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Production of Biogas from an Agro-industrial Waste and its Characteristics

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Abstract

Molasses is a significant by-product of sugar industry and can be used as substrate in anaerobic digestion process for biogas production. Molasses was diluted ten time; inoculated by methane producing bacteria, mixed thoroughly in 2 liter batch bioreactor, kept at 37^0C for 15 days under anaerobic conditions for biogas yield. pH in the process was monitored three times a day. Total solids, volatile solids and COD were measured at alternate days. The gas production was measured by water displacement method. Ten times diluted molasses under anaerobic conditions, in the presence of methane producing bacteria was converted to 6.55 dm^3/kg of biogas or 3.93 dm^3/kg CH_4 and 0.144 kWh electricity.

Keywords: Agro industrial waste; Molasses; Methanogen; Anaerobic digestion; Biogas production.

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1. Introduction

Biogas is the mixture of gases, mainly methane, CH_4, and carbon dioxide, resulting from the anaerobic digestion of organic matter. Biogas contains 50-70% methane that can be used as a fuel for heating or electrical power generation. Production of biogas from biomass offers several environmental benefits including production of renewable energy and the nutrient rich stabilized liquor containing NH_4^+ that is directly available for plants. Biogas production has the advantage when compared to other biomass energies, that it can be produced from specially grown energy crops as well as from organic waste products. Biogas production units provide a decentralized fuel supply and waste management system both of which are becoming increasingly attractive particularly in rural areas of developing countries. The European Union is striving for more renewable energy sources, in efforts to decrease the use of fossil fuels and meet the Kyoto Protocol [1]. In 2005 bio-energy represented 78% of all renewable energy produced, making it by far the most important renewable energy source used to date. On average, bio energy accounts for 33% of energy use in developing countries, with large variations between different regions:

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60% in Africa, 34% in Asia and 25% in Latin America. Anaerobic digestion process has long been applied to the treatment of domestic and industrial wastes/ wastewater but these days, the process has been frequently employed to produce renewable bioenergy [2]. Anaerobic digestions can breakdown waste to produce a useful by-product of biogas. Further, anaerobic digestion process assisted to reduce and destroy the pathogens to acceptable levels and also help to reduce odors often associated with storing and handling of liquid wastes. The used technology can help to reduce the emission of greenhouse gases by replacement of fossil fuels, reduce methane emission from landfills and displace industrially produced chemical fertilizers [3]. The process reserves nutrient and structure of biomass, opposite to incineration because anaerobic digestion requires less energy, highly efficient in utilizing all types of organic materials, relatively simple and straight forward to implement [4] .

Numerous studies were carried out on the application and benefit of this process to produce renewable bio-energy from different energy crops [5] like industrial wastes, manure and molasses [6]. Anaerobic Digestion is a suitable method for treatment of wastes to yield biogas [7]. In anaerobic system, majority of the chemical energy contained within the starting material is released as methane by methanogen [8]. Many developing countries like India, Nepal and many African countries are using biogas energy especially for stoves and lighting in rural areas. In industrialized countries, bio energy makes a lower but still a significant contribution in the range of 3–4% of energy use [9]. The effect of total solids concentration on biogas production under anaerobic conditions was also studied [10]. The effect of bio-stimulant combined residues of cattle dung and kitchen waste (15%). on biogas yield was studied by Carrucci *et al.* [11].

Sugarcane is an important industrial crop as raw material to 84 sugar mills in Pakistan. Pakistan has the 5th largest sugarcane growing area (around a million hectares) in the world and 15th biggest global producer of sugar. The annual production of molasses is more than 4.6 million metric ton per annum [12]. Molasses is the mother liquor left out from sugar industry after sugar crystallization. It is commercially available, cost effective and contains high concentration of sugars as well as other nutrients [13]**.** The disposal of this excessive agricultural waste needs a simple and viable eco-friendly solution and can be used as substrate for biogas production in anaerobic digestion process. In the presence of specialized methanogens, the intermediates are converted to the 'final' end products of methane, carbon dioxide, and trace levels of hydrogen sulfide .

The objective of the study was to comprehensively examine the potentiality of molasses as a sole carbon source for batch anaerobic digestion process aimed at the production of methane. In this study, TS, VS and COD were optimized in term of percentage removal efficiency.

2. Material and Methods

All feedstock and molasses used in this study were obtained at once, diluted freshly before the initiation of each experiment and kept at 4^{0}C. All the parameters including pH, COD, TS and VS were determined each day by withdrawing 20 ml of digestion mixture from the reaction flask.

Fifteen batch experiments (Fig. 1) were carried out with different dilutions. The pH in the bioreactor was adjusted in between 6.9–7.5 with saturated solution of sodium hydroxide. Every day the gas pressure was measured by water displacement method. Out of eleven batches, the last three were optimized under best conditions for biogas yield and confirmed by operating four more batches under same conditions presented in Table 3. The average result was found to be 6.55 $dm^{3/}$kg of substrate as a biogas yield. In order to maintain the process stability in bioreactor, an occasional and very slow mixing in bioreactor was performed that was beneficial to homogenize the feed stock with inoculum, to avoid temperature gradients, for no scum formation and to prevent the formation of dead spaces that reduced the effective volume in bioreactor.

Fig. 1. Working model of bioreactor for biogas production.

2.1. *Substrate (molasses)*

In order to study the biogas potential, twenty liter pure cane molasses generated from Pattoki Sugar Mills Private Limited during sugar manufacturing process was obtained and used for biogas production.

2.2. *Physical and chemical characterization*

Pure molasses was characterized for physical and chemical parameters (Table 1). The pure molasses was diluted with chlorine free distilled water and used as substrate in the said experiment. The nutrients in substrate were determined according to APHA [14].

Substrate composition is a major factor to determine the methane production rates from the digestion of biomass.

Table 1. Characteristics of ten times diluted molasses as substrate.

Parameters	Results*	Units
pH at 25 ^{0}C	5.90	-
COD	67,860	mg / L
Brix	8.4	degree
Total sugar	4.85	%
Total solids	6.71	%
Volatile solids (as such basis)	5.35	%
Organic carbon	2.69	%
Nitrogen	0.09	%
C/N ratio	29.89	-

*Results are average of four values.

2.3. ***Preparation of inoculum***

The substrate was inoculated to make the process more efficient, effective, to enhance the biogas yield and to reduce the amount of waste at the end because the population of anaerobic microorganisms take a significant period of time to establish themselves to be fully effective [15]. Therefore, the common practice is to introduce anaerobic microorganisms from materials with existing populations, a process known as "seeding" including with different types of agro-industrial, sewage sludge and animal wastes (manure) or cattle slurry used for inoculation. Animal waste (manure) provides nutrients, trace metals and vitamins necessary for the growth of microorganisms with respect to gas formation. The use of manure is a valuable source of affordable, sustainable and renewable bio-energy. Manure also neutralized the pH and improved the buffering capacity in bioreactor [16]**.**

To prepare inoculum, all the ingredients (Table 2) for methanogens specific media were weighed and dissolved in one liter distilled water. The pH of media was adjusted at neutral and autoclaved. Fresh cow dung (10g) was added to sterilized media as source of methanogens. The media was used for stimulation, viability, growth and rapid multiplication of pre-existing methanogens in cow dung. The mixture of cow dung plus nutrients was incubated anaerobically at 37^{0}C and is used as inoculum in the substrate for biogas production.

Methanogens have unique ability to convert organic matter to methane gas. Each species is quite restrictive with regard to organic acid and alcohol they use as a carbon source. Oxidation of substrate by single species is incomplete and no single bacterium is able to produce the products alone. In order to check the growth profile and viability of methanogens, bacterial count test was conducted. In this study, 10% inoculums size was optimized for the production of biogas from substrate [14].

Table 2. Composition of specific synthetic culture media for methanogens [17].

Substance	Composition g / L	Substance	Composition g / L	Substance	Composition g / L
Yeast extract	2.0	Copper sulphate ($CuSO_4.5H_2O$)	0.001	Sodium molybdate ($Na_2MoO_4.2H_2O$)	0.001
Peptone	2.0	Nickle chloride ($NiCl_2.6H_2O$)	0.001	Sodium tungstate ($Na_2WO_4.2H_2O$)	0.001
Ammonium chloride (NH_4Cl)	1.0	Sodium sillicate (Na_2SiO_3)	0.001	Zinc sulphate ($ZnSO_4.7H_2O$)	0.001
Magnesium sulphate ($MgSO_4.7H_20$)	0.4	Nitrilo tiacetic Acid	0.015	Aluminium chloride ($AlCl_3.6H_2O$)	0.001
Calcium chloride ($CaCl_2.2H_2O$)	0.4	Potassium iodide (KI)	0.01	Ferrous chloride ($FeCl_2.4H_2O$)	0.001
Ferrous ammonium Sulphate $Fe(NH_4)_2(SO_4)_2.6H_2O$	0.002	Sodium bicarbonate ($NaHCO_3$)	5.0	Sodium acetate (CH_3COONa)	1.36
Cobalt chloride ($CoCl_2.6H_2O$)	0.001	Potassium chloride (KCl)	0.04	Cysteine / boric acid	0.01
Manganese chloride ($Mn Cl_2.4 H_2O$)	0.001	Diammonium biphosphate $(NH_4)_2HPO_4$	0.4	Sodium sulphide ($Na_2S. 9H_2O$)	0.3

Note: Dissolved each component separately in water and mix them all to make one liter. Culture media employed for enumeration, isolation and enrichment of methane producing bacteria.

3. Results and Discussion

After characterization of the provided molasses sample, it was diluted with chlorine free water for efficient working of microorganism to be converted into biogas. All the experiments were conducted with different dilutions but reported results were based of 10 times diluted molasses. It is a batch study and whole experiment was performed in a closed anaerobic bioreactor on lab scale. It was observed that if the dilution was > 13 times, the total solid concentration reduced and the solid particles settled down into the bioreactor that made the reaction slow. If the dilution was < 4 times, thick slurry was formed and the particles in slurry impedes the flow of gas formed at the lower part of bioreactor resulted slow release of biogas [18]. The present study showed biogas production from ten times diluted molasses containing total sugar (4.82%), total solids (11.24%), volatile solids (9.88%), carbon content (2.69%), nitrogen contents (2.7%) with C/N ratio 30 (Table 3).

Table 3. Operational and performance data for biogas production from molasses-an industrial agro waste.

Batch Volume	2000 ml					
C/N	30					
Batch composition	Molasses (10 times diluted) 1800 ml and inoculum 200 ml.					
	Parameters					
Days	pH (observed)	pH (adjusted)	T.S (%)	V.S (%)	COD (%)	Water displaced (L)
1	5.94	7.51	11.24	9.88	20.54	-
2	5.15	7.45				1.66
3	4.87	7.41	10.74	9.32	17.42	1.61
4	5.63	6.95				1.58
5	5.91	7.39	10.15	8.63	14.51	1.52
6	5.71	6.98				1.45
7	5.72	7.35	9.47	8.23	11.79	1.39
8	5.69	7.41				1.33
9	5.92	7.35	9.02	7.67	9.67	1.27
10	5.99	6.88				0.65
11	6.33	7.11	8.53	7.37	8.15	0.44
12	6.58	6.99				0.16
13	6.82	7.22	8.15	6.99	7.04	0.03
14	6.91	7.35				0.01
15	6.89	7.25	7.74	6.62	5.42	0.00
Total water displacement						13.1
Biogas yield						13.1 dm^3

- Each value is result of four replicates.
- The reduction in total solids was 11.24% to 7.74%, volatile solids 9.88% to 6.62% and COD 20.54% to 5.42% within fifteen days.
- The initial pH in bioreactor was 5.94, dropped to 4.87 on third day and last day it was 6.89.
- The batch study was continued for 15 days where the water displacement was 13.1 liter after fourteen days and on last day, the water displacement was zero.
- The biogas yield of one batch was 13.1 dm^3.

A number of factors such as temperature, pH, C/N ratio, total solids and volatile solids and COD affect the metabolic activity involved in anaerobic digestion for biogas production and the effect of each factor was observed during operation and performance of process (Table 3).

3.1. *Effect of temperature*

During study, it was observed that methane producing bacteria are extremely sensitive to temperature changes, even 1–2°C temperature change significantly reduced the biogas production [19]. The most efficient temperature range for working of mesophilic bacteria without energy input was 30–37°C. The stability and growth of bacteria in bioreactor at mesophilic conditions made the process more balanced, and resistant to chemicals, so the optimum temperature for biogas production was kept at 37°C throughout the study period [20].

3.2. *Retention time*

All experiments were performed in a closed anaerobic bioreactor so the retention time was considered as the total time in days from start up to the end of the reaction because in a batch process. In a continuous batch process, the mean retention time is approximated by dividing the bioreactor volume to daily input depending upon the vessel geometry and means of mixing. Therefore a continuous process can only accurately defined the retention time.

3.3. *Effect of pH*

The pH was adjusted in the range of 6.9-7.5 by the addition of saturated solution of sodium hydroxide one times a day without disturbing the digestion process by injecting alkaline solution with a medical syringe of 10ml through airtight inlet/outlet valve as shown in Fig. 1. It is because methane producing bacteria live at best under neutral or slightly alkaline conditions. The variation in pH over the period of digestion was 4.7 – 7.0 as shown in Fig. 2. There was an initial decrease in pH which reached minimum values

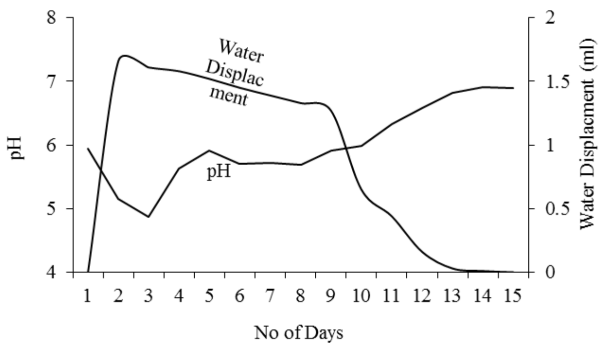

Fig. 2. Variation of pH with time and production of gas during the period of study.

after 48 hours and generally increased over the remaining period of digestion. In the present study, initially the pH of the molasses was found to be 5.9 and adjusted to 7.0 by saturated solution of sodium hydroxide to enhance the biodegradation of organic matter and the practice was made to maintain the pH between 6.0 to 7.0 The observed decrease in pH was probably due to increase of un-dissociated volatile fatty acids (VFA) in an anaerobic digestion process [21]. The activity of methanogenic bacteria becomes very slow below pH 6.3 and above pH 7.8 [22] as the lower pH results in growth of filamentous bacteria and a higher pH results in buildup of unionized ammonia [23]. It is noted that pH of the medium tends to drop up to 3rd day, then began to rise gradually as the experiment proceeds up to 6th day, stabilized from 6th to 8th day and again going to rise and stabilized on 13th day. Maximum biogas production was observed in the pH range between 6.0 to 7.5, A pH > 8.5 will start showing toxic effect on methanogenic population [24].

3.4. *Biogas potential of molasses*

Biogas yield against displacement of water was noted with respect to pH and shown in Fig. 2. The displacment of water in mL was direclty related to the production of biogas adjusted at 25 ºC and 1 atm pressure. The water displacement was recorded to increas on 2nd days and increased upto 4-5 days. It gradually started to decrease on 6th day, decreased more on 7th upto 9th day and stoped after13 th day.Same trend was observed in another study presented by [25], where the biogas increase on 1st and 2nd day and decrease on 3rd day but the reduction was more or less costant in our experiment contrary to [20] which increase on 4th day and gradually increase upto 7th day and after that going to decrease gradually.

3.5. *Effect of chemical parameters on biogas production*

The efficiency of anaerobic digestion was evaluated in terms of total solids (TS), volatile solids (VS) and COD reduction throughout the study. At the beginning of anaerobic digestion process the decompostion of reactant was low so the values of COD, TS and VS perecntage reduction was also low. As the process became fast, the water displacment increased indirectly showed the gas formation and the values of TS, VS and COD decreased.

Total solids concentration in bioreactor, started to decrease slowly on 2nd day and reduced at value not more than 2% on last day, although it decreased across the whole process but stability in results was observed in Fig. 3. The effect of time on total solids was studied in concentration range between 3.9 % – 13.26%. Total solids less than 13% produced more gas and enabled the bacteria to more readily access the substances on which they are feeding resulted to increases the rate of gas production.

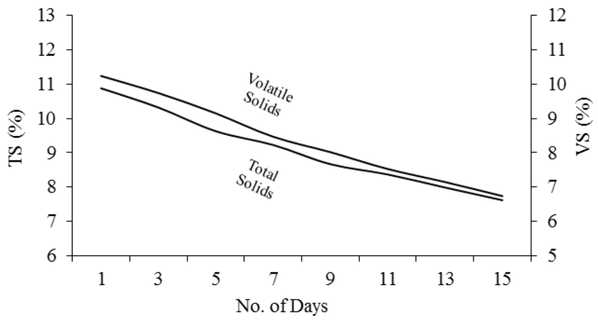

Fig. 3. Effect of time on total solids and volatile solids during anaerobic digestion of molasses.

The lowest concentration of volatile fatty acids (VFA) also did not correspond to the highest methane production. While data was difficult to interpret with respect to the total solids (TS) and volatile solids (VS), it was found that a higher ratio of TS/VS matched a higher value of methane production.

The volatile fatty acids (VFA) produced by acidogeic bacteria are used as a part of an intermediat step for the metabolic activity of methane producing bacteria. If the VFA's are not utilized at the rate they are produced, then it can kill the methanogenic activity due to lower pH that is why it is necessary to monitor and adjust the pH during the whole process. In present study, The effect of time on volatile solids was observed and found a similar decreasing trend like total solids as shown in Fig. 3 and the results are supported by the study of Cortsen *et al.* [26]. It was observed that higher the volatile solid contents in a unit volume fresh dung higher the gas production.

At initial stage of the biogas production process, due to intense mineralization of the reactants a considerable decrease in COD occured. By the end when microoganism does not exibit a living behaviour and the process stoped, the COD perenctages decreased. COD was measuremed on alternate days and the effect of time on COD was observed like in Fig. 4.

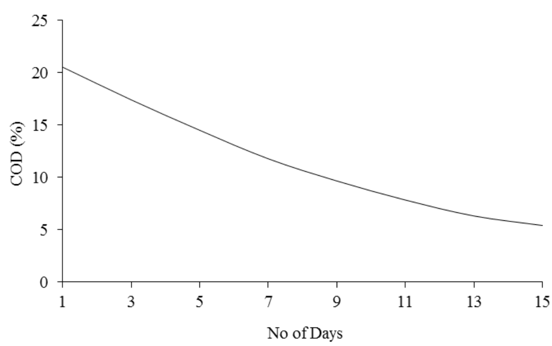

Fig. 4. Effect of time on COD during anaerobic digestion of molasses.

In a single-stage digestion system (one-stage), all of the biological reactions occur within a single, sealed reactor which reduced construction costs, but resulted in less control of the biological reactions of the different species in a single-stage reactor that can be in direct competition with each other.

Biogas production from molasses is an economically feasible process on lab scale as well as commercial scale. Difference between input and output cost is given in the Table 4.

Table 4. Cost approximation of input and output material in the present study.

Materials	Cost (Rs/L batch)
Input	
Molasses	0.63
Inoculum	0.001
NaOH	0.22
Total cost of input (Rs/L batch size)	0.851
Output	
Methane (as electrical energy)	0.399
Carbon dioxide	0.458
Total cost of output (Rs/L batch size)	0.857

4. Conclusion

The production of biogas from sugar cane molasses is an economically feasible process. In our study, biomass (10 times diluted molasses) produced about 6.55 m^3 biogas per ton of biomass at optimum conditions of total solids 11.24%, volatile solids 9.88%, C/N ratio 30.0 at pH 6.0–7.5 and temperature 37^0C. The quantity of biogas is equivalent to 0.144 kWh electricity as a source of electrical energy.

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