Synergetic Effect of Temperature and Partial Digestion of Cellulose on Conventional Biogas Production Rate

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Abstract- In India and several other developing countries, small towns and village are using biogas for fulfillment of their daily household burning and electricity production. In biogas plant cattle dung is a key ingredient, along with other organic feedstocks. Cellulose containing feedstocks are one of the most commonly used and highly accepted substrate for methanogenesis. Degradation of cellulose under anaerobic conditions is a very slow process and results into lower production rate of gas. Therefore, an attempt was made to increase the rate of biogas production by providing partially digested cellulose rich feedstock to the fermentor vessel. Digestion was carried out by extracellular enzymes produced by thermophiles. The vessels were incubated at different temperatures to determine the effects of temperature on rate of methanogenesis. As a result of the study, it was found that fermentor vessels fed with partially digested cellulose and thermophiles have great potential for production of significantly higher quantity of biogas at 55^{O}C in half the incubation time as required by conventional method. Rate of gas production under such conditions was found more than double in many cases. Not only this, a direct relationship between the rate of cellulose degradation and production of biogas was also observed.

Keywords- Two step methanogenesis, enzymatic digestion, rapid production method, double rate of production

1. Introduction

Biogas, one of the most commonly used renewable and alternate energy sources in rural area of India and other developing countries. It is produced by anaerobic digestion of organic matters and used for household burning and electricity generation. The major steps involved in the process of methanogenesis (biogas formation) are i) conversion of polymers into monomers (if feedstock is polymer) commonly known as degradation ii) formation of acetate or lactate from monomers commonly known as acidogenesis and finally iii) formation of biogas from acetate or lactate commonly known as methanogenesis.[1] Cattle dung is the key initiator for biogas production which provides microbes required for methanogenesis and also act as a buffering agent for maintaining the pH throughout the process.[2], [3] Along with cattle dung, other feedstocks are also being added in fermentor tank which promote gas production. Polymers (cellulose, hemicelluloses), alcohols and lactate are examples of such feedstocks.[1], [4], [5] Preference is given to polysaccharides, as they provide larger quantity of monomers to provide sufficient energy and carbons required for the growth of microbes and biogas production for prolong period.[1], [5]

Cellulose is one of such polysaccharide which is abundant and easily accessible as plant fibers. It constitutes a major fraction of urban solid waste, paper recycling, and agricultural residues. It is biodegradable and can be degraded by the synergetic effect of a group of extracellular enzymes collectively known as cellulase, which ultimately produce glucose monomers.(Leschine 1995; Smiti N. Ollivier B. and

Garcia J.L. 1986) These enzymes can be easily obtained from certain microorganisms.[7], [8] The rate limiting step for cellulose in methanogenesis is its slow degradation rate under anaerobic condition, which makes the process slow and results into lower gas production. Degradation of cellulose is highly dependent on many other abiotic factors like temperature, size of feedstock particles, moisture and pH.[1], [2], [6] In this experiment, thermophilic microorganisms capable of producing extracellular cellululase were isolated and used for degradation of cellulose at different temperature. This digested feedstock was directly fed into the vessel to study the rate of methanogenesis. Reasons behind selecting thermophilic species are higher stability of enzymes produced by them and faster rate of enzymatic reaction which makes the process much faster for long period.[9]

The main objective of this study is to increase the rate of biogas production by decreasing degradation phase of cellulose degradation. If this could be reduced significantly then production of higher quantity of biogas is possible in comparatively shorter period of time. This was achieved by providing partially digested cellulose and thermophiles in the fermentor vessel.

2. Materials and Methods

2.1. Isolation of Thermophilic Microbes

Hot water samples were collected in sterilized bottles from hot spring of "Lasundra", in the small district of Kheda, Gujarat, India. Temperature at the time of sample collection was 52^{O}C. Collected samples were immediately transferred to the laboratory under cold condition and immediately proceeded for isolation. Samples were serially diluted and spreaded on N-agar plates and incubated in an incubator at 55^{O}C still visible colonies appear on the plate.

2.2. Screening of Extracellular Cellulase Producing Microbes

Media containing either CMC or cellulose filter paper, as a sole carbon source was used for selective screening of microbes. Extracellular production of enzymes was confirmed by applying congo red stain on CMC containing plates and activity of enzyme was measured by DNS method.[10] Enzyme activity was defined as amount of glucose liberated (µg) per mL by extracellular enzymes produced by thermophiles per minute at 55^{O}C.

2.3. Apparatus

A special kind of apparatus was designed in the laboratory for this study. Detail of the apparatus is shown in the figure 1, where 'A' is methanogenesis vessel filled with slurry, 'B' is inlet of apparatus for addition of components if required any afterwards, 'C' is gas collection vessel, filled with colored fluid whose displacement indicates the amount of gas produced in mL and 'D' is effluent tube for removal of excess fluid. All the junctions and ends were checked for leakage before starting the experiment.

2.4. Statistical Analysis

All the statistical analysis was done on SPSS 16, where $p \leq 0.001$ was considered as significant.

3. Experimental Design

Whole experiment was divided into two phases. First phase was degradation of cellulose by the enzymes produced by thermophiles at different temperatures and second phase was conventional methanogenesis using partially digested feedstock at the same temperature maintained for digestion of cellulose.

In the first phase cellulose degrading microbes were incubated with plant extract (10% w/v) for 3 days on a rotary shaker at 120 rpm at different temperatures: 37^{O}C, 45^{O}C, 55^{O}C and 65^{O}C. Quantity of librated glucose was measured by dinitrosalicylic acid (DNS) method. After the first phase, the second phase of experiment was set up where, digested feedstock was mixed with cattle dung (1:1 ratio) and incubated for 30 days as done in conventional methanogenesis at the same temperature stated for phase I. Total 5 strains were selected for this study, based on their cellulose degradation rate which are designated as *Bacillus subtilis* xrf14 (C5), *Bacillus licheniformis xrf18* (C4), *Bacillus sonorensis* xrf19 (C1), *Paenibacillus dendritiformis* xrf20 (C2) and *Brevibacillus parabrevis* xrf21 (C9). Results obtained from this experiment were analyzed using SPSS 16.0, where $p \leq 0.001$ was considered as significance.

3.1. Gas Chromatographic Analysis

HP PLOT Q column, Column gradient 60ºC - 240oC, TCD Detector, detection temp. 250˚C, injection volume-0.25cc with spilt ratio 1:20.

4. Results and Discussion

On CMC plates, colonies producing extracellular cellulase showed yellow zone after destaining with 0.1N NaOH.[10] Zone of clearance helped in determination of enzyme activity of the organism but exact activity was determined by DNS method. Rate of enzyme reaction for different microorganisms are given in Table I with total gas production. From the results, it was found that higher enzyme activity leads to more biogas production. This is because higher activity produces more amounts of glucose monomers which are directly available for acidogenesis. Plant extract used for experiment was comprised of very small particles as it was observed that size of particles also played significant role on rate of biogas production in many previous studies.[8]

Result of the experiment is given in Table II, where it is shown that vessels incubated at 55^{O}C temperature have

produced maximum quantity of gas followed by 45^{O}C and 37^{O}C, while vessels incubated at 65^{O}C have showed very less gas production as compared to the control.(Figure 2) The maximum production was obtained at 55^{O}C, because of optimum temperature for both the growth of thermophiles as well as the activity of cellulase enzymes. Production was slightly lower in case of 45^{O}C where generation time was higher and activity of enzyme was lower. At 65^{O}C, no microorganisms were able to reproduce; consequently there was no production of enzyme, resulted into lowered gas production.

Another important observation was, production of gas was started as early as on the seventh day of incubation in case of partially digested cellulose fed vessel incubated at higher temperature which is approximately ten to fifteen days in other conventional methods.(Table III, Figure 3)[11] Similar kind of observation was made by Miah *et al*, where they have shown the relationship between the temperature and rate of gas production.[12] This faster production rate and higher quantity of gas is because of shorter initial degradation phase (phase I) of methanogenesis which is generally longer in other conventional methods.[11] At the end of incubation period of 30 days, significant quantity of gas was accumulated into the vessels as compared to the control ($p \leq 0.001$), which is found almost double in quantity in many cases.(Table IV)

During the experiment, it was also noticed that temperature alone didn't have any significant effect on methanogenesis. This is because cellulose is a polymer and is highly stable; it is very difficult to degrade polymers using only single physical factor like temperature. So, one should use other physical, chemical or biological methods along with temperature to break down cellulose polymers.[6][8] However, a little increase in rate of production was observed with elevated temperature.(Figure 2) This may be due to higher activity of endogenous microbes present in the dung. Similarly in case of undigested cellulose fed vessel, there was no significant amount of gas produced at normal as well as elevated temperature.(Figure 2) However, not all the glucose molecules librated from cellulose were utilized for methanogenesis, a few of them were utilized by microbes for their own growth resulting into production of biomass.

The experiment was restricted for 30 days only to determine the effect of partially degraded cellulose and temperature on initial rate of gas production. However, continuous production of biogas is possible by addition of partially degraded cellulose with microbes at regular intervals and simultaneously collecting produced gas under favorable conditions.

Produced biogas when ignited, it burned with blue (oxidizing) flame with minimum quantity of black fumes, which indicate presence of sufficient quantity of methane in the biogas which is above 60.0%. Quality and composition of produced gas was further determined by gas chromatography. As a result of gas chromatography, three major peaks were obtained in chromatogram along with a peak of air/N2. These three peaks were of methane, carbon dioxide and H_2S when compared with the standards. Based on peak intensity it was found the biogas mixture contains about 65.0% of methane, 30.0% of carbon dioxide, 2.0% of H_2S and 3.0% of other gases including water and oxygen. (Figure 4) This composition comes within the standard biogas limits.

From this experiment, it was concluded that the rate of methanogenesis could be expedited by addition of partially digested cellulose with microbes and by incubating at suitable temperature.

Tables

Table 1. Rate of glucose libration by DNS method and quantity of biogas accumulated in mL

Strain	**Rate of glucose Liberation at 55^{O}C (enzyme activity U/mL/min)**	**Biogas accumulated after 30 days (in mL) at 55^{O}C**
Bacillus licheniformis xrf18	0.36±0.03	121.2±1.95
***Bacillus subtilis* xrf14**	0.32±0.04	118.1±0.9
***Brevibacillus parabrevis* xrf21**	0.26±0.02	97.46±1.20
***Bacillus sonorensis* xrf19**	0.31±0.03	117.04±0.96
***Paenibacillus dendritiformis* xrf20**	0.18±0.04	79.5±1.05

Table shows the relationship between enzyme activity and biogas production. This indicates that as higher activity of enzymes leads higher production of biogas.

Table 2. Effect of temperature on different environmental factors affecting rate of biogas production

Conditions	**Temperature**			
	37^{O}C	**45^{O}C**	**55^{O}C**	**65^{O}C**
Cd (Control)	41.20±0.70	56.23±0.45	53.86±0.75	25.40±0.96
Cdc	52.10±0.45	63.50±0.45*	70.00±1.31	33.20±0.40
Cdc + Bacillus licheniformis xrf18	58.44±0.15*	86.13±0.80*	121.20±1.95*	39.93±0.51*
***Cdc + Bacillus subtilis* xrf14**	56.4±0.15*	81.70±0.52*	118.10±0.90*	37.20±0.26
***Cdc + Brevibacillus parabrevis* xrf21**	54.50±0.26	71.50±0.60*	97.46±1.20*	38.20±0.36
***Cdc + Bacillus sonorensis* xrf19**	58.30±0.40*	88.16±0.55*	117.06±0.96*	36.50±0.36
***Cdc + Paenibacillus dendritiformis* xrf20**	52.60±0.30	65.40±0.41	79.50±1.05*	37.60±0.40

Cd=dung Cdc=dung+cellulose, * p≤0.001 (compared to respective controls)

When effect of different parameters were studied to determine their effects on biogas production, it was found that vessels containing dung, partially digested cellulose and thermophiles and maintain at 55° C has produced maximum quantity of gas. In all the other cases production was comparatively less because of several reasons.

Table 3. Accumulation of biogas by different microorganism at regular intervals

Strain	**DAYS**			
	0	**7**	**15**	**30**
Cd	ND	ND	17.1±0.5	53.83±0.75
Cdc	ND	ND	26.3±0.6	70.3±1.31
Cdc + Bacillus licheniformis xrf18	ND	16.70±0.45	53.16±0.40	121.20±1.95
***Cdc + Bacillus subtilis* xrf14**	ND	17.70±0.55	51.33±0.60	118.10±0.90
***Cdc + Brevibacillus parabrevis* xrf21**	ND	12.26±0.41	41.10±0.50	97.46±1.20
***Cdc + Bacillus sonorensis* xrf19**	ND	16.66±0.55	50.80±0.36	117.06±0.96
***Cdc + Paenibacillus dendritiformis* xrf20**	ND	10.80±0.40	35.10±0.20	79.50±1.05

Cd=dung Cdc=dung+cellulose

Here the production of biogas was started on the seventh day of incubation in all the cases which is generally twelve to fifteen days in conventional methods. This indicates that use of partially digested cellulose can increase the efficiency of biogas production.

Table 4. Rate of biogas production as evident from total accumulated gas in vessel

Conditions	**Temperature**			
	37^{O}C	**45^{O}C**	**55^{O}C**	**65^{O}C**
Cd	1	1	1	1
Cdc	1.264563	1.12929	1.299666	1.307087
Cdc + Bacillus licheniformis xrf18	1.418447	1.531745	2.250278	1.572047
***Cdc + Bacillus subtilis* xrf14**	1.368932	1.452961	2.192722	1.464567
***Cdc + Brevibacillus parabrevis* xrf21**	1.322816	1.271563	1.809506	1.503937
***Cdc + Bacillus sonorensis* xrf19**	1.415049	1.567846	2.173413	1.437008
***Cdc + Paenibacillus dendritiformis* xrf20**	1.276699	1.16308	1.476049	1.480315

Cd=dung Cdc=dung+cellulose

In many cases rate of production was found more than double as compare to conventional plants.

Figures

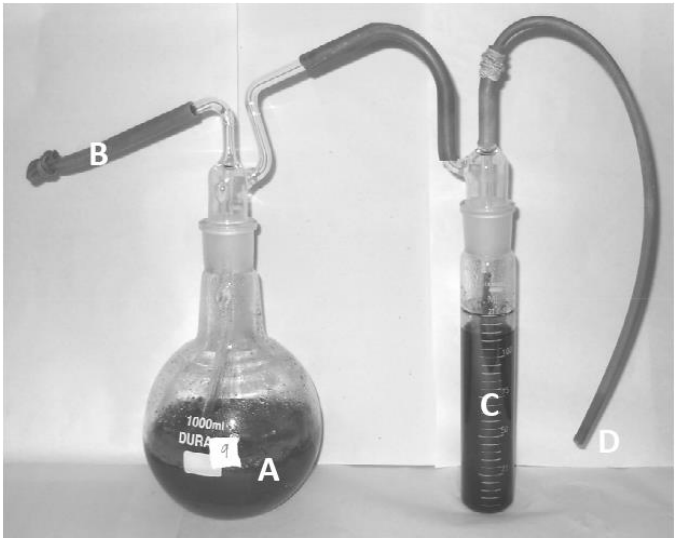

Fig. 1. Apparatus for biogas production

'A' is methanogenesis vessel, filled with slurry, 'B' is inlet of apparatus for addition of components, if required, 'C' is gas collection vessel, filled with colored fluid whose displacement indicates the amount of gas produced (in mL) and 'D' is effluent for removal of excess fluid.

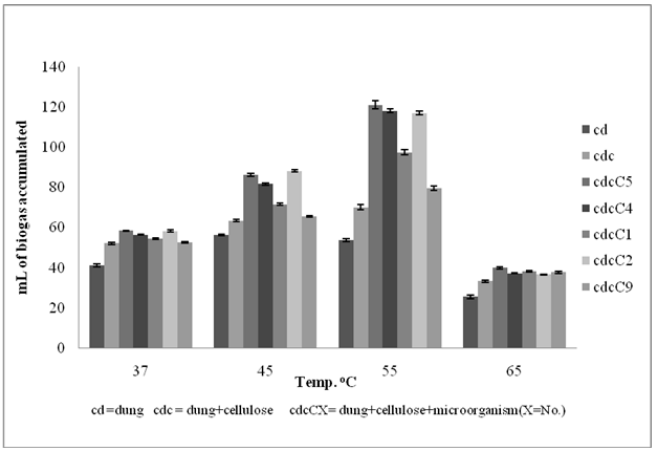

Fig. 2. Effect of temperature on different environmental factors involved in biogas production rate

Optimum production of biogas was achieved at the temperature of 55ºC, as it is an optimum temperature for the activity of the enzymes. While above and below this temperature production was not so high. At extremely high temperature of 65ºC microbes are not able to grow resulted into lower gas production compare to control.

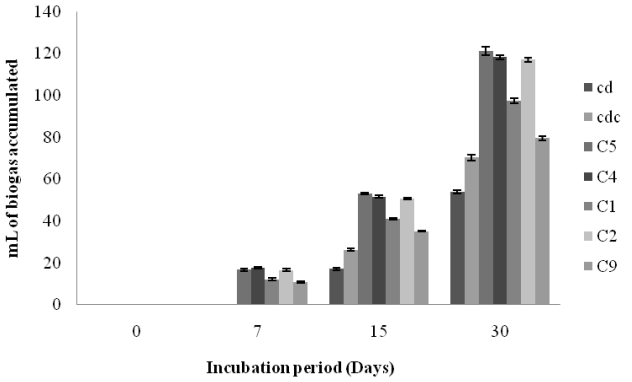

Fig. 3. Biogas accumulation at regular interval at 55°C

Figure shows the graphic representation of the data obtained for the effect of different incubation time on the rate of biogas production. Production was started as early as seventh day onwards which is generally 10 – 15 days. This is because of minimizing the initial degradation time of polymer for monomer production by providing partially digested feedstock which provides monomers directly in the medium for acidogenesis.

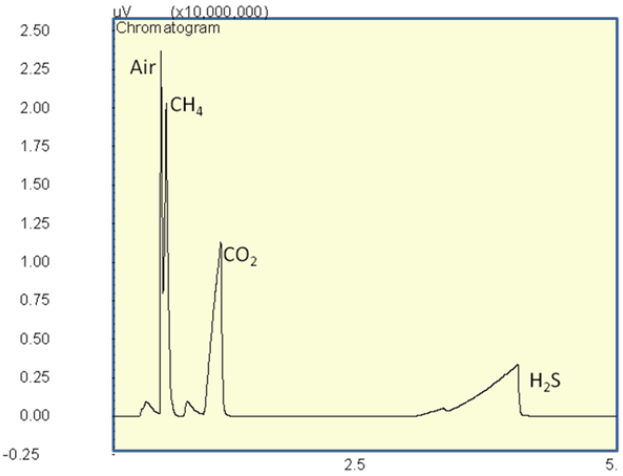

Fig. 4. Gas chromatogram of produced biogas

GC analysis shows presence of methane (65.0%), carbon dioxide (30.0%) and H2S(2.0%) in the produced gas by methanogenesis. This combination is one of the ideal combinations of biogas produced from plant based feedstock.

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