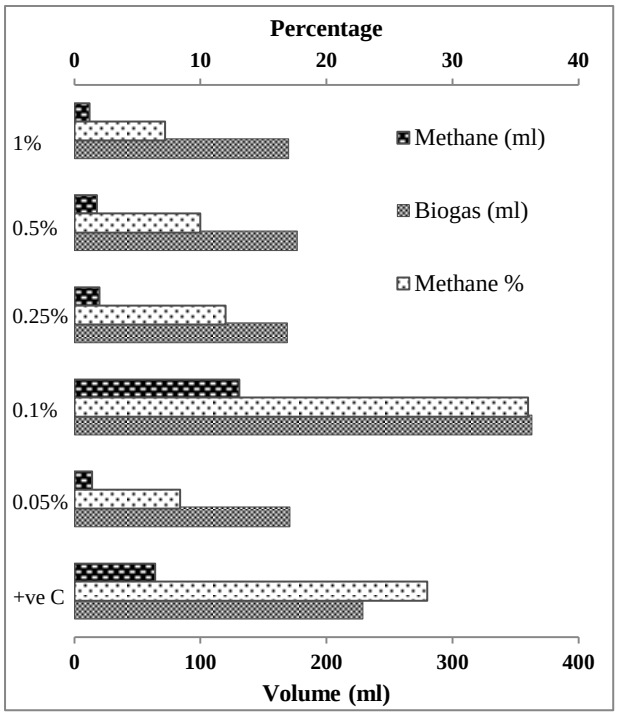


Figure 3 Comparison of the biogas and CH_4 yield between the different test samples

Discussions

Substrate analysis revealed very high chemical oxygen demand for both GS and CL, indicating that both these substrates are suitable as feed stocks for AD systems and ideal substrates for further studies.

Previous studies carried out by the authors (unpublished data) using GS and CL as substrates (singly) resulted in 86 l CH_4/kg VS and 49 l CH_4/kg VS methane yield for GS and CL respectively. These studies with single substrates were done without enzyme treatment. Mix feed (2:1GS:CL) without enzyme was kept as a positive control and produced 27.2 l CH_4/kg VS. Here, co-digestion of the substrates yielded a lesser methane volume compared with single substrate. Similar observation was made in a study carried out by Zhang *et al*., 2013, co-digestion ratios of 90:10 and 10:90 of goat manure to wheat straw resulted in lower methane than single substrates.

In comparison the 0.1% (w/v) enzyme treatment described in this study resulted in the production of 59 l CH_4/kg VS, more than twice that produced by mix feed alone and 10 l CH_4/kg VS higher than CL (fig. 3)

Other treatments did not show any enhancement in biogas or methane productivities. Enzyme treatment with 0.05%, 0.25%, 0.5% and 1% resulted in lower biogas and methane volume when compared with positive control (fig 3). While 0.1% appears to be the optimum treatment, the reason behind the reduction in gas volumes for lower and higher treatments is unknown. In this study, enzyme treatment is a synergistic action of a cocktail of enzymes including cellulase, xylanase, and betaglucanase. Higher enzyme concentrations seem to have an inhibitory effect on the reaction thus causing lower methane production. Thus, activity increases as percentage is increased up to 0.1% beyond which it starts decreasing again. A similar trend was observed by Vidya *et al*. 2014 where enzyme treatment with lipase and protease enhanced biogas productivities at a single specific concentration within a range.

It should be noted that GS utilised as a single substrate yielded a higher methane yield (86 l CH_4/kg VS) when compared to both enzyme treated co-digested samples and non-enzyme supplemented co-digestion samples. This is not of great concern as the objective of the current study was not to achieve comparable methane yields as GS but to asses if GS, which in itself is a valuable animal feedstock product, can be used to facilitate the co digestion of a waste product such as CL. Future studies will determine the lowest ratio of GS that can be used in the co-digestion using increasing volumes of CL and the effect this has on methane yield. Furthermore, the effect of enzyme supplementation will be assessed using these amended co-digestion volumes.

Conclusions

The following conclusions can be inferred from this study: (1) the enzyme treatments applied to the co-digested (CL

and GS) enzyme supplemented BMP assays displayed optimum activity at a specific concentration; (2) the supplementation of batch assays with 0.1% (w/v) enzyme treatment enhanced methane production when compared all other enzyme supplemented BMP assays; (3) co-digested enzyme supplemented BMP assays also recorded higher methane yields when compared to BMP assays containing non-enzyme supplemented chicken litter alone; (4) co-digestion of CL and GS could represent a viable option for the treatment of waste generated from the poultry industry while generating a gaseous fuel in the process, thus making it economically viable and eco-friendly.

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