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### **Energy for Sustainable Development**



## Optimal ratio for anaerobic co-digestion of poultry droppings and lignocellulosic-rich substrates for enhanced biogas production



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#### ABSTRACT

The poultry industry is a progressive and prospective agro-based sector in Bangladesh. Poultry droppings (PD) make an excellent and abundant raw material for anaerobic co-digestion (AD) because of its high nitrogen content. Two sets of comparative assays were conducted on the anaerobic co-digestion of PD with two lignocellulosic co-substrates (LCSs), namely wheat straw (WS) and meadow grass (MG), under five different mixing ratios to optimize substrate composition and C:N ratio for enhanced biogas production. All digesters were run simultaneously under a mesophilic temperature of  $35 \pm 1$  °C with an identical volatile solids (VS) concentration. The results showed that the co-digestion of PD with LCSs was significantly higher in terms of biogas yield and biomethane potential (BMP) than those obtained by mono-digestion of PD and LCSs. Co-digestion of PD and MG produced a higher cumulative biogas production, biogas yield and BMP than from respectively PD and WS. The highest methane contents found were 330.1 and 340.1 Nl kg<sup>-1</sup> VS after digestion for 90 days at a mixing ratio of, respectively, 70:30 (PD:WS) with a C:N ratio of 32.02 and a mixing ratio of 50:50 (PD:MG) with a C:N ratio of 31.52. The increases were 1.14 and 1.13 times those of the LCSs alone, respectively. Predicted optimum ratio for PD:LCSs and C:N ratios, maximum BMP and percentage volatile solids destruction (PVSD) were calculated by using software MINITAB-17 according to the best fit regression models for co-digestion of PD with LCSs.

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#### Introduction

Poultry farming is now an up-and-coming agro-based industry in Bangladesh with more than 0.1 million households and commercial farms (Gofran, 2016) and a total of 3122 million birds (BBS, 2015) generating 114 million tonnes of raw poultry droppings (PD) annually. Of these droppings, 20% is not used (discharged), 40% is sold at markets after sun-drying for a set time, 30% is used as fertilizer for crops and 10% is used for fish culture (Sarker et al., 2009). The current application of poultry droppings (PD) is not sustainable in the long run due to environmental problems such as deterioration of soil quality, buildup of phosphorus in soil (Shih, 1987; Chastain et al., 2012) and air, and soil and water contamination resulting from both chemical (such as ammonia emission to the air) and biological pollutants (such as pathogens proliferating in soils and water bodies), which can lead to adverse effects on aquatic and human health.

Anaerobic digestion might be considered as a potential treatment method for PD for the following reasons: (1) the production of energy (bio-methane) is renewable, which can offset the operating costs of the anaerobic digestion process (Singh et al., 2010); (2) maintenance of nutrient components of PD to soils (Kelleher et al., 2002); (3) nuisance odors would be eliminated and (4) the content of pathogens in the digested effluent would be reduced and there would be as well as better management of waste disposal (Horan et al., 2004).

However, due to the low C:N of PD (less than 10) (Singh et al., 2010), it is often necessary to add carbon-rich lignocellulosic co-substrates such as crop residues to PD to raise the C:N ratio and improve methane yield. The benefit of co-digestion lies in balancing the C:N ratio in the cosubstrate mixture as well as balancing macro and micronutrients, pH, inhibitors/toxic compounds and dry matter content (Hartmann and Ahring, 2005). The C:N ratio is an important indicator for controlling biological treatment systems. Studies show that crop residues containing low levels of nitrogen (high C:N ratio) are characterized by a low pH in the substrate, poor buffering capacity and the possibility of a high volatile fatty acid (VFA) accumulation in the digestion process (Banks and Humphreys, 1998; Campos et al., 1999). Co-digestion of manure and other co-substrates overcomes those problems by maintaining a stable

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pH within the methanogenesis range due to their inherently high buffering capacity. In addition, droppings that have low C:N ratios contain relatively high concentrations of ammonia, in excess of what is needed for microbial growth and risking inhibiting the anaerobic digestion (Hansen et al., 1998; Prochazka et al., 2012).

Tong et al., 2014; Shen and Zhu, 2016 measured the methane yields from a mixture of PD with cereal residues and wheat straw, respectively, but did not specify any optimal mixing combinations of the different substrates between PD and straw. A critical review of literature reveals no comprehensive study on the effect of composition on the biodegradation process in order to optimize the co-digestion process and thus the gas yield. As the biogas and bio-methane yield from organic waste depends on its composition, an attempt has been made in the present investigation to optimize via substrate composition and C:N ratio the biodegradation of volatile solids (VS) and the bio-methane quantity and generation patterns of a mixture by using a best fit regression model.

#### Materials and methods

#### Substrates and inoculum

The poultry droppings (PD) used in this experiment were collected from the poultry farm "Spring Source Bio Aps", Horsens, Denmark. After collecting from the farm, the PDs were put in cool storage (-18 °C)and kept at ambient temperature one day prior to utilization as a feedstock. Briquetted wheat straw without additives and briquetted wheat straw with additives (2% KOH) were used as co-substrates and collected from the Foulum Research Center (Aarhus University, Denmark), where they had previously been prepared and stored in a barrel at ambient temperature. The inoculum was obtained from a mesophilic post-digester at the full-scale biogas plant at Foulum Research Center. This reactor was operated at an elevated total solids level of 8-9%, because it was fed with high levels of extruder-pretreated (MSZ B 110e, Lehman Maschinenbau GmbH, Germany), lignocellulose-rich biomass. The inoculum was stored for three weeks at 35 °C to minimize the biogas production from the inoculum. The inoculum was sieved to remove large particles. The average TS and VS of the inoculum were 4.8% (wb) and 3.0% (wb), respectively. The average pH of the inoculum was 7.7, ammonium nitrogen was 4.55 g  $l^{-1}$  and volatile fatty acid (VFA) content was 47.0 mg  $l^{-1}$ .

#### Analytical method

All the feedstocks selected for the digestion were analyzed for their physical and chemical properties. Total solids (TS), volatile solids (VS), pH and total ammonium nitrogen (TAN), were analyzed by using standard methods (APHA, 2005). To determine the TS in the substrates, samples were kept in an oven at 105 °C for 24 h and weighed before and after this period. To determine the VS in the samples, the ovendried crucibles were kept in a muffle furnace at 550 °C for 5.5 h. The crucibles were removed from the furnace and cooled in air until most of the heat had dissipated. The sample was then weighed and the result for calculation of VS. Samples for fiber analysis were dried (48 h at 60 °C) and milled to a particle size of 0.8 mm using a Cyclotec TM 1093 mill (FOSS, USA). Fiber fractions (neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL)) were analyzed according to the Van Soest (1991) procedure. From these fractions, hemicelluloses, cellulose and lignin were calculated by using the procedure described by Xavier et al. (2015). The total volatile fatty acid (TVFA) and biogas compositions were analyzed by using gas chromatography (7890 A, Agilent Technologies, USA) (Møller et al., 2004). These parameters were analyzed for the feedstock mixtures used in the batch reactors before and after digestion. All the measurements were performed in triplicate and the averages were taken for further interpretation. All the chemicals used for the analysis were of analytical grade.

#### Experimental design, setup and calculations

The batch test was performed as described by Møller et al. (2004). A total of 200 g of inoculum was added in each 500 ml infusion bottle, followed by the addition of substrate with a ratio of 1:1 (VS<sub>substrate</sub>: VS<sub>inoculum</sub>). A control with only inoculum was included. Two sets of experiments were performed: poultry droppings with briquetted wheat straw (WS) for set A and poultry droppings with briquetted meadow grass (MG) for set B. Five different mixing combinations of PD and LCS (lignocellulosic substrates) for both sets were tested separately to obtain the best mixing ratio for maximum methane production. Mixing combinations are shown in Table 1. The total masses of raw samples of five mixtures with two single as controls were calculated on the basis of VS by using Eq. (1):

$$P_i = \frac{m_i \times C_i}{m_s \times C_s} \tag{1}$$

Where,  $P_i$  is the VS mass ratio and the calculations were done to achieve a fixed  $P_i$  equal to 1;  $m_i$  is the amount of inoculum (g);  $C_i$  is the concentration of VS(%) in the inoculum;  $m_s$  is the amount of substrate (g) and  $C_s$  is the concentration of VS(%) in the substrate.

The mass of a feedstock ( $m_{feedstock}$ ) of the mixture was calculated separately by using Eq. (2):

$$m_{feedstock} = \frac{m_i \times VS_i}{\left[\left\{VS_{pd} \times r\right\} + \left\{VS_s \times (1-r)\right\}\right]} \times r$$
(2)

where,  $m_i$  is the amount of inoculum (g);  $VS_i$ ,  $VS_{pd}$  and  $VS_s$  are the volatile solids concentrations of the inoculum, poultry droppings and other substrates of the mixture, respectively (%), and r is the percentage of the individual co-substrate added in the mixture composition.

Digestion of PDs and LCSs on their own was also conducted as controls. All the treatments were repeated in triplicate to determine the biogas production and methane yield as response variables. The bottles were incubated at  $35 \pm 1$  °C for 90 days. In order to maintain anaerobic conditions, the headspace in the bottles was purged with pure nitrogen gas for two minutes and the bottles were closed with airtight butyl rubber stoppers. The bottles were static throughout, except for gentle manual mixing during gas measurements. The measurement of biogas volume was made by inserting a needle connected to a tube with inlet to a column filled with acidified water (pH < 2) through the butyl rubber. The produced biogas was measured by water displacement until two pressures (column and headspace in bottles) were equal (Møller et al., 2004). Methane produced from each sample was corrected by subtracting the volume of methane produced from the inoculum serving as control. The specific methane yield was calculated using Eq. (3):

$$BMP_{observed} = \frac{V_{(ino+feedstock)} - V_{ino}}{mVS_{feedstock}}$$
(3)

where,  $BMP_{observed}$  is the observed biochemical methane potential (ml CH<sub>4</sub> (g VS)<sup>-1</sup>), V (ino + feedstock) is the volume of methane produced by inoculum and substrate (ml CH<sub>4</sub>),  $V_{ino}$  is the volume of methane produced by the inoculum alone (ml CH<sub>4</sub>) and  $mVS_{feedstock}$  is the mass of volatile solids in the substrate (g VS) added.

**Table 1**Mass of each substrate for each mixing ratio.

Mixtures	PD:LCS ratio	100:0	90:10	70:30	50:50	30:70	10:90	0:100
Set A (PD/WS)	Mass of PD (g)	25.5	18.28	10.09	5.58	2.73	0.77	0
	Mass of WS (g)	0	2.03	4.32	5.58	6.38	6.93	7.1
Set B (PD/MG)	Mass of PD (g)	25.53	18.80	10.73	6.05	3.00	0.85	0.00
	Mass of MG (g)	0	2.09	4.60	6.05	7.00	7.66	7.93

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