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**ORIGINAL ARTICLE**

# PERFORMANCE CHARACTERISTICS OF SEMI-DRY ANAEROBIC DIGESTION OF COW DUNG AND PIG MANURE MIXTURE UNDER PSYCHROPHILIC CONDITION

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**ABSTRACT:** Low-ambient temperatures, <20°C, are known to cause drastic reduction in the efficiency of anaerobic process due to low growth rate of the constituent bacterial consortium. 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 objective of this study was to evaluate the effect of inoculum percentages on semi-dry anaerobic digestion of cow dung and pig manure mixture at 15±1°C in single-stage batch reactors for 84 days. The specific biogas production obtained for 15%, 30%, 45% and 60% inoculum based on wet-weight were 13.53, 20.85, 25.09, and 22.88 L/kg with methane yield 8.63, 13.36, 15.80 and 14.33 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.

**KEYWORDS**: Psychrophilic Temperature, Semi-Dry Anaerobic Process, Methane, Organic Materials Removal

## 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.*, 2011; 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°C (Lettinga *et al.*, 2001; Dhaked *et al.*, 2010). Jha *et al.*, (2011) presented that solid retention time for anaerobic process is increased twice to thrice under psychrophilic condition, compared to mesophilic fermentation process. 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.*, 2010). 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).

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 (Wellinger and Kaufmann, 1982; Sutter and Wellinger, 1985; Lettinga *et al.*, 2001; Massé *et al.*, 2003; Dhaked *et al.*, 2010). Rieradevall *et al.*, (1983) investigated that the psychrophilic anaerobic digestion of swine manure under HRT of 100 days yielded 0.03-0.09 $m^3$ of biogas per cubic meter of the bio-digester. Similarly, another previous study (Chandler *et al.*, 1983) found that 0.66-0.92 $m^3/m^2$/day of biogas with 70% methane content was produced at 10-11°C from a lagoon under HRT of 50 days. 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. Our previous study (Unpublished) showed that a mixture of cow dung and pig manure provides

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better results compared to cow dung alone or pig manure alone. The objective of this study was to evaluate the effects of various amounts of low temperature acclimatized inoculum on methane yields and organic materials removal from a mixture of cow dung and pig manure.

## MATERIALS AND METHODS

*2.1. Experimental set up and procedure*

The experiments were performed in triplicate in single stage batch lab-reactors. Each bioreactor has 2.5 L working volume with an internal diameter of 13 cm, and height of 25 cm. The capped bioreactors were kept in a water bath of operational temperature 15±1°C. The temperature of the water bath was kept constant at 15±1°C by continuous flow of cooled water from a refrigerator (DTY-15A, Beijing Detianyou Technology Development Co. Ltd.). The bioreactor consists of four ports. The two ports were positioned on the cover while other two ports were situated on the wall. One of the cover ports was utilized for quantifying biogas yields. The sample for analysis of methane content in biogas was taken out from the transfer pipe from the same port to the graduated cylinder. Another cover port was positioned aside as spare. One of the wall ports was positioned above 5 cm above from the bottom. This port was utilized to take out the sample for the analysis of various parameters while pH meter was set up at the other side port. The samples were stored at -4°C in a refrigerator before analysis. The analysis was normally performed within one week.

*2.2. Characteristics of feedstocks and inoculum*

The cow dung and pig manure, collected from a livestock farm of Harbin, China were mixed in the proportion of 2:3 based on wet-weight. 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. The average values of the physico-chemical characteristics of the manures and inoculum are presented in Table 1. The high proportion of volatile solid (VS) to total solid (TS) in the manures depicts that a large fraction of the substrates was biodegradable and could serve as an important feedstocks for biogas production. Table 2 exhibits the composition of the substrates and inoculum with their characteristics. Each digester was purged with nitrogen for 15-20 minutes to create complete anaerobic environment. The contents of the reactors were slowly shaken once daily for 3-5 minutes to make homogeneous substrate avoiding stratification and production of a surface crust and allocating microorganisms all through the reactor.

**Table 1:** Characteristics of substrates and inoculum.

| Type of analysis | Cow Dung | Pig Manure | Inoculum |
|---|---|---|---|
| pH | 7.76 | 7.81 | 7.73 |
| Total solid (g/kg) | 16.01±0.2 | 18.79±0.2 | 10.12±0.2 |
| Volatile solids (% of TS) | 84.80±0.3 | 78.05±0.3 | 68.31±0.3 |
| Chemical oxygen demand (g/L) | 150.54±0.5 | 163.63±0.5 | 74.53±0.4 |
| Total phosphorus (g/L) | 1.55±0.1 | 1.76±0.1 | 1.35±0.1 |
| Total Kjeldahl Nitrogen (g-N/L) | 2.72±0.1 | 4.84±0.2 | 2.33±0.1 |
| Ammonia nitrogen (g-N/L ) | 1.49±0.1 | 2.25±0.1 | 1.28±0.1 |
| Free ammonia | 0.10 | 0.18 | 0.11 |

**Table 2:** Composition and condition of the reactors.

| Reactor | Feedstocks | Inoculum (g) | Temp. (°C) | pH | TS (%) | VS (% TS) |
|---|---|---|---|---|---|---|
| R1 | 600 g cow dung + 400 g pig manure | 150 | 15±1 | 7.75 | 16.20±0.3 | 79.84±0.2 |
| R2 | 600 g cow dung + 400 g pig manure | 300 | 15±1 | 7.78 | 15.49±0.2 | 77.77±0.2 |
| R3 | 600 g cow dung + 400 g pig manure | 450 | 15±1 | 7.81 | 15.05±0.3 | 76.27±0.2 |
| R4 | 600 g cow dung + 400 g pig manure | 600 | 15±1 | 7.79 | 14.51±0.3 | 75.05±0.2 |

*2.3. Analytical methods*

The parameters such as temperature, pH, TS, VS, chemical oxygen demand (COD), volatile fatty acids (VFAs), total phosphorus (TP), total Kjeldahl nitrogen (TKN), ammonia nitrogen and free ammonia were determined accordingly as explained in APHA (1995). The pH was measured with 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 by graduated one litre cylindrical jar for each reactor. The quality of biogas ($CH_4$, $CO_2$ and $H_2$) were measured by means of Gas Chromatography (SP-6800A, Shandong Lunan Instrument Factory, China) equipped with a thermal conductivity detector and a 2 m stainless column packed with Porapak TDS201 (60-80 mesh). Nitrogen was employed as the carrier gas at a flow rate of 40 ml/min. The operation temperatures for the injection port, oven and detector all were 80°C.

The samples taken from the batch cultures were centrifuged at 6000 rpm for 15 min, and then acidified with hydrochloric acid and filtered through a 0.2 μm membrane for the analysis of VFAs. The concentrations of the VFAs were determined using an another gas chromatograph (SP6890, Shandong Lunan Instrument Factory, China) equipped with a flame ionization detector and a 2 m stainless (5 mm inside diameter) column packed with Porapak GDX-103 (60/80 mesh). The operational temperatures of the injection port, the column and the detector were 220, 190 and 220°C respectively. Nitrogen was used as carrier gas at a flow rate of 50 ml/min.

## RESULTS AND DISCUSSION

### *3.1. Evolution of pH, ammonia nitrogen and VFAs*

The pH of the feedstocks was around 7.70. It was decreased due to the increase in VFAs production by acidogenic bacteria during the start up phase of each experiment. The eagerly biodegradable proportion of the substrates was hydrolyzed and converted to fatty acids. The pH value did not drop off much lower because the substrates were able to buffer themselves and avoid the acidification happening due to appropriate alkalinity of the manures to keep up most favorable biological activity and stability of the anaerobic digestion system. The pH value for all the experiments commenced to rise gradually as the VFAs were consumed by methanogens and transferred to the methane. 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 1.55 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. Afterwards, the concentration of ammonia nitrogen was decreased since it was used as nitrogen source for methanogens growth. 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 supposed to be high enough to make inhibition because though it can inhibit anaerobic digestion, the ammonia nitrogen concentration that can be tolerated was reasonably high. 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. However, an inhibitive threshold of 1.1 g-N/l of free ammonia was reported (Hansen *et al.*, 1998). The free ammonia levels for all the reactors during the digestion period were remained much lower than the inhibitive levels reported (Hansen *et al.*, 1998). Figure (1) depicts total VFAs accumulation and consumption patterns in all the bioreactors during the digestion period. VFAs are usually generated due to the biodegradation of the complex organic polymers during hydrolysis and acidogenic stages.

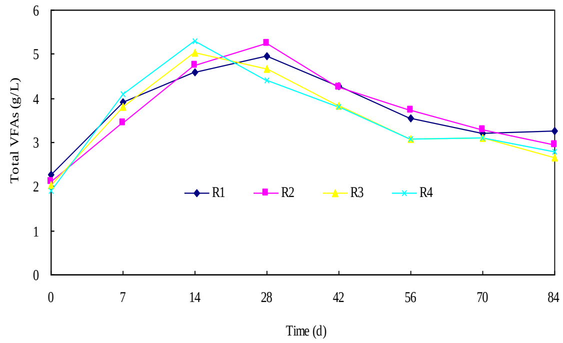


**Figure 1:** Total VFAs trends.

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 principal volatile acids were acetic, butyric and propionic acids. Acetic acid was the dominant 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 share of propionic and butyric acids was observed low. 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 anaerobic reactor. The ratio varied between 0.21-0.45 and so the process seemed 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 VFA and no drastic fall in pH also support that the process was not inhibited extensively.

*3.2. Biogas yields and methane content*

Figure (2) depicts the daily biogas yield, percentage methane content and cumulative methane yields in the bioreactors R1-R4 during the digestion period. The most quick start-up was in the bioreactor containing greatest quantity of inoculum.

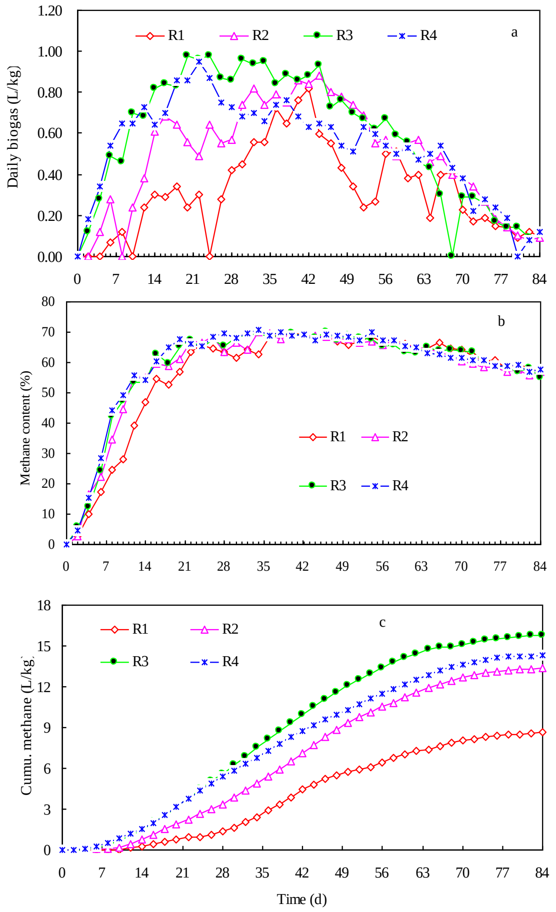


**Figure 2:** (a) Daily biogas yields (b) Percentage methane contents and (c) Cumulative methane.

The initial biogas production was reduced with the decrease of inoculum percentage. Hence, 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 of daily biogas and methane yields were attained for all the bioreactors. 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 generation was due to eagerly biodegradable organic materials in the substrates and existence of methanogens in the inoculum. The total biogas yields of the reactors R1, R2, R3 and R4 were 13.53, 20.85, 25.09 and 22.88 L/kg with 8.63, 13.36, 15.80 and 14.33 L/kg methane contents, respectively. The biogas generation turn 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 higher percentage increase of inoculum (> 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. The methane content was low during start up period and increased steadily in all the functional reactors. The average methane contents were 63.77%, 64.09%, 62.97%, and 62.64 % in the reactors R1-R4, respectively. There were no considerable variations of methane content among different treatments. The percentage of carbon dioxide has increased and stabilized in between 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), hydrogen gas was detected in very small percentage.. This might be happened due to all the accessible hydrogen gas was quickly combined with $CO_2$ to generate acetate and acetate was then converted into methane.

*3.3. 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 high in the beginning and gradually decreased due to consumption by fermenting and methanogenic bacteria. Table 3 presents the organic materials removal efficiency and methane yield per $gVS_r$ and $gCOD_r$ in bio-methanization processes of cow dung at psychrophilic temperature. The VS removal efficiency was calculated higher in R3 (32.08%) followed by R4 (30.28%), R2 (28.56%), and R1 (20.0%). A similar trend was for COD removals. The increment in inoculum amount up to 45% could enhance VS and COD losses linearly. The specific methane yields was determined to be 0.101, 0.154, 0.177 and 0.162 $LCH_4/gVS_r$ in the bioreactors R1-R4 whereas in terms of $LCH_4/gCOD_r$ were 0.091, 0.132, 0.165 and 0.144, respectively. It can be observed that the highest methane yield and organic materials removal were achieved in R3 compared to the other treatments. 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.

**Table 3:** Organic matter degradation and methane yields.

| Reactor | 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 | 129.74±0.4 | 20.0 | 155.39±0.6 | 21.25 | 0.101 | 0.091 |
| R2 | 120.62±0.4 | 28.56 | 146.34±0.7 | 29.16 | 0.154 | 0.132 |
| R3 | 115.26±0.4 | 32.08 | 140.55±0.6 | 33.53 | 0.177 | 0.165 |
| R4 | 108.91±0.4 | 30.28 | 132.05±0.6 | 30.281 | 0.162 | 0.144 |

i: initial, r: removal efficiency

### *3.4. 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 20.0-32.08% 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 (2.32-3.17 g-N/l) and phosphorus (1.30-1.85 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 10.0-11.65%, handling of the digestate to the farms is convenient and economical.

## CONCLUSION

Psychrophilic semi-dry anaerobic digestion has the potential to be an efficient, reasonable and easy-to-use technology to biodegrade a mixture of cow dung and pig manure 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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