EFFECT OF BIOMASS HYDROLYSIS ON BIOGAS PRODUCTION

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1 INTRODUCTION

Background: Co-digestion is defined as the anaerobic treatment of a mixture of at least two different waste types. Different feedstock types can provide more biogas than the sum of the two or less, which can be due to a range of factors some are known such as C:N ratio. Therefore we need to establish the best blend in order to maximise methane production and economic output.

Research objectives: Currently, most full-scale biogas plants are co-digestion livestock slurry and other biomass material in Europe. A main research topic for co-digestion is to achieve the maximum methane volume from a biogas plant. Various factors affect methane productivity these include pre-treatment of the feedstock, microbial biomass activity, the C:N ratio, pH, volatile fatty acids (VFAs), chemical parameters of biomass substrates and dry matter. This study investigated how to optimise biogas production through an improved hydrolysis procedure by establishing the best blend in order to maximise methane production.

2 MATERIALS AND METHODS

2.1 Bioreactor reactors

Ten bench-scale bioreactors were constructed from 5 L glass culture vessels (Fig 1). A 48 cm· 6 mm diameter stainless steel tube is bent in a gentle J-shape with a bend of 30^0 from the vertical over the lower 15 cm of its length and any sharp edges are removed from the lower end by grinding. The bent length of the stir rod is lightly coated with silicone grease and inserted into a 38 cm length of 12.5 mm ID · 17 mm OD flexible plastic tubing, which is sealed at the end with a 14/18 neoprene bung. The open end of the tubing is inserted through a 19/26 hole in the center of the reactor lid such that it flush with the top of the lid's central port, with the steel rod extending 11 cm above the tubing. The protruding rod is coupled to a 60-rpm motor (W.W. Grainger, Inc., Lake Forest, IL). Gas is collected and measured by water displacement in a calibrated, inverted cans. (Wilkie et al 2004)

2.2 Analysis

Hydrolysis was monitored by total sugar estimations by phenol-sulfuric assay (Dubois, 1956) and VFAs. VFAs were analyzed with Thermo Electron High Performance Liquid Chromatography HPLC using a Bio Rod Column (125-0115). H_2SO_4 (1mM) was used as a mobile phase with a flow rate of 0.5mL/min. The detection was carried out at ambient temperatures with a diode array UV detector at 220nm.

2.3 Methods

We monitored the hydrolysis of pig feed, grass silage, maize, and wheat straw using the Taguchi factorial designed experiment to establish the optimum conditions and to understand the effect of individual factors. We studied six parameters which were pre-treatment, fermentation temperature, substrate concentration, mixing, enzymes and inoculum volume. Three sets of experiments to understand pre-treatments of heating the substrate at 70^0C, a mix of enzymes and the effect of the fermentation parameters on biogas production. Set 1 experiment with grass silage was performed to understand the effect of pre-treatments and fermentation conditions of temperature and inoculum load. Set 2 experiments was to understand the effect of pre-treatments of different substrates in co-digestion and set 3 experiments was to understand the effect of pre-treatments with variation in substrate to livestock slurry ratio in co-digestion (Table 1).

Samples from the bioreactors were monitored for sugars and VFAs content and their effect on biogas production for all three sets of experiments. Standard methods were used to determine, biogas composition, C:N ratio, pH and other chemical parameters. Xylanase and cellulose enzymes were used as per manufacturer (Biocatalysts, UK) instructions. Inoculum was collected from a commercial biogas plant (Holsworthy, Devon, UK).

Biogas production was used to assess the effectiveness of hydrolysis and a Crowcon Triple plus InfraRed gas detector was used to measure the percentage of methane, CO_2 and hydrogen. Biogas production was measured by water displacement. Pig feed, grass silage, wheat straw and maize were procured from local farms in Devon, UK. Pig feed was (wheat 4.44%, wheat feed 16%, barley, sunflower fibre, and rape extract) procured from Crediton Milling Co Ltd, Crediton, Devon, UK. Grass silage from North Wyke farm contained grasses, *Lolium perenne, Holcus lanatus,Phleum pratense, and Poa trivialis* mix. The chemical composition of grass silage was sugars as sucrose 5.44%, Neutral Detergent Fibre (NDF) 54.6%, Acid Detergent Lignin (ADL) 10.64%, Crude Firbe (CF) 28.3% and Protein 10.7%.

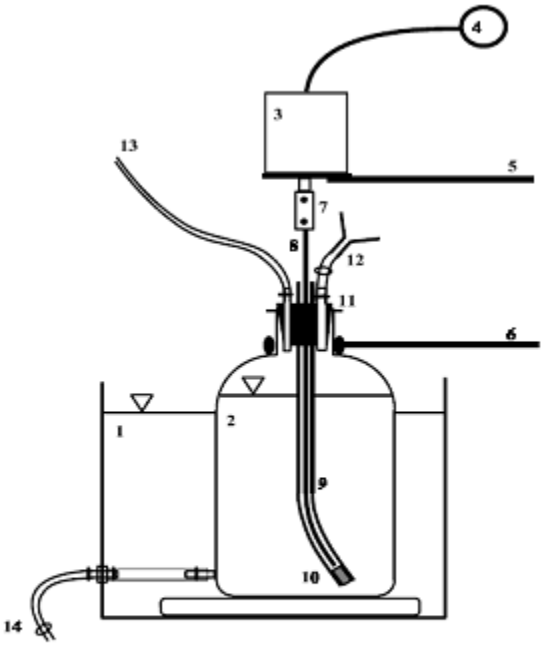

Fig. 1. Schematic of bioreactor with Bordeaux stirrer. Components are: (1) water bath with temperature control; (2) reactor vessel (5 L); (3) stirrer motor; (4) timer; (5) motor support; (6) reactor support; (7) coupler with set screws; (8) bent stir rod; (9) stirrer tube; (10) stirrer tube stopper; (11) reactor stopper; (12) feed inlet with funnel and clamp; (13) gas outlet to gas collector; (14) effluent outlet with clamp.

FIGURE 1 **Schematic of small scale bioreactor**

3 RESULTS AND DISCUSSION

3.1 Biogas production with different substrates

Different fermentation experiments were performed using a factorial design using cattle slurry (CS), grass silage, maize, wheat straw and pig feed (Table 1). Different parameters were varied in the co-digestion experiments in order to evaluate the effect of temperature as well as using two pre-treatments i.e heat treatments at 70^0C and enzymatic additions. The samples from bioreactors were analysed to determine total and volatile solids, total carbohydrates and VFA content during fermentation (Table 2).

Notable results were that the maize silage with CS gave the maximum biogas yield in the co-digestion experiments with up to 4.90 l/day. Also pretreatment influenced substrate biodegradation and methane potential and temperature and continuous mixing were factors that influenced biogas production. Propionic acid accumulation occurred with all the substrates during the methanogenesis stage and acetic acid accumulation occurred during the hydrolysis stage and iso- butyric acid accumulated at the end of both fermentations.

TABLE 1 **Biogas production as a measure of hydrolysis for different experimental designs**

	enzyme mix	Stirring and inoculum	Biogas production (l/day)
(set 1 without CS)			
Grass +70^{0}Cpre treatment	200mg xylanase+2ml Cellulase, 30 ^{0}C	continuous stirring and low inoculum	2.90
Grass +70^{0}Cpre treatment	50mg enzyme, 45 ^{0}C	continuous stirring and high inoculum	2.30
Grass +70^{0}Cpre treatment	200mg xylanase+2ml Cellulase, 30 ^{0}C,	continuous stirring and high inoculum	2.70
Grass +70^{0}Cpre treatment	200mg xylanase+2ml Cellulase, 30 ^{0}C,	on and off stirring and high inoculum	0.80
Cattle slurry	200mg xylanase+2ml Cellulase, 45 ^{0}C	continuous stirring and high inoculum	1.50
Co-digestion (set 2 with cattle slurry 2.1L) +70^{0}C pre treatment in all experiments.			
Pig feed	200mg xylanase+2ml Cellulase, 45 ^{0}C	continuous stirring and high inoculum	3.00
Pig feed	With out enzyme at 45 0C	continuous stirring and high inoculum	3.10
Maize	200mg xylanase+2ml Cellulase, 45 ^{0}C	continuous stirring and high inoculum	3.20
Maize	With out enzyme at 45 ^{0}C	continuous stirring and high inoculum	3.25
Grass	200mg xylanase+2ml Cellulase, 45 ^{0}C	continuous stirring and high inoculum	1.50
Grass	With out enzyme at 45 ^{0}C	continuous stirring and high inoculum	2.10
Wheat straw	200mg xylanase+2ml Cellulase, 35 ^{0}C	continuous stirring and high inoculum	2.50
Co-digestion and Substrate variation in dry wt (25%DM) (set 3) +70^{0}C pre treatment in all experiments			
Grass silage	42g +CS 2.5l at 40^{0}C	continuous stirring	3.00
	83g +CS 2.1l at 40^{0}C	continuous stirring	3.40
	97g +CS 1.9l at 40^{0}C	continuous stirring	3.40
Maize	45g +CS 2.5l at 40^{0}C	continuous stirring	4.00
	90g +CS 2.1l at 40^{0}C	continuous stirring	4.20
	105g +CS 1.9l at 40^{0}C	continuous stirring	4.90

3.2 Effect of pre-treatment and fermentation parameters on hydrolysis and biogas production.

In set 1 experiments contribution to the optimum conditions for grass silage hydrolysis was identified by methanogenesis as follows; (Significant levels at their levels given in %) Temp 20% (at 45 ^{0}C), Enzyme 28.8% (high enzyme concentration, 200mg xylanase+2ml Cellulase), inoculum 51% (high inoculum, 1 L), to give the maximum biogas production of 2.9Ld^{-1}. An on and off stirring sequence for the hydrolysis stage reduced hydrolysis as perceived by the subsequent biogas production.

Set 2 and 3 experiments investigated the effect of variation of substrate/CS ratio on hydrolysis. Higher biogas production occurred with an increase in the substrate from 40 to 100g of grass silage.

The reducing sugars concentration was high during the hydrolysis stage with a concentration range between 300 to 800 $\mu g.ml^{-1}$ and during the fermentation stage between 155- 200 $\mu g.ml^{-1}$. At the end of the fermentation reducing sugars were below 160 $\mu g.ml^{-1}$. VFAs also shown a different trend during the hydrolysis and fermentation stage Acetic acid was higher during the hydrolysis stage and propionic acid was present during the hydrolysis and fermentation stage between 17mM to 72mM (Table 2). N-butyric acid was high during hydrolysis and iso-butyric acid was present at the end of fermentations. During the hydrolysis stage pH varied between 5.0 to 7.0 while during the methanogenesis fermentation the pH was between 7.4 and 7.8.

Biogas production was high with maize as a co-digestion substrate compared to other substrates. In co-digestion experiments with maize more than 3.0L/day of biogas was produced. Methane concentration varied between 40-60% in biogas produced during the co-digestion experiments.

TABLE 2 **Biogas production and analysis of reactor contents during fermentation experiments**

Substrate	**Biogas (l/day)**	**Sugars µg/ml (day 3)**	**Acetic acid (day 2) and propionic acid in mM concentration (day 5)**
Pig feed + CS	3.000	222	6.1 and 34.20
Maize +CS	3.250	273	16.0 and 32.54
Grass + CS	2.100	250	10.7 and 32.84
Wheat straw + CS	2.500	270	7.82 and 72.80
Cow CS	1.400	288	5.0 and 17.00

4 CONCLUSIONS

More complex understanding of biogas production was derived by these multiparameter experiments conducted to evaluate the effect of pre-treatment using heat and enzymes mixtures on hydrolysis of biomass as measured by the effects on biogas production. Main conclusions were:

- A 70 ^{0}C pre-treatment increased hydrolysis with a mixture of enzymes for substrates containing grass silage.
- Continuous stirring was necessary for hydrolysis as switching the stirring on and off reduced hydrolysis.
- Additions of enzymes in co-digestion experiments had little effect on the hydrolysis of grass silage and biogas production.
- Co-fermentation…, positive effect compared to single or not ? or inhibition? How we can find best blend?
- High vs. low inoculum…

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