



## 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 bio-methane 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  $\text{Nl kg}^{-1}$  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 co-substrate 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 ( $-18\text{ }^{\circ}\text{C}$ ) 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  $35\text{ }^{\circ}\text{C}$  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  $4.55\text{ g l}^{-1}$  and volatile fatty acid (VFA) content was  $47.0\text{ mg l}^{-1}$ .

### 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  $105\text{ }^{\circ}\text{C}$  for 24 h and weighed before and after this period. To determine the VS in the samples, the oven-dried crucibles were kept in a muffle furnace at  $550\text{ }^{\circ}\text{C}$  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  $60\text{ }^{\circ}\text{C}$ ) 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 ( $VS_{\text{substrate}}:VS_{\text{inoculum}}$ ). 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):

$$P_i = \frac{m_i \times C_i}{m_s \times C_s} \quad (1)$$

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 ( $m_{\text{feedstock}}$ ) of the mixture was calculated separately by using Eq. (2):

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

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  $35 \pm 1\text{ }^{\circ}\text{C}$  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 ( $\text{pH} < 2$ ) 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):

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

where,  $BMP_{\text{observed}}$  is the observed biochemical methane potential ( $\text{ml CH}_4\text{ (g VS)}^{-1}$ ),  $V_{(\text{ino}+\text{feedstock})}$  is the volume of methane produced by inoculum and substrate ( $\text{ml CH}_4$ ),  $V_{\text{ino}}$  is the volume of methane produced by the inoculum alone ( $\text{ml CH}_4$ ) and  $mVS_{\text{feedstock}}$  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

The resulting specific methane yields were normalized to standard conditions (0 °C and 1.013 bar) by using Eq. (4):

$$V_s = \frac{V_m \times T_s \times P_m}{T_m \times P_s} \quad (4)$$

Where,  $V_s$  is the volume of measured gas at STP (ml),  $V_m$  is the volume of measured gas at ambient condition (ml),  $T_m$  is the ambient temperature (°K),  $P_m$  is the ambient pressure (atm),  $T_s$  is the standard temperature (0 °C or 273°K) and  $P_s$  is the standard pressure (1 atm).

### Statistical analysis

Significant differences between each mixing combination were determined using analysis of variance (ANOVA). Fisher's least significant difference (LSD) multiple range tests were used for multiple comparison tests using SAS version 9.4. Standard forms of the mixture models and best-fit regression equations were analyzed for optimization of the response variables using ANOVA with the software MINITAB-17.

### Response analysis

The conversion efficiency of the feedstock was estimated from the biochemical methane potential (BMP) and percent volatile solids destruction (PVSD) of the response variables. The BMP was obtained from the specific volume of total methane generated from the feedstock during the entire hydraulic retention time (HRT). At the end of the experimental period, the value was calculated for all the mixtures in the study. PVSD is a very important parameter which indicates the conversion efficiency of volatile solids into biogas. PVSD was calculated using Eq. (5):

$$\% \text{VS destruction (PVSD)} = \frac{VS_{\text{initial}} - VS_{\text{final}}}{VS_{\text{initial}}} \times 100 \quad (5)$$

## Results and discussions

### Characteristics of substrate

The chemical composition of substrates such as total solids (TS), volatile solids (VS), pH, total organic carbon (TOC), total nitrogen (TN), fiber content (lignin, cellulose and hemicellulose), P, K, S and the C:N ratio used in this study are listed in Table 2.

The TS, VS and TOC of PD were below those of LCSs, but the TN of PD was significantly higher than those of LCSs ( $p < 0.01$ ). The C:N ratios of the different substrates and substrate mixtures in AD greatly influence biogas production (Wang et al., 2012; Kayhanian, 1999). A higher carbon content provides more carbon for  $\text{CH}_4$  production, whereas a lower nitrogen content limits microbial activity because microbes

need a considerable amount of nitrogen to maintain growth (Zhu, 2010). Maishanu and Hussani (1991) stated that the optimum C:N ratio is 25–30 for biogas production. Siddiqui et al. (2011) showed that the metabolic activity of methanogens was optimized by a C:N ratio range of approximately 9–30. The C:N ratio of WS (92.72) and MG (57.03) was much higher than that of PD (6.01). This finding indicates that the addition of LCSs reduced the C:N ratio and increased biogas as well as methane production when co-digested with PD.

### Comparison of biogas production and methane content at different PD:LCS combinations

For comparison of the difference of mono-digestion and co-digestion of PD with LCSs, the final cumulative biogas yields obtained by mono-digestion and co-digestion under mesophilic conditions are shown in Fig. 1. The biogas yield and BMP after 90 days were significantly higher with co-digestion than with mono-digestion ( $p < 0.05$ ). The highest cumulative biogas productions and specific biogas production rate for both set A and set B were obtained at the ratio of 50:50. The values of cumulative biogas production and specific biogas production rate for set A and set B were 3850 ml and 4013.3 ml, corresponding to 585.90  $\text{NI kg}^{-1}$  VS and 610.70  $\text{NI kg}^{-1}$  VS, respectively (Fig. 1). However, the highest BMP was obtained at the ratio of 70:30 for set A (330.10  $\text{NI kg}^{-1}$  VS) and at the ratio of 50:50 for set B (340.10  $\text{NI kg}^{-1}$  VS) (Fig. 2). These results show similar trends to the results from the co-digestion of goat manure with LCSs by Zhang et al. (2013), indicating that mixing goat manure with LCSs improves biogas production.

Biochemical methane potential at 30 days (BMP-30), the results were statistically significant difference between mono-digestion of PD and co-digestion of PD with WS at only combination of 70:30, produced highest BMP for set A and for set B, significant differences have between mono-digestion of MG and co-digestion of PD with MG at combination of 50:50 which produced highest BMP and also has significant difference between co-digestions of PD and MG at combinations of 50:50 and 10:90 (Fig. 2A).

At BMP-90, showed the results statistically significant differences between mono-digestion of WS and in co-digestions of PD and WS for set A and for set B, significant differences have between mono-digestion of MG and co-digestion of PD with MG at combination of 50:50 which produced highest BMP and also has significant difference between co-digestions of PD and MG at combinations of 50:50 and 90:10 (Fig. 2B).

The BMPs for set A at co-digestion ratios of PD:WS (90:10, 70:30, 50:50, 30:70 and 10:90) were calculated as 298, 330, 322, 310, 299 and 291  $\text{NI kg}^{-1}$  VS, respectively, throughout the entire digestion process (90 days). The results show statistical significant improvements of 17.2, 30.0, 26.9, 22.0 and 17.7% compared to mono-digestion of PD (254  $\text{NI kg}^{-1}$  VS) and an improvement of 2.3, 13.5, 10.7, 6.5 and 2.7% compared to mono-digestion of WS (291  $\text{NI kg}^{-1}$  VS). A similar tendency was noticed for set B (PD:MG) where an improvement of 15.8, 23.6, 33.9, 28.0, 22.0 and 18.2% compared to mono-digestion of PD (254  $\text{NI kg}^{-1}$  VS) and an improvement of 0, 4.7, 13.4, 8.4 and 3.3% compared to mono-digestion of MG (300  $\text{NI kg}^{-1}$  VS). These results are in line with other research (Callaghan et al., 2002; Ardic and Taner, 2005), although the values obtained in this study were lower than the 345  $\text{NI kg}^{-1}$  VS obtained in their studies but higher than the results obtained by Abouelenien et al. (2010), which was less than 200  $\text{NI kg}^{-1}$  VS. These differences may be caused by differences in substrate composition, inocula and digestion temperature.

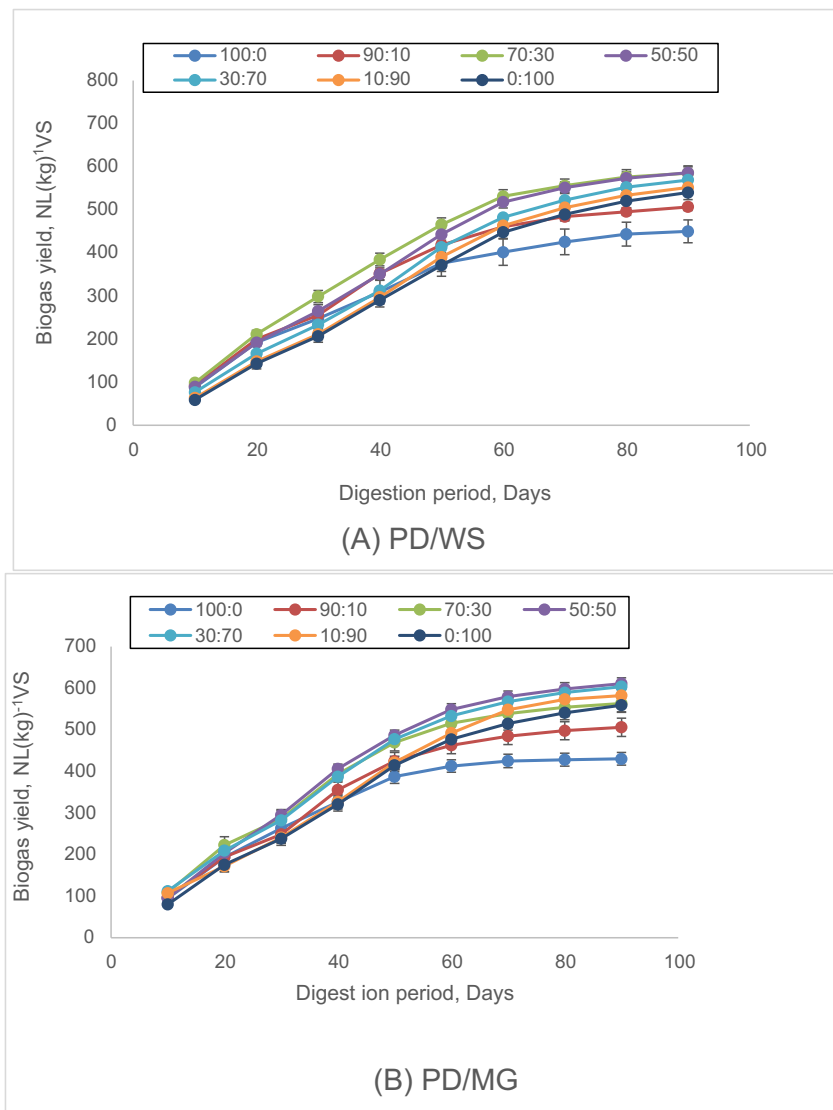
### Effect of the C:N ratio on the co-digestion process

The C:N ratio is an important process parameter in the co-digestion process (Wang et al., 2012). A relatively high C:N ratio means fast nitrogen degradation by microbials and results in low biogas yields and vice versa. A low C:N ratio can result in inhibition of methanogens (Verma,

**Table 2**

Basic characteristics of substrates used in the anaerobic co-digestion.

	PD	WS	MG	Inoculum
pH	6.5	ND	ND	7.7
TS (%)	32.36	88.95	87.82	4.8
VS [wb](%)	23.50	83.97	75.68	3.0
TOC ( $\text{g kg}^{-1}$ )	116	420.0	418	ND
TN ( $\text{g kg}^{-1}$ )	19.30	4.53	7.33	ND
Lignin (%)	5.08	6.53	5.51	ND
Cellulose (%)	24.29	44.44	42.21	ND
Hemicellulose (%)	4.90	32.59	28.45	ND
P ( $\text{g kg}^{-1}$ )	4.85	0.82	1.49	ND
K ( $\text{g kg}^{-1}$ )	8.38	10.70	8.42	ND
S ( $\text{g kg}^{-1}$ )	1.39	0.99	0.67	ND
C:N ratio	6.01	92.72	57.03	ND



**Fig. 1.** Biogas production yield from co-digestion of PD with (A) WS and (B) MG for different mixing ratios. Mean values of three independent replications. Vertical bars represent standard errors.

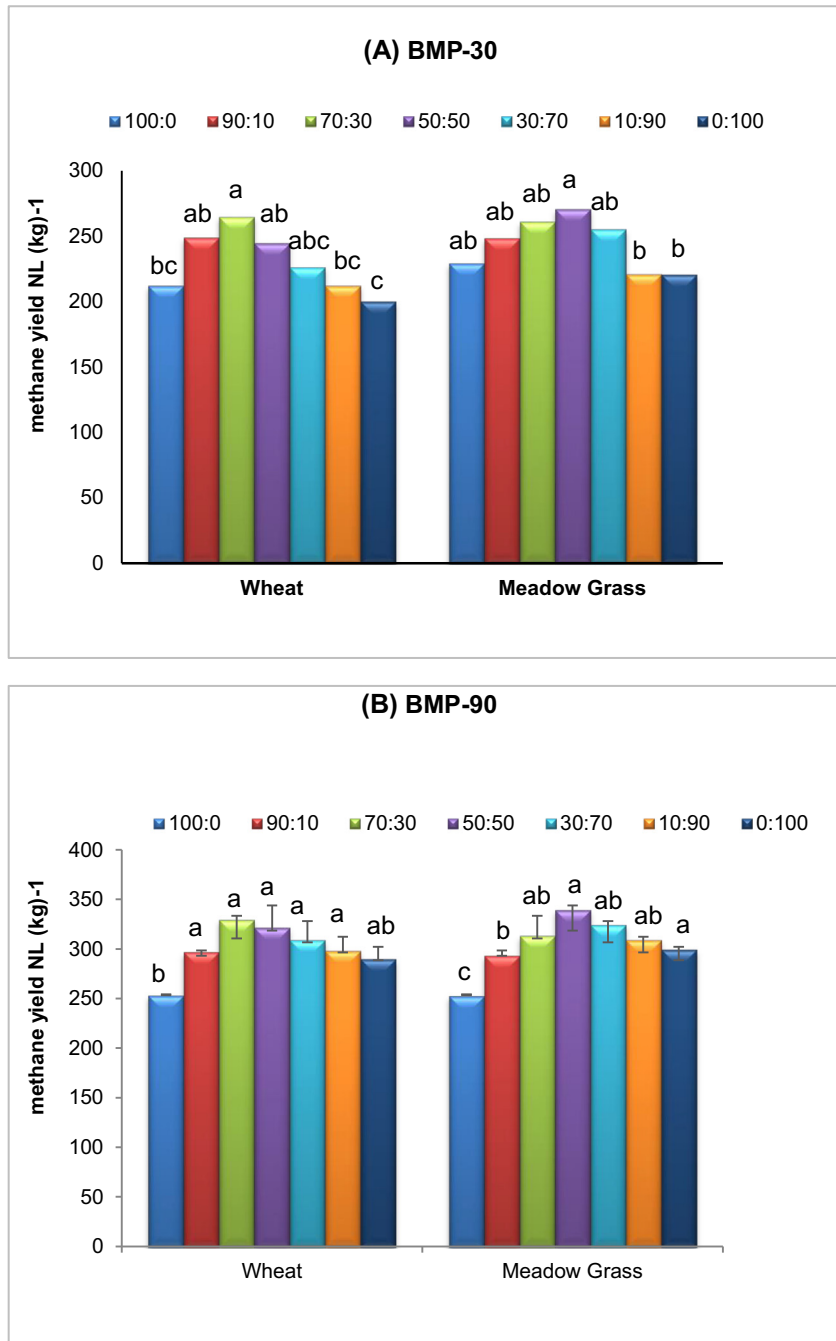
2002). In this study, the range of C:N ratios for each co-digestion and mono-digestion was between 6.01 and 92.72 (Table 3). The C:N ratios of each LCS were much higher than for the co-digestions, thus co-digestion could be maintained at low C:N ratios during the AD process. Cumulative biogas production results indicated that the co-digestion treatments produced higher biogas yields than the corresponding mono-digestion (Fig. 1). The highest methane yields achieved were 330.1 and 340.1  $\text{NL kg}^{-1} \text{VS}$  at a mixing ratio of 70:30 for set A (C:N 32.02) and 50:50 for set B (C:N 31.52), which were 1.14 and 1.13 times higher than that of LCSs alone (Fig. 2). These results suggest that C:N ratios from 30 to 33 were ideal for the co-digestion of PD but were not consistent with the results obtained by Tong et al. (2014) and Zhang et al. (2013), who found that the optimum C:N ratio in the AD co-digestion of chicken manure with crop residues (CRs) was 15–25 and of cattle manure with food waste was 15.8. Wu et al. (2010) revealed that the optimal C:N ratio for the co-digestion of swine manure with CRs was 20. Zhong et al. (2013) also revealed that the ideal C:N ratio for the digestion of blue algae and corn stalks was 20. The most likely explanations for this are that (i) the TN content of fresh PD ( $19.3 \text{ g kg}^{-1}$ ) is much higher than for other livestock and poultry manures, and (ii) a higher C:N ratio can result in fast nitrogen consumption

by microorganisms, leading to a lower biogas production (Verma, 2002).

#### Model fitting and regression analysis

The interactions between the components in a mixture with regard to maximizing the response were studied using a mixture design approach. In a mixture design experiment, the total amount of a material (VS) is held constant and the total proportion is 1 (one) because the response depends on the relative proportions of the component (ingredients) in the mixture and not on the amount of the mixture. The response data based on the independent variables are recorded in Table 6. All the independent variables were fitted to linear, quadratic and full cubic models. Model summary statistics are given in Table 4. Standard error of regression (S) was used as a measure of model fit in regression and analysis of variance (ANOVA). For a given study, the better the equation predicts the response, the lower the value of S. Another parameter which was considered for evaluating the model was  $R^2$  (co-efficient of regression) as this reflects its relationship with one or more predictor variables. For the present study, the regression models included a percentage of the PD of the mixture and the C:N ratio of





**Fig. 2.** Biochemical methane potential (BMP) from co-digestion of PD with WS and MG at different mixing ratios for (A) 30 days and (B) 90 days. Mean values of three independent replications. Vertical bars represent standard errors. Values with the same letters indicate no significant difference at  $p < 0.05$ .

each mixture separately as independent variables and BMP and PVSD as response variables. The best model was selected using the criteria of the lowest standard error of regression (S) and the highest co-efficient of regression ( $R^2$ ). After applying the criteria, the full cubic model was found to be the one best suited for both sets A and B. The values of  $R^2$ ,

which is a measurement of goodness of fit of regression equations, were all more than 95% (97.8% for set A and 95.9% for set B) (Table 4 and Fig. 3).

**Table 3**  
Average C:N ratios in the co-digestion of each mixture before digestion.

	100:0	90:10	70:30	50:50	30:70	10:90	0:100
PD/WS	6.01	14.68	32.02	49.36	66.70	84.04	92.72
PD/Grass	6.01	11.11	21.32	31.52	41.72	51.92	57.03

**Table 4**  
Model summary statistics for set A and set B.

Model	Set A (PD/WS)		Set B (PD/MG)	
	Standard error of regression, S	Regression, $R^2$ (%)	Standard error of regression, S	Regression, $R^2$ (%)
Linear	26.3102	6.5	26.0566	25
Quadratic	13.3103	80.9	8.2537	94
Cubic	5.2383	97.8	7.8263	95.9

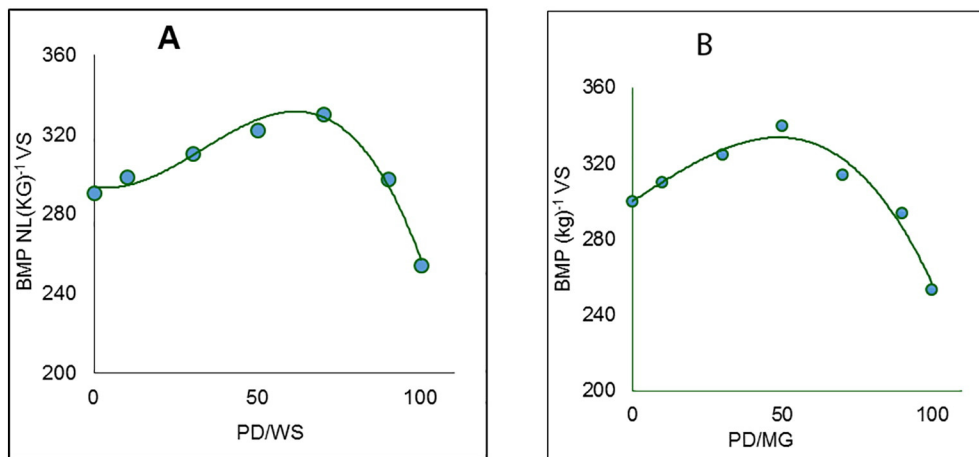


Fig. 3. Regression (polynomial) curves of BMP in the co-digestion of PD with (A) WS and (B) MG.

#### Optimization of mixing and C:N ratios of PD and LCSs

Response optimization of mixture proportions is used to identify the combination of input variable settings that jointly optimizes a single response or a set of responses and is useful for determining the operating conditions that will maximize the yield response. Joint optimization must satisfy the requirements for all the responses in the set, which is measured by composite desirability. Using MINITAB, optimal solutions were obtained and plots were drawn accordingly. The predicted optimum PD:LCS proportions, optimum C:N ratios and maximum methane

yield were estimated on the basis of the presented models (Table 5). The models were subsequently used to calculate the optimum BMP and PVSD from seven different combinations of PD and LCSs. The optimum mixture ratio obtained from the analysis was 69.69% PD to 30.31% WS for set A and 65.66% PD to 34.34% MG for set B. The predicted maximum BMP and PVSD at optimized mixing ratios were 328.78 NI kg<sup>-1</sup> VS and 0.6296 for set A and 326.97 NI kg<sup>-1</sup> VS and 0.6223 for set B, respectively (Fig. 4). The composite desirability of the respective mixtures was found to be 0.9690 and 0.8813 for sets A and

Table 5  
Best-fit regression equations (polynomial, cubic model).

Predictor	Response variable	Regression equations	
		Set A	Set B
Mixing ratio	BMP	$Y = 293.7 - 0.317x + 0.04x^2 - 0.0004x^3$	$Y = 300 + 1.019x + 0.00075x^2 - 0.00015x^3$
Mixing ratio	PVSD	$Y = 46 - 0.097x + 0.011x^2 - 0.00009x^3$	$Y = 51.42 + 0.25x + 0.0003x^2 - 0.00002x^3$
C:N ratio	BMP	$Y = 92.72 - 0.8676x + 0.000012x^2$	$Y = 57.03 - 0.5106x + 0.000013x^2$

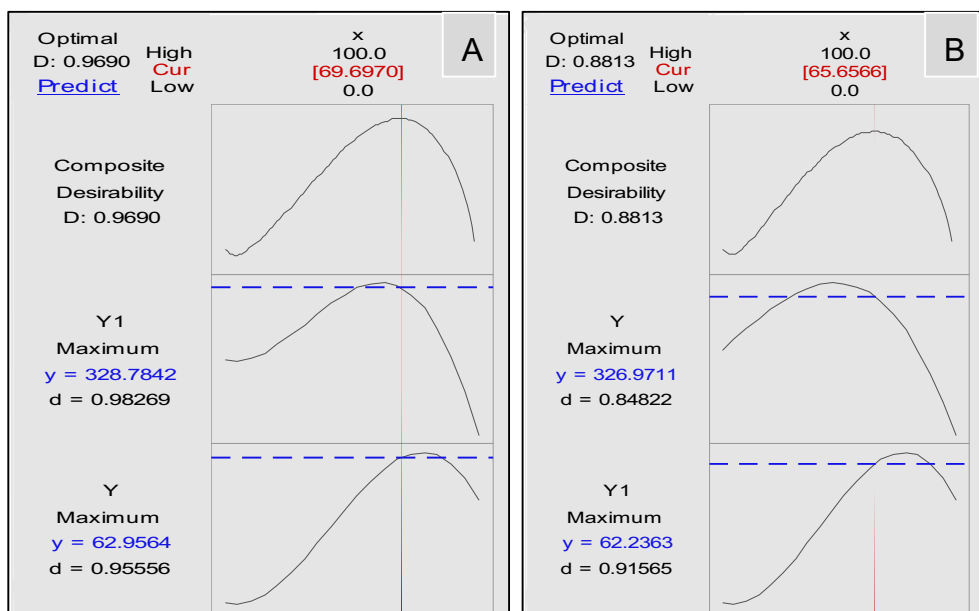


Fig. 4. Optimization plot for BMP and PVSD depending on mixing ratios of sets A and B.

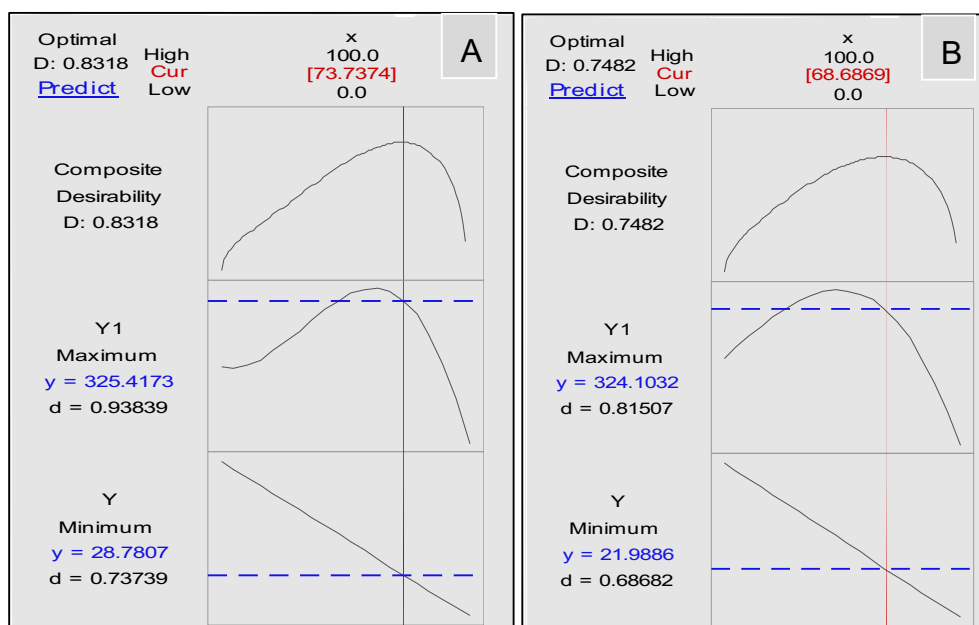


Fig. 5. Optimization plot for BMP and C:N ratios of sets A and B.

B, respectively, which is a significant factor that needs to be taken into account when considering the optimum composition. The composite desirability close to 1 indicates a positive effect on maximizing the response variables.

The optimum C:N ratios obtained from the composite analysis of co-digestion ratios and C:N ratios of co-substrates were 28.78 for set A and 21.98 for set B. The predicted maximum BMP obtained at the optimal C:N ratio was 325.42  $\text{NI kg}^{-1}$  VS for set A and 324.10  $\text{NI kg}^{-1}$  VS for set B (Fig. 5). The composite desirability of the respective mixtures was found to be 0.8318 and 0.7482 for sets A and B, respectively.

#### Model validation

The results obtained from the experiment were found to correlate well with the predicted values listed in Table 6. The experimental data show values for BMP of 330  $\text{NI kg}^{-1}$  VS for set A and 340  $\text{NI kg}^{-1}$  VS for set B and maximum values for PVSD of 63.8% for set A and 63.2% for set B, corresponding to a proportion of PD in the mixture of 70% PD for set A and 50% PD for set B. In order to validate the model, experiments were conducted with the optimum compositions obtained. The results obtained from predictions using 69.69% PD were 329  $\text{NI kg}^{-1}$  VS for BMP and 63.0% for PVSD, and using 65.65% PD they were 334  $\text{NI kg}^{-1}$  VS for BMP and 63.1% for PVSD for sets A and B. A small variation in the experimental results from predicted data were further confirmed by the consistency between the obtained and predicted data.

Table 6

Actual and predicted values of BMP and PVSD at different mixing ratios for set A and set B.

Mixing ratio PD/LCSs	BMP ( $\text{NL}(\text{kg})^{-1}$ VS)				PVSD (%)			
	Set A		Set B		Set A		Set B	
	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
100:0	254	257	254	257	58.7	58.1	56.1	55.9
90:10	298	294	294	287	60.9	62.1	60.9	60.0
70:30	330	329	314	322	63.8	63.0	61.2	61.6
50:50	322	327	340	334	58.2	57.8	63.2	63.1
30:70	310	309	325	327	49.6	50.7	57.9	57.3
10:90	299	294	310	310	47.1	46.1	54.2	53.4
0:100	291	294	300	300	45.7	46.1	51.3	52.2

#### Conclusion

Anaerobic co-digestion of PD with LCSs is a promising way of improving biogas and biochemical methane production compared to mono-digestion, as it solves imbalances in C:N ratios. In this study, the highest BMPs of 330.1 and 340.1  $\text{NI kg}^{-1}$  VS were obtained at a 70:30 mixing ratio for PD with WS (C:N 32.02) and 50:50 ratio for PD with MG (C:N 31.52) throughout the digestion process. Calculated optimum PD:LCS proportions were 69.69:30.31 for PD with WS (C:N ratio 28.78) and 65.65:34.35 for PD with MG (C:N ratio 21.98) using the best fit regression model.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.esd.2017.04.004>.

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