



Co-digestion of ossein factory waste for methane production in batch

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ABSTRACT

Primary clarified bone waste (PCBW) from ossein factory was anaerobically co-digested with cattle dung in relative proportions at ambient temperature. A substitution of PCBW (60%) with cattle dung (40%) reported herein to be appropriate for a maximum biogas production yield with 68-71% methane content. The pH of the slurry was intensively maintained until the course of digestion. The best degradation of organic matter was achieved at a low proportion of PCBW used in the digesters. Thus, co-digestion of PCBW and cattle dung at a moderate proportion is a more noteworthy for producing maximum biogas yield with high quality methane content and to control odor in ossein factory.

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Introduction

Any biodegradable organic material can be used as feedstock for processing inside the anaerobic digesters. If easily available biodegradable wastes are used as inputs, then the benefits could be of two folds in industrial sectors. Although various plant feedstocks have been considered for biomethanation process in an economical way, industrial based wastes, particularly proteinaceous wastes, are the most important to treat anaerobically for obtaining renewable energy in the form of methane. The important noteworthy of treating such wastes is that they do not have lignin-cellulose complex and other structural integrity like plant materials (Chellapandi et al., 2007; Chantrakant and Chellapandi, 2008). An anaerobic digestion strategy for exploiting industrial protein wastes have been developed for biomethanation process, however, biomethanation potential of ossein factory wastes has not yet been studied elaborately (Kalavathy et al., 2001; Braun et al., 2003; Resch et al., 2006; Chellapandi et al., 2008; 2010; Chellapandi and Uma, 2012).

After collecting flesh and hides from cattle in slaughterhouse the bones are brought to ossein factory wherein they are soaked in water for at least ten days. Then, the same bones are clarified with alums in a clarification tank to separate out the minerals (calcium) from bones and neutralize it before gelatin extraction. During this process, a huge amount of bone wastes having hairs, greases and small bones is discharged out. Primary clarified bone waste (PCBW) is one of the major biological wastes discharged from this process that can be caused odor due to deamination reactions occurred in bulk storage. Chellapandi et al. (2010) have recently revealed the application of a simple methanogenic activity assay to evaluate reactor biomass and its substitution in terms of relative production levels of methane by using ossein factory wastes (PCBW and sinews) and cyanobacterial biomass. Thus, the present work was aimed to evaluate PCBW as a potential substrate for biogas production with different proportions of cattle dung at ambient temperature. It would be reinforcing the

pilot-scale strategies for methane production in industrial sectors.

Materials and Methods

Feedstock collection

The test substrate PCBW in wet form was directly collected from discharging output of primary clarification tank at Pioneer-Myagi Indo-Japan Company, Cuddalore, Tamil Nadu, India. It was immediately stored in plastic container at 4°C until the use.

Biomethanation process

Both cattle dung and PCBW (one Kg wet weight, w/w) were blended together with equal volume of tap water. The relative proportion of each digester was set as; D₀: 100% cattle dung; D₁: a mixture of 80% cattle dung and 20% PCBW; D₂: a mixture of 60% cattle dung and 40% PCBW; D₃: a mixture of 40% cattle dung and 60% PCBW; D₄: a mixture of 20% cattle dung and 80% PCBW. Every bottle digester (2.4 L) was fed with two litre of the above mixtures. The contents of the mixtures were mixed as thoroughly as possible daily. All set-ups were placed at ambient conditions in the field lab. Ambient shade temperature was throughout the trial and the temperature range was 30-35°C. Gas production was measured daily from day 0 to day 60 by saline water displacement method (20% NaCl, w/v, pH 4.0) and results were worked out using five-day totals. The pH of digested slurry was measured at weekly intervals. Physicochemical properties of feedstock and digested slurry were analyzed according to standard methods described in APHA (APHA, 1989).

Gas chromatographic analysis

The percentage of methane in gas phase was determined by gas chromatograph (Hewlett Packard-5090 Model) equipped with flame ionization detector and a data integrator. The sample gas (100 µl) was injected through a 2 m Poropak T (80-100 mesh) steel column. Nitrogen and hydrogen were used as a mobile phase and as a fuel, respectively. The flow rate was used as 30 ml/min. Column, injection and detector temperatures were set at 75, 110, 120°C, respectively. The retention times of sample injected were calibrated with retention times of standard

gas mixture and then calculated methane in per cent (Kalavathy et al., 2001).

Results and Discussion

Physiochemical properties of PCBW

During cattle bone processing in primary clarification tank, ossein factory generating huge quantity of bone wastes wherein alum and lime were used to treat the bones. The PCBW was seemed to be a semi-solid waste (total solids 48 g/L) consisting a more proportion of proteins (72%) and low quantity of lipids (2.6%) in an oven dried waste, and 28% minerals in a wet form as obtained from factory. Physiochemical property of this waste has already reported by us (Chellapandi et al., 2010). It suggested that due to more concentration of proteins and minerals PCBW alone may not be suitable as a potential source for biomethanation process, but its substitution with cattle dung may compensate to maintain an effective anaerobic digestion.

Biomethanation of PCBW

As shown in Figure 1, the biogas production was started substantial level at 16 days and then attained to maximum on 23 to 25 days, when a digester fed with a low proportion of PCBW (20-40%). The cumulated biogas volume was obtained maximally (15.6 ± 1.5 L after 40 days) using a mixture of 40% cattle dung and 60% PCBW. It also revealed that a low proportion of PCBW was supported for biogas generation, but at high proportion (80%) of it yielded significant methane content (65-71 %) after 35 days (Figure 2). As similar to that a mixture of coir pith and cattle waste at 3:2 ratio gave a better biogas production with methane content of 80-85 per cent (Radhika et al., 1983).

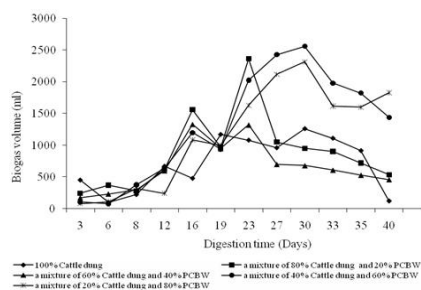


Figure 1 Effect of a mixture containing cattle dung and PCBW at different proportions on biogas production in batch digester

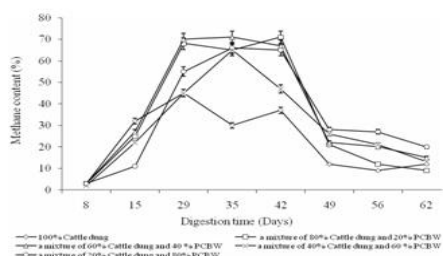


Figure 2 Effect of a mixture containing cattle dung and PCBW at different proportions on methane production in batch digester

The nature of a substrate that determines the type and extent of the fermentative bacteria present in the digesters. An enhanced methane yield has achieved by existence of higher proteolytic organisms in cow dung-fed digesters and other animal waste-fed digesters (Ramasamy and Pullammanmappallil, 2001). The potential proteolytic organisms may be highly established in the digesters. They can be contributed on degrading protein contents in PCBW and then converted into amino acids. Stickland pathway is a predominant metabolic route to convert 40% amino acids, as coupled with other amino acids reactions, into methane from proteinaceous

wastes and rest of the amino acids are utilized, as uncoupled reactions with molecular hydrogen by hydrogen consuming methanogens (Ramasamy et al., 1990). The electron donor amino acid (glycine, alanine etc.) in Stickland reactions is oxidized to a volatile carboxylic acid (acetate), one carbon atom shorter than the original amino acid, which can further be converted into methane. Thus, our findings on ossein factory wastes for methane production are agreed to earlier investigations (Kalavathy et al., 2001; Chellapandi et al., 2008; 2010; Chellapandi and Uma, 2012).

Effect of biomethanation on pH of the digester

The pH of the digester is a function of the concentration of volatile fatty acids produced, bicarbonate alkalinity of the system, and the amount of carbon dioxide produced. The balance of inconsistent pH and buffering $\text{NH}_4\text{-N}$ reported to run intensive and stable digesters loaded with partly hydrolyzed organic wastes and protein rich slaughterhouse waste (Resch et al., 2006). Apart from the decreased methanogenic activity due to lower pH, the population of cellulolytic bacteria, amylolytic organisms, and proteolytic organisms may be reduced by 4 and 2 log order, respectively. Anaerobic growth of microbial consortia could be more progressive because pH of each digester almost ranged to neutral (6.20-7.80) during digestion period as shown in Table 1. Angelidaki and Ahring (1993) were observed the reduction in methane yield, when acetate concentration increased from 1 to 3 g/L as compared to control. They also noted a stable anaerobic digester performance even at high concentration of ammonia (6 gN/L) after 6 months of operation. It suggested that a stable digester performance could possibly to be undertaken, when PCBW was used as a substrate in ossein factory.

Table 1 Change in pH of digested slurry during anaerobic batch digestion of a mixture of cattle dung and PCBW

Period (Days)	Do	D1	D2	D3	D4
0	7.60	7.10	7.50	7.12	7.60
1	6.20	6.70	7.84	6.92	6.93
7	6.85	6.90	6.80	7.23	6.98
14	6.71	6.96	7.25	7.16	7.50
21	6.67	7.11	6.93	6.86	7.01
28	6.97	7.04	6.94	7.18	7.17
35	6.95	7.82	8.00	7.40	7.43
42	6.75	6.56	6.73	7.00	6.95
49	6.77	6.80	7.02	7.12	7.05
56	7.36	7.35	7.33	7.73	7.50

Do: 100% cattle dung; D1: a mixture of 80% cattle dung and 20% PCBW; D2: a mixture of 60% cattle dung and 40% PCBW; D3: a mixture of 40% cattle dung and 60% PCBW; D4: a mixture of 20% cattle dung and 80% PCBW

Effect of biomethanation on total solids reduction in PCBW

The best reduction in total solids was found to be 65% in digester loaded with cattle dung alone (control, 100%), and later on the digester with a mixture of 20% cattle dung and 80% PCBW shown 46% (Table 2). Nevertheless, a mixture of 60% PCBW and 40% cattle dung suited to show the maximum biogas production yield (21.29 L biogas/Kg TVS added). It may be resulted due to more bioavailability of minerals to methanogens. Nickel, iron and cobalt form relatively strong organic complexes with proteins in which bioavailability of these essential metals in anaerobic batch digesters is dramatically increased. An enhanced production of biogas has been reported earlier in the presence of cobalt, nickel, magnesium, calcium, iron and manganese in the digesters (Preeti and Seenayya, 1994; Gonzalez-Gil et al., 2003). Generally, metals are essential one for the growth of methanogens during anaerobic digestion in which they carry out catalytic activity of key enzymes (coenzyme M, format dehydrogenase, methylamine dehydrogenase, carbon monoxide dehydrogenase etc.) involved

in methanogenesis at optimal concentrations. Thus, as a result of good metal ions composition in PCBW it is more opted to boost the growth of anaerobic microorganisms in the digesters, suggesting that advantageous of using it as an alternative feedstock for biogas production.

Table 2 Effect of a mixture of cattle dung and PCBW at different proportions on biomethanation process in batch digesters after 40 days (digester volume 2.4 L)

	D0	D1	D2	D3	D4
Cumulative biogas volume (L)	7.58±1.4	10.55±0.9	7.89±1.3	15.60 ±1.5	13.95±0.7
Biogas production yield (L biogas/Kg TVS added)	9.98±1.3	14.23±1.1	10.63±1.2	21.29±1.6	19.27±0.9
Specific biogas production rate (L biogas/Kg TVS added/day)	0.249±0.05	0.355±0.06	0.265±0.05	0.532±0.07	0.481±0.04
Best methane content (%)	45±5	71±7	68±6	65±4	66±5
Total solid reduction (%)*	65±4	45±5	44±5	37±6	46±4.6

*Determined at 49 days of digestion

D0: 100% cattle dung; D1: a mixture of 80% cattle dung and 20% PCBW; D2: a mixture of 60% cattle dung and 40% PCBW; D3: a mixture of 40% cattle dung and 60% PCBW; D4: a mixture of 20% cattle dung and 80% PCBW

Conclusions

Biomethanation should be certainly the simplest, potentially lowest cost, biological energy production process, compared with other technologies. In batch mode, it is important to keep the digestion time as low as possible and to maintain acclimatization of anaerobic microbial consortiums involved on using alternative feedstocks (Kalavathy et al., 2001; Chantrakant and Chellapandi, 2008; Chellapandi et al., 2008; 2010; Chellapandi and Uma, 2012).. Co-digestion of PCBW and cattle dung at a moderate proportion is a more considerable for producing maximum biogas yield with high quality methane content. This approach is also an efficient way for degrading solid bone wastes so as to control odor in ossein factory. Obviously, it can be useful to compensate industrial energy needs from methane in eco-friendly manner.

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