Dry Anaerobic Co-digestion of Cow Dung with Excess Sludge at Low Temperature

Ajay Kumar Jha

Abstract— **Low evolution rate of anaerobic microbes during psychrophilic condition (<20oC) affects anaerobic digestion process severely. It is also observed that the constituent bacterial consortiums are less active at low ambient temperatures and consequently it causes the drop off in the efficiency of anaerobic fermentation process. The performance of bioreactors even more decreases with the increment in solid content in the feedstocks. It is therefore great challenge to stabilize organic solid waste economically in cold and hilly regions. The purpose of this single-stage batch cultured investigation was to assess the effect of increment in inoculum amount on semi-dry anaerobic digestion process of cow dung with excess sludge at 15±1oC for 84 days. The biogas yields for 15%, 30%, 45% and 60% inoculum based on wet-weight were 13.33, 19.37, 23.99, and 21.64 L/kg with methane of 8.41, 12.19, 15.15 and 13.66 L/kg, respectively. Moreover, the specific methane yield was highest when the psychrophilic semi-dry anaerobic digestion process was inoculated with a mass of 45% of the substrate.**

Index Terms— **Psychrophilic Temperature, Semi-Dry Anaerobic Process, Methane, Organic Materials Removal**

I. INTRODUCTION

Manure and other organic solid wastes stand for a valuable resource, if utilized aptly, can substitute considerable quantities of both fossil fuels and chemical fertilizers whereas the impact of mishandling would be detrimental. Anaerobic digestion process is a successful tool to treat organic matters for recovering biogas and bio-slurry under mesophilic or thermophilic conditions (Li *et al.*, 2010; Zhao and Viraraghavan, 2004). Due to slow hydrolysis and shrink in the population, growth and activity of microbial consortia, it is challenging to stabilize organic wastes below 20^{o}C (Lettinga *et al.*, 2001; Dhaked *et al.*, 2010). The solid retention time for anaerobic process is increased twice to thrice under psychrophilic condition, compared to mesophilic fermentation process (Jha *et al.*, 2011). Moreover, the process becomes instable. Regardless of this, the most of the parts of the world have low-ambient temperatures and disposal of the wastes is a severe hitch. It is also inevitability to stabilize the solid wastes without dilution or with limited liquid since it is tough to collect water in the hilly regions of the developing world due to be short of infrastructure. Dropping off in percentage of moisture of the bio-wastes affects anaerobic process and consequently decrease in biogas production (Jha *et al.*, 2012). Though such wastes contain high quantity of biodegradable compounds, it is immense challenge to stabilize the wastes economically because a large amount of energy is necessary to maintain the bioreactor temperature up to the mesophilic range (Kashyap *et al.*, 2003).

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Knowing the importance of anaerobic digestion under psychrophilic condition, several researchers are concentrated in this filed. They reported that though methanogenesis is sensitive to temperature, psychrophilic anaerobic digesters could successfully degrade organic matters to produce significant amount of biogas (Dhaked *et al*., 2010, Wellinger and Kaufmann, 1982; Massé *et al.*, 2003; Sutter and Wellinger, 1985; Lettinga *et al*., 2001). Rieradevall *et al*. (1983) investigated that the psychrophilic anaerobic digestion of swine manure under HRT of 100 days yielded biogas of 0.03-0.09 m^3 per m^3 of the bioreactor. Similarly, Chandler *et al.* (1983) noted biogas yield of 0.66-0.92 m^3/m^2/day at 10-11^{o}C from a lagoon under HRT of 50 days. The methane content in biogas was superior (70%). Moreover, Zeeman *et al.* (1988) reported that no methane was yielded from fresh manure in batch culture up to five months without inoculation at 5, 10 and 15°C. Biogas generation at low ambient temperatures is feasible with proper amount of temperature-adapted inoculation. Li *et al*. (2011) found that a mixture of cow dung and excess sludge provides better results compared to cow dung alone or excess sludge alone under mesophilic condition. The aim of this paper is to present the effects of various amounts of low temperature acclimatized inoculum on methane yields and organic materials removal from a mixture of cow dung and excess sludge.

II. MATERIALS AND METHODS

Experimental set-up and procedure

The experiments were conducted in triplicate in single stage batch lab-reactors. The volume of each bioreactor was 2.5 L while its internal diameter and height were 13 cm and 25 cm, respectively. The temperature of the capped bioreactors were maintained at 15±1^{o}C by keeping them in a water-bath. The hotness of the water bath was kept constant at 15±1^{o}C by continuous flow of cooled water from a refrigerator (DTY-15A, Beijing Detianyou Technology Development Co. Ltd.). The bioreactor consists of four ports. Among them, two ports were located on the cover of the reactor whereas rest of the ports were placed on its wall. One cover port was connected with a graduated cylinder in order to note daily biogas yields. The another cover port was positioned aside as spare. Regarding the wall ports, one of them was set 5 cm higher than the bottom of the bioreactor in order to take out sample sludge for determining various physico-chemical parameters. Another wall port was for setting up pH meter.

Characteristics of feedstocks and inoculum

The cow dung was collected from a livestock farm while excess sludge from a wastewater treatment plant. The cow dung and excess sludge were mixed in the proportion of 2:3 based on wet-weight as discussed in Li *et al*. (2011). The inoculum was obtained from a psychrophilic anaerobic bioreactor treating cow dung and stored at 15±1°C inside an anaerobic environment. The feedstocks for the bioreactors, R1-R4, were inoculated with 15%, 30%, 45% and 60%, respectively. The substrates were then fed into airtight bioreactors for 84 days at specified operational conditions.

Table 1 Characteristics of substrates and inoculum.

Type of analysis	Cow Dung	Excess Sludge	Inoculum
pH	7.77	7.89	7.83
Total solid (g/kg)	161.13±0.2	178.90±0.2	101.26±0.2
Volatile solids (% of TS)	84.82±0.3	62.78±0.3	68.37±0.3
Chemical oxygen demand (g/L)	150.51±0.5	134.33±0.5	74.59±0.4
Total phosphorus (g/L)	1.53±0.1	1.67±0.1	1.38±0.1
Total Kjeldahl Nitrogen (g-N/L)	2.70±0.1	4.18±0.2	2.36±0.1
Ammonia nitrogen (g-N/L)	1.48±0.1	2.02±0.1	1.29±0.1
Free ammonia	0.11	0.19	0.12

Table 1 presents the mean values for the physico-chemical parameters of cow dung, excess sludge and inoculum. The high proportion of volatile solid (VS) to total solid (TS) in the feedstocks illustrates that a great proportion of the influents was recyclable and easy to degrade to yield gas.

Table 2 Composition and condition of the reactors.

Reactor	Feedstocks	Inoculum (g)	Temp. (°C)	pH	TS (%)	VS (% TS)
R1	400 g cow dung + 600 g excess sludge	150	15±1	7.82	16.24±0.3	71.19±0.2
R2	400 g cow dung + 600 g excess sludge	300	15±1	7.80	15.51±0.2	70.87±0.2
R3	400 g cow dung + 600 g excess sludge	450	15±1	7.85	15.0±0.3	70.62±0.2
R4	400 g cow dung + 600 g excess sludge	600	15±1	7.83	14.52±0.3	70.39±0.2

Table 2 discloses the composition of cow dung, excess sludge and inoculum. Anaerobic environment was made in each bioreactor by purging with nitrogen gas for 20 min. The bioreactors were gradually shaken for 5 min everyday in order to make homogeneous contents, evade stratification and creation of a float up layer and distributing microorganisms inside the bioreactor.

Analytical methods

The physico-chemical parameters including temperature, pH, TS, VS, volatile fatty acids (VFAs), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen, free ammonia, total phosphorus (TP) were determined accordingly as explained in APHA (1995). The values of pH were noted using a digital pH meter (Seven Multi SK40, Switzerland). The free ammonia was determined by formulae stated by (Østergaard, 1985). The biogas was quantified using downward water displacement method at atmospheric pressure per day using graduated cylindrical jar of 1 L for each bioreactor. The methane content in biogas was quantified by means of Gas Chromatography (SP-6800A, Shandong Lunan Instrument Factory, China) as explained in Li *et al.* (2011). The concentrations of the VFAs were calculated with a different gas chromatograph (SP6890, Shandong Lunan Instrument Factory, China) as enlightened in Li *et al.* (2011).

III. Results

Evolution of pH, ammonia nitrogen and VFAs

The pH of the influent was about 7.8 and it was dropped off in each bioreactor because of the raise in VFAs concentrations during the beginning of the process. The eagerly biodegradable proportion of the substrates was hydrolyzed rapidly and converted into volatile fatty acids due to presence of the acidogenic bacteria. The pH value did not drop off much lower because both cow dung and sludge have ability to buffer themselves and evade the acidification occurrence because of the apt alkalinity of the substrates for providing the most encouraging biological activities and constancy of the anaerobic digestion process. The pH value for all the experiments commenced to go up progressively because the concentration of the VFAs were dropping off with their consumption by methanogens in order to yield methane, carbon dioxide and other gases. The pH range noted seemed appropriate for anaerobic digestion process. In addition, there was no apparent effect on pH due to variation in percentage of inoculum as the trend of pH variation was observed alike in all the operating reactors.

The preliminary ammonia nitrogen of the feedstocks was around 1.7 g-N/l. In this study, average ammonia nitrogen concentration was increased to some extent in all the reactors during the start up period. The additional ammonia nitrogen was produced due to hydrolysis of amino acids and proteins. Then, the ammonia nitrogen concentration was decreased since it was utilized as the source of nitrogen for the growth of methanogens. It was again increased since the protein-containing hard biodegradable proportion began to hydrolyze after some days of the beginning of the digestion process. As a result, fluctuated ammonia nitrogen variation patterns were observed for all the tests during the digestion period. The ammonia nitrogen values attained were not assumed to be very high to create inhibition as despite the fact that it can hinder anaerobic digestion process, the concentration of ammonia nitrogen which can be tolerated was higher than the practically observed vales. The concentration of 2.80 g-N/l has been reported as critical value for ammonia nitrogen inhibition in the anaerobic digestion process (Poggi-Varaldo *et al.*, 1997). The ammonia concentrations were noted much lower than the above inhibition value. In addition, free ammonia is considered more inhibitive constituent if its value exceeds 1.1 g-N/l (Hansen *et al.*, 1998). The free ammonia levels for all the reactors during the digestion period were stayed behind much lesser than the inhibitive ranges stated ((Hansen *et al.*, 1998).

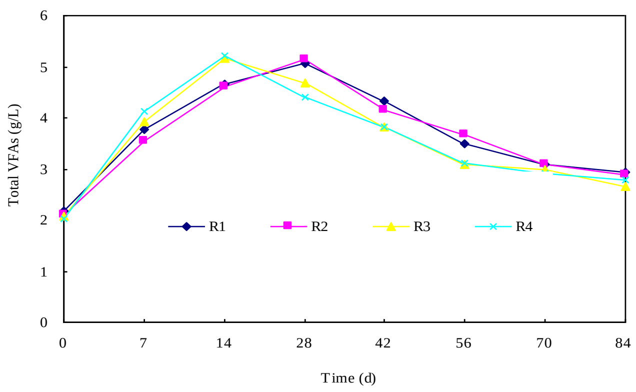

Fig. 1 Total VFAs trends.

Fig. 1 depicts total VFAs accumulation and consumption patterns in all the bioreactors during the digestion period. During hydrolysis and acidogenic stages, VFAs are typically produced as a result of the biodegradation of the organic polymers. The hydrolysis is considered as a rate-limiting step in anaerobic process and the rate of hydrolysis becomes slower at low temperature due to decline in microbial activities under psychrophilic conditions (Dhaked *et al.*, 2010). Therefore, the generation of VFAs was observed slow in all the bioreactors compared to our previous experiments for mesophilic and thermophilic dry anaerobic fermentation processes of cow dung (Li *et al.*, 2011). They are relatively high in the reactor R3 and R4 followed by R2 and R1. The VFAs have been increased gradually to a higher concentration. The major volatile fatty acids were acetic acid, butyric acid and propionic acid. The concentration of acetic acid was 60-70% and it was considered as the foremost volatile fatty acid. At low temperatures, H_2/CO_2 was converted into acetate and methane is then formed from the acetate (Kotsyurbenko *et al.*, 2007). The distribution of propionic acid and butyric acid were comparatively stumpy. The residual VFAs were observed higher in R1 followed by R2, R4 and R3. This result suggested that methanogenic activities have been increased with the percentage increase inoculum up to 45%. The propionic acid was not degraded significantly even the percentages of inoculum had been increased.

VFAs and alkalinity simultaneously are the good indicators for appraising the process stability of the bioreactor. As the ratio ranged between 0.2-0.5, it can be assumed that the process was stable because the anaerobic digestion is not remarkably inhibited if the VFAs to alkalinity ratios are below 0.8 (Zhao and Viraraghavan, 2004). No accumulation of VFAs and no drastic fall in pH also support that the process was not inhibited extensively.

Biogas yields and methane content

Fig. 2 depicts the daily biogas yield, percentage methane content and cumulative methane yields in the bioreactors R1-R4 during the digestion period.

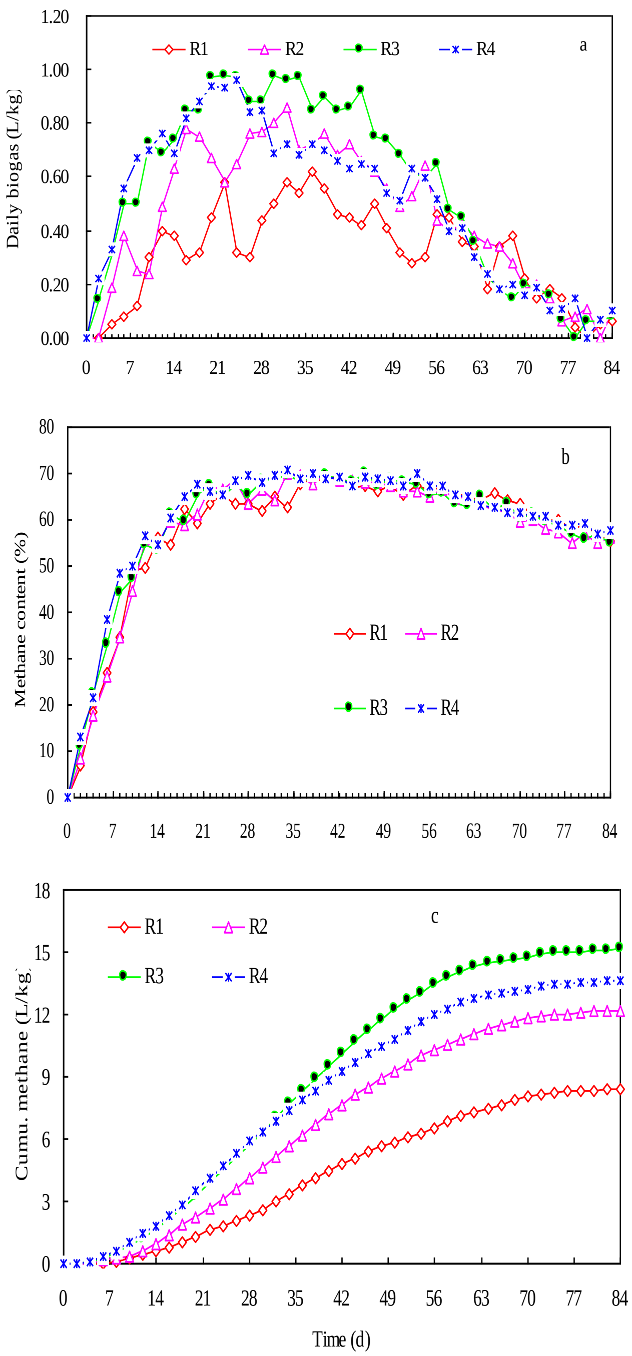

Fig. 2 (a) Daily biogas yields (b) Percentage methane contents and (c) Cumulative methane.

The most quick start-up was in the bioreactor containing greatest quantity of inoculum. The initial biogas production was reduced with the decrease of inoculum percentage. The biogas production was commenced with seeding, remained rising until getting the peak, and then to instigate decrease but two or more peaks were noted during the digestion period. The preliminary biogas production was the result of the keenly biodegradable organic matters in the feedstocks and subsistence of methane producing bacteria in the inoculum. Therefore, the bioreactor with higher amount of inoculum yielded higher quantity of biogas in the beginning of the process. The total biogas yields of the reactors R1, R2, R3 and R4 were 13.33, 19.37, 23.99 and 21.64 L/kg with 8.41, 12.19, 15.15 and 13.66 L/kg methane, respectively. The production of biogas turned into lower than one percentage of the total biogas production during the closing stages. The maximum sum of biogas and methane yields was in R3, followed by R4, R2 and R1. The methane content was low during start up period and increased steadily in all the functional reactors. The average methane contents were 63.12%, 62.95%, 63.15%, and 63.17 % in the reactors R1-R4, respectively. The proportion of carbon dioxide in biogas was being increasing since the beginning of the process until it was stabilized. Its range during the stabilized phase was 15-30%; which is lower than mesophilic and thermophilic anaerobic digestion processes (Li *et al*., 2011). As like mesophilic and thermophilic anaerobic fermentation processes (Li *et al.*, 2011), negligible amount of hydrogen was observed. This might be happened due to all the accessible hydrogen was promptly converted into acetate after combining with CO_2 and the acetate was then converted into methane.

Organic materials removal efficiency

The organic content of the waste is reduced with simultaneous generation of biogas in an anaerobic process. The efficiency of semi-dry anaerobic digestion was assessed in terms of biological conversion of the substrates with VS and COD removals. The values of VS and COD were observed high during the beginning of the process. Due to biodegradation by the presence of fermenting and methanogenic bacteria, they were progressively declined.

Table 3 Organic matter degradation and methane yields.

R	Organic matter & its removal				Methane yield	
	VS_i (g/kg)	VS_r (%)	COD_i (g/L)	COD_r (%)	LCH_4/gVS_r	$LCH_4/gCOD_r$
R1	115.61±0.4	21.04	132.15±0.6	22.12	0.101	0.091
R2	109.91±0.4	29.85	125.46±0.7	30.29	0.154	0.132
R3	105.93±0.4	33.20	120.51±0.6	34.35	0.177	0.165
R4	102.20±0.4	31.02	115.95±0.6	31.92	0.162	0.144

R: Reactor, i: initial, r: removal efficiency

Table 3 exhibits the organic materials removal efficiency and methane yields in the terms of gVS_r and $gCOD_r$ during the bio-methanization processes of the mixture of cow dung and excess sludge at psychrophilic temperature. The VS removal efficiency was calculated higher in R3 (33.20%) followed by R4 (31.02%), R2 (29.85%), and R1 (21.04%). A similar trend was for COD removals.

IV. Discussion

Effect of Inoculum percentage on Energy recovery

A percentage increase in the inoculum could noticeably enhance microorganism activity and process efficiency during start-up phase. This result is consistent with the previous research for common wet anaerobic digestion under psychrophilic environment (Zeeman *et al.*, 1988). In the present study, comparable patterns for biogas/methane yields were achieved in all the bioreactors. Both biogas and methane have been increased with the increase of percentage of inoculum upto 45%. Increasing the percentage of the inoculum more than 45% failed to yield higher amount of methane because the reactor with high amount of inoculum contained more inorganic carbon and accordingly make bigger the volume of the bioreactor. Regarding quality of biogas, there were no considerable variations of methane content among different treatments. Li *et al.* (2011) also found the same results during the co-digestion of cow dung and sludge. As like production of biogas, the increment in inoculum amount up to 45% could enhance VS and COD losses linearly. The specific methane yields for the bioreactors R1-R4 were calculated to be 0.132, 0.163, 0.181 and 0.163 LCH_4/gVS_r whereas in terms of $LCH_4/gCOD_r$ were 0.114, 0.142, 0.157 and 0.142, respectively. It can be observed that the highest methane yield and organic materials removal were achieved in R3 compared to the other treatments. It can be noted that the higher quantity of inoculum could improve the performance and biodegradability of the substrates up to 45% inoculum but more than this percentage of the inoculum failed to enhance linearly due to high loading and presence of more non-carbon matters.

Digestate characteristics and its reuse

The mass balance for batch bioreactors discloses that the bio-slurry consists of high organic materials in psychrophilic anaerobic process compared to mesophilic and thermophilic anaerobic processes (Li *et al*., 2011) as the organic materials removals efficiency was achieved relatively lower, in between 21-31% in term of VS. The psychrophilic semi-dry anaerobic process produces a lower outcome of leachate and generates the bio-slurry with reasonably lower liquid constituent. The bio-slurry is useful as organic fertilizer due to conservation of the nutrients during psychrophilic semi-dry anaerobic process. The nutrients, especially nitrogen (2.3-3.1 g-N/l) and phosphorus (1.2-1.8 g/L), in the bio-slurry were found high. Bio-fertilizers, which enhance soil and boost crop productivity with no detrimental effects on the environment, are economical and eco-friendly supplements than chemical fertilizers. As the total solid for the semi-dry bioreactors was in between 10-11.5%, handling of the digestate to the farms is convenient and economical.

V. Conclusions

Psychrophilic semi-dry anaerobic digestion process has the potential to be a competent, economical and eco-friendly method to biodegrade a mixture of cow dung and excess sludge for methane production under psychrophilic condition. A percentage increase for inoculum up to 45% significantly enhanced the microbial activities, anaerobic process efficiency, methane yield and organic materials removal efficiency linearly. However, its larger mass (60%) failed to increase methane yield and organic materials removal efficiency.

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