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Enhancement of Biogas Production by Cellulytic Bacteria from Bagasse Using Methanogenesis

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Abstract: Energy is essential to meet the basic needs of life, to increase amenities and modernization. The main sources of energy that are met our energy demands are mineral oil, coal, natural gas and firewood. These conventional energy sources are being depleted day by day. So renewable, alternative and effective energy sources should be explored for our country as well as whole world. The production of biogas serves as an alternative energy source. The main objective of our research work was enhancement of biogas production by cellulytic bacteria from bagasse using methanogens. Five liters capacity glass reactors were used. Five sets of batch modes anaerobic digesters were used under laboratory condition. Bagasse was used as feed materials. Bagasse is the by-product of sugar mill and it was used as raw materials for paper production in our paper mills. Now it is discarded and creates a problem of sugar mills to use and manage bagasse. The raw materials were diluted with supply water in the ratio of 1 to 9 for bagasse. The characteristics of the influent slurry in term of Total Solid (T.S)%, Volatile Solid (V.S)%, P^H and temperature ranges were determined every 7 days intervals for bagasse. The percentage of methane of biogas obtained from bagasse was 80%. The S_1 strain (Monococcus sp.) and S_3 strain (Streptococcus sp.) of cellulytic bacteria produced 3.45×10^{-3}(m^3/day/kg feedstock) biogas and 3.85×10^{-3} (m^3/day/kg feedstock) biogas at 22^{th} day respectively whereas control produced 2.85×10^{-3}(m^3/day/kg feedstock) biogas at 34^{th} day by using bagasse as feedstock. The results clearly demonstrated that the rate of biogas production was increased by S_1 strain and S_3 strain of cellulytic bacteria. The cumulative biogas production was found $54.20\times10^{-3}m^3$, $66.21\times10^{-3}m^3$ and $61.59\times10^{-3}m^3$ for control, S_1 strain and S_3 strain of cellulytic bacteria, respectively. In conclusion, results obtained from the present research work can be used to design biogas reactor in the field conditions to operate batch and semi-continuous mode for disposal management of sugar mills and thereby contribute a lot of in our fuel and fertilizer sectors.

Keywords: Biogas, Alternative Energy, Bagasse, Cellulytic Bacteria, Biogas Plant

1. Introduction

As the time being passed the population of the world is increasing gradually and the requirement of energy is also increasing. The increasing energy requirement is due to fulfill basic needs of the population of developing countries and to increase amenities and modernization of the population of the developed countries. But the main energy sources that are met energy demands are mineral oils, coal and natural gas. So it is suspected that this deposited natural energy may be depleted one day. Hence now a day a burning question has been arisen to find out a renewable alternative energy source. An anaerobic digestion process or biogas production has already been identified as an alternative energy source. The fraction of renewable energy forms for energy supply is constantly increasing since fossil fuels are running short and energy production from fossil fuels brings about emissions of the greenhouse gas carbon dioxide which has implications on the climate and environments. In this context the production of biogas by means of fermentation of biomass becomes more and more important because biogas is regarded as a clean, renewable and environmentally compatible energy

source [1, 2]. Moreover, generation of energy from biogas relies on a balanced carbon dioxide cycle. In Germany biogas is mainly produced from energy crops such as maize and liquid manure in medium-sized agricultural biogas plants [1]. The microbiology of biogas formation from organic matter is complex and involves interaction of different microorganisms. In the first step of the digestion process, organic polymers of the substrate such as cellulose, other carbohydrates, proteins and lipids are hydrolyzed to low-molecular weight compounds [3–5]. Subsequently, fermentative bacteria convert low molecular weight metabolites into volatile fatty acids, alcohols, and other compounds which are then predominantly metabolized to acetate, carbon dioxide and hydrogen by syntrophic bacteria. These latter compounds are in fact the substrates for methane synthesis which is accomplished by methanogenic bacteria. The present work deals with reviewing the pretreatment processes used in ethanol and biogas processes from waste materials [6]. Anaerobic digestion can be carried out under ambient (<25°C), mesophilic (25~45°C) and thermophilic (>45°C) conditions [7]. According to EC directives the renewable energy share in gross energy consumption should increase from 32.5% in 2005 up to 40% in 2020 for Latvia [8]. The renewable energy share in gross energy consumption was only 29.9% in 2008 in Latvia, therefore new alternative energy resources should be utilized to reach the appointed targets in the energy sector. Additional renewable energy supply can be provided by increasing of the area of intensive energy crops providing high dry matter (DM) yields [9]. Energy crops used for biogas production should provide high dry matter yield and high methane output per area unit. Energy crops should be easy to cultivate, i.e., to be tolerant to weeds, pests, diseases, drought and frost, have good winter hardiness and be able to grow with low nutrient input. For the agro ecological conditions of Latvia such energy crop can be maize, having the annual dry matter yields from 10 to 16 ton/hectare. Maize is preferable for energy production as it belongs to C4 type plants (e.g. Food crops corn, Sorghum, Sugarcane and Millet) featuring less need for plant nutrients (nitrogen, phosphorus, potassium) uptake per unit of dry matter produced compared to C3 type plants (e.g. Sunflower, Tobacco, Spinach, Soybeans and Sugar beet) [10]. The investigated photosynthetic efficiency for C4 plants was 2.0% compared to 1.4% for C3 plants [11]. The advantage of C4 crops is less water requirement for plants dry mater production, for example, maize, sorghums (*Sorghum spp*), sudangrass and others had a mean transpiration ratio of 300 kg water kg-1 dry biomass, as compared to ratios 500-900 kg water kg-1 in the C3 crops [12-13]. Energy crops are one alternative for how to diversify agricultural production and enhance the business of a farm. Biogas energy can be used to improve the energy balance of a farm itself, or the excess energy can be offered for sale (e.g. to an electricity network). Maize, which in a form of silage offers interesting yields (about 30 tons of total solids - TS per hectare [14-16], there is only a very little information on anaerobic digestion of maize silage as an only substrate. Generally, it may be said that studies focusing on anaerobic digestion of fresh or ensiled materials did not show significant differences in biogas production, which is discussed, e.g. in Zubr [17]. Conservation qualities are an advantage when using silage, i.e. it may be used year round regardless to the season. Negligible differences in biogas production from fresh or silage material also are presented in the work of Gunaseelan [17]. Most efficient utilization of maize is supply of green maize biomass directly to biogas plants for heat and power energy production. The periods of fresh raw material supply to biogas plants can be prolonged by introduction of early ripe and late ripe sorts in the crop rotations, and by different harvest time of maize. The purpose of the investigation is biogas yield obtainable in anaerobic digestion process from fresh maize biomass harvested in different plant vegetation periods [18]. The anaerobic digestion (AD) of sugarcane waste can be considered a promising strategy, since the digestate could still be used to partially replace the mineral fertilizers on the sugarcane fields and the produced biogas could be upgraded to biomethane and sold as a new energy product by the sugarcane plants [19-20].

1.1. Cellulytic Bacteria

Cellulase refers to a family of enzymes which act in concert to hydrolyze cellulose. Jaksevac *et. al*., (1982) showed that when bacteria and fungi of some cultivation conditions grown on lactose as sole source of carbon, it synthesizes the cellulytic complex which is able to degrade native cellulose [21]. Kurakake *et al*., (2007) studied the biological pretreatment of office paper with two bacterial strains, *Sphingomonas paucimobilis* and *Bacillus circulans*, for enzymatic hydrolysis. Biological pretreatment with the combined strains improved the enzymatic hydrolysis of office paper from municipal wastes [22]. The bacterial species *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* represent promising candidates for cellulase production because they are thermophilic (less contamination problem and faster rate at a high temperature), anaerobic (no oxygen transfer limitation), and ethanologenic (conversion of cellulose to ethanol via glucose with a single culture). In general, different species of microorganisms produce different cellulolytic enzymes. Agricultural and industrial wastes are among the causes of environmental pollution. Their conversion into useful products may ameliorate the problems they cause. These wastes which include cereals, straw, leaves, corncobs etc are highly underutilized. These materials are mainly used as animal feeds. Large quantities left on farmlands to be decomposed by microorganism such as bacteria and fungi [23]. Economically, the most important industrial material other than foodstuffs affected by microorganisms is cellulose and wood products including the wood itself. Production of wood products such as pulp, paper, textiles from natural fibers such as cotton flax and jutes are enhanced by microorganisms. Cellulase (a complex multienzyme system) acts collectively to hydrolyze cellulose from agricultural wastes to produce simple glucose units [24]. Cellulases are synthesized from

bacteria, including species are *Bacillus sp. Cellulomonas sp. Clostridium acetobutylicum, Clostridium thermocellium*etc.

1.2. Characterization of Cellulytic Bacteria

All the cellulase-producing bacterial strains were isolated by their degrading capabilities of filter paper strip, were characterized according to the biochemical tests described in the "Bergey's Manual of Determinative Bacteriology, Eighth edition"[25], "Text book of "C. H. Collins, and Monica Cheesbrough" [26-27]. Characteristics of cellulytic bacteria are given in Table 1. On the basis of morphological and biochemical test, it may be concluded that cellulase producing bacterial strains were identified. The S_1 strain was *monococcus* sp. but the S_3 strain was *streptococcus* sp.

1.3. Characteristics of Methanogenic Bacteria

The methanogenic bacteria are a large and diverse group that is united by three features: 1) They form large quantities of methane as the major product of their energy metabolism. 2) They are strict anaerobes. 3) They are members of the domain Archaea or archaebacteria and only distantly related to the more familiar classical bacteria or eubacteria. Like the photosynthetic eubacteria, the methanogenic bacteria are related to each other primarily by their mode of energy metabolism but are very diverse with respect to their other properties. Methanogenic bacteria obtain their energy for growth from the conversion of a limited number of substrates to methane gas. The major substrates are H2 + CO2, formate, and acetate. In addition, some other C-1 compounds such as methanol, trimethylamine, and dimethylsulfide and some alcohols such as isopropanol, isobutanol, cyclopentanol and ethanol are substrates for some methanogens. All of these substrates are converted stoichiometrically to methane [28].

2. Materials and Method

2.1. Raw Materials

The raw materials used in this research work were bagasse, inoculum (cow dung), cellulytic and methanogenic bacteria.

Bagasse was collected from Rajshahi sugar mills. Cellulytic bacteria were collected from Microbiology Laboratory Department of Biochemistry and Molecular Biology, university of Rajshahi.

2.2. Preparation of the Samples

The bagasse was collected from different location and cut into tiny pieces. These tiny pieces were mixed with water, culture of cellulytic bacteria and 10% inoculum (cow dung) of total weight and were placed in the digester bottles. The digesting system was consisted of three digesters (A, B and C) and three gas collecting bottles (X, Y and Z). Digester bottle (A) for control was connected with the gas collecting bottle (X) by a rubber pipe. Digester bottle (B) for S_1 strain of cellulytic bacteria was connected with the gas collecting bottle (Y) by a rubber pipe. Another digester bottle (C) for S_3 strain of cellulytic bacteria was connected with the gas collecting bottle (Z) by a rubber pipe. The each digester was five liters capacity respiratory glass bottle closed by a rubber bung. Two pipes, one for gas removal and another for feeding and removal of slurry were fitted through the rubber bung. The slurry feed pipe was placed through the bung in such a way that the lower end of the pipe reached near the bottom of the digester. Therefore, no air could pass into the digester through pipe. The slurry pipe was also closed by a small rubber bung. The photograph of apparatus used in this study are shown in Figure-1.

2.3. Batch Reactor

One set of anaerobic digesters was used for bagasse. The system was started as batch process and collected gas for consecutive days from starting of gas production. The daily gas production, cumulative gas production and temperature were measured daily and TS%, VS% and PH were determined once for a week carefully.

Table 1. *Characteristics of cellulytic bacteria.*

Characteristics			**S_1 strain bacteria**	**S_3 strain bacteria**
Microscopic test	Gram-staining		+	+
	Cell shape		Cocci	Cocci
	Arrangement		Single	Chain
	Motility test		+	+
Visual observation	Colony form		Circular	Circular
	Colony type		Wet	Wet
	Colony colour		Red	Red
	Growth in nutrient broth medium		Surface	Surface
	Fermentation test	Glucose	-	-
		Galactose	+	+
		Sucrose	+	+
Biochemical test	Citrate utilization test		-	-
	Indole test		+	+
	Catalase test		+	+
	Urease Test		-	-
	H_2S production test		-	-
	Methyl red test		-	+
	Voges-proskauer test		+	-

+ = Positive test, - = Negative test

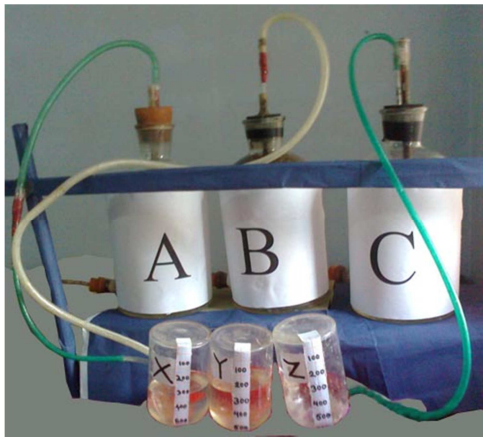

Figure 1. *Photograph of apparatus used in this study.*

2.4. Experimental Procedure

One set of experiment (Photograph) were carried out at room temperature. One was run by using bagasse and another was run by using maize straw as feed materials. All the sets were run as batch operation mode all over experimental periods. The p^H, total solid and volatile solid were measured carefully at the beginning of the experiments and during operational period, at 7 days interval. The amount of gas produced per day was measured daily from the graduation of gas collecting bottles almost at the same time.

2.5. Measurement of Methane Percentage

A 10 ml glass pipette was bent accordingly to the saccharometer of Dr. Einhorn. The sharp end of the pipette was sealed by heating. A disposable hypodermic syringe of 10 ml capacity and having 6 cm. niddle was used for removal of gas formed digester and injected it into pipette (A) containing concentrated solution of KOH. A concentrated saturated solution of KOH was prepared by dissolving KOH pellet in the distilled water. The gas sample was withdrawn from the gas collector bottle (Y) through opening two ways key. The syringe was filled with biogas and emptied it 3 to 5 times to ensure that only biogas would be sampled. Then the syringe was immediately placed in the bent pipette and the gas was injected into the solution. After waiting for 1-2 minutes the reading was taken. The percentage of CH_4 was calculated using the following formula.

1. Percentage of $CH_4 = \frac{X-Y}{X} \times 100$

Where X=Volume of biogas injected into the saccharometer in ml.

Y= Volume of CO_2 dissolved by KOH solution in ml.

Then the percentage of CO_2 was calculated by subtracting the percentage volume of CH_4 from 100 since other gas constituent present in the biogas was considered negligible. The accuracy of this procedure for determination of CO_2 ± 3% with respect to gas chromatographic analysis.

2.6. Measurement of P^H

Since the P^H has an important effect on over all the digestion process. So, the patterns of P^H change of the digester content were measured at 7 days interval with P^H paper. The measurement of P^H for bagasse, S_1 strain and S_3 strain are shown in Table 2, 3 and 4 respectively. In case of batch mode of operation a certain amount of slurry was removed from the digester through the feeding pipe and after P^H measurement the slurry was inserted back into the digester.

2.7. Measurement of Total Solid (TS)

A definite amount of slurry was weighted with a digital balance and then placed it in the oven at 105°C ± 5°C temperature about 12 hours. The reduced weight of the sample was measured and then it again introduced into the oven at the same temperature about 1 hour and weighted again. In this way the process was continued until the weight of the sample remained constant. The same procedure was followed for both the influent slurry, effluent slurry and total solid content of the sample was calculated by using the following equation.

2. Percentage of total solid (% of TS) $= \frac{A-B}{A} \times 100$

Where A = weight of sample in gm before drying.

B = weight of sample in gm after drying.

The measurement of TS for bagasse, S_1 strain and S_3 strain are shown in Table 2, 3 and 4 respectively.

2.8. Measurement of Volatile Solid (VS)

A known volume of sample was weighted, dried and measured its total solid as mention 2.7. Then the sample was placed in a furnace at 550°C to 600°C for one hour. The volatile solid content of the sample was calculated by using the following equation.

3. Percentage of volatile solid (% of VS) $= \frac{A-B}{A} \times 100$ (on dry basis)

Where A = weight of sample in gm before combustion.

B = weight of sample in gm after combustion.

The measurement of VS for bagasse, S_1 strain and S_3 strain are shown in Table 2, 3 and 4 respectively.

Table 2. *Measurement of TS, VS and P^H for bagasse.*

Day	TS (Total Solid)%	VS (Volatile Solid)%	P^H
1-18	8.2	7.02	8.1
25	8.1	6.98	7.9
32	7.8	7.8	8.0
39	7.6	7.9	7.8
46	7.3	7.6	7.7
53	7.2	7.8	7.7

Table 3. *Measurement of TS, VS and P^H for S_1 strain with bagasse.*

Day	TS (Total Solid)%	VS (Volatile Solid)%	P^H
1	8.7	7.0	8.0
7	8.3	6.5	8.2
14	8.1	7.3	8.1
21	7.9	7.1	8.0
28	7.6	7.0	7.9
35	7.6	6.9	8.0

Table 4. *Measurement of TS, VS and P^H for S_3 strain with bagasse.*

Day	TS (Total Solid)%	VS (Volatile Solid)%	P^H
1	8.2	7.2	8.1
7	8.0	7.0	8.0
14	8.1	7.3	8.2
21	7.8	7.2	8.1
28	7.9	7.4	7.9
33	7.7	7.3	7.9

3. Result and Discussion

The daily gas production (m^3/day/kg feedstock) in the digester from bagasse using S_1 strain, S_3 strain of cellulytic

bacteria and control was shown in Figure-2. It was found that the gas production was started from 4th day, 2nd day and 20th day for S_1 strain, S_3 strain of cellulytic bacteria and control respectively. Methanogenic bacteria produced 3.45×10^{-3} m^3/day/kg feedstock and 3.85×10^{-3} m^3/day/kg feedstock biogas at 22th day in the presence of S_1 strain and S_3 strain of cellulytic bacteria respectively whereas control produced 2.85×10^{-3} m^3/day/kg feedstock biogas at 34th day. The results clearly demonstrated that in presence of S_3 strain of cellulytic bacteria the rate of biogas production was maximum than in the presence of S_1 strain of cellulytic bacteria. Whereas in absence of cellulytic bacteria the production rate of biogas was significantly minimum and needed more time. The comparison of cumulative gas production (m^3) in the three sets of digesters using bagasse as feedstock in presence of S_1 and S_3 strain of cellulytic bacteria with control was represented in Figure-3. In presence of S_1 strain of cellulytic bacteria produced 66.21×10^{-3} m^3 biogas for 32 days from the starting period. The presence of S_3 strain of cellulytic bacteria produced 61.59×10^{-3} m^3 biogas for same days whereas control produced 54.20×10^{-3} m^3 biogas for 53 days.

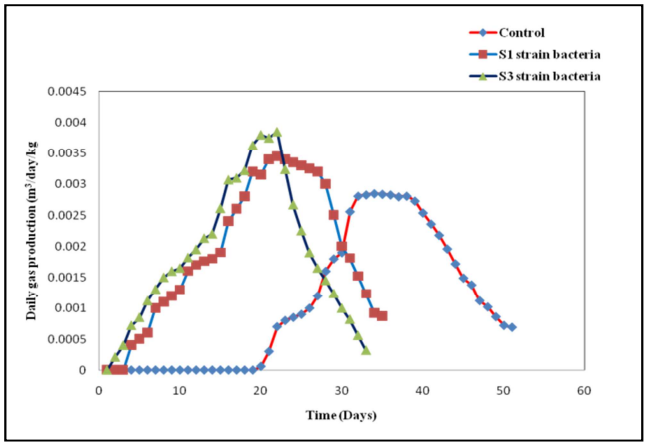

Figure 2. *Comparison of daily gas production (m^3/day/kg feedstock) versus time (days) in presence of S_1 strain and S_3 strain of cellulytic bacteria with control.*

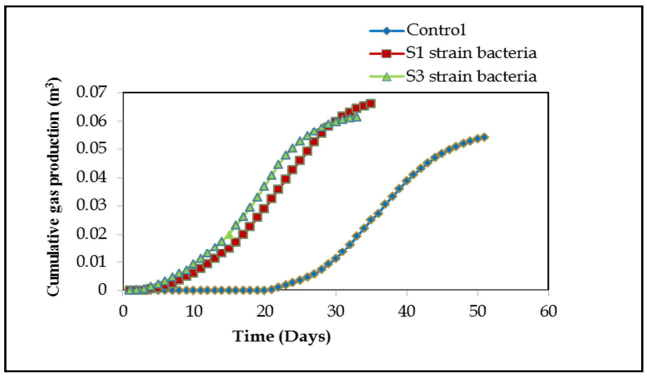

Figure 3. *Comparison of cumulative gas production (m^3) versus time (days) for control, S_1 strain of cellulytic bacteria and S_3 strain of cellulytic bacteria.*

4. Conclusion

In conclusion, the results obtained from the laboratory based present research can be used to predict and design biogas reactor in field condition. We can get higher amount of biogas from bagasse by cellulytic bacteria together conventional methanogenic bacteria within short period of time. Anaerobic treatment of bagasse for biogas and biofertilizer may be a new sustainable technology for disposal management of Sugar Mills of Bangladesh. So it will be an alternative renewable energy source of Bangladesh and thereby our people and country will be benefited economically and environmentally.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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