Biogas Production from Cow Dung By Using Methanogen Bacteria

Sundarassu, Sumithra, P* and Benila smily, J. M

Department of Microbiology, Srimad Andavan college of Arts and Science

Thiruchirappalli-620 005, Tamil Nadu, India

E.mail:benila.jm@gmail.com

*Corresponding author

ABSTRACT

Rapid urbanisation and depletion of fossil fuels lead us to search for an alternative means of fuel production from renewable resources. Substituting fossil fuel through biofuel has become crucial for constant or green energy development. Since being produced from renewable resources, biofuel is better for the environment with lesser emissions compared to petroleum fuel. In the present study, a new, eco-friendly approach for biogas production from food waste was attempted. For the purpose, the methanogenic bacteria isolated from cow dung was used as inoculum for the production of biogas. The slurry was further characterized for the presence of bacteria and the isolated bacteria were characterized biochemically. The gas chromatography was used to quantitate the amount of biogas produced. The results of the present study enabled in identifying a new, eco-friendly and cost-effective means of producing biogas from food waste, using methanogenic bacteria isolated from cow dung.

KEYWORDS: Biogas, Food waste, Cow dung, Gas chromatography

INTRODUCTION

The increasing demand for petroleum and coal around the world leads to the research to find renewable energy resources including thermal, solar, wind energy and biogas. Of them all, biogas is found to be distinct from others in terms of their characteristics. Biogas does not requires any advanced technology or instrumentations for producing energy. Biogas can be defined as the mixture of methane, carbon dioxide and trace gases, which can be produced by the degradation of organic substances using microbes under anaerobic environment (Insam et al 2010). The composition of these gases varies depending on the substrate as well as the microbes and process conditions (Al Seadi et al 2008). Anaerobic digestion of organic matter comprised of four stages including: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Insam et al 2010; Weiland, 2010).

Kitchen waste possesses high calorific and nutritive value to microbes, which can be used as substrate for biogas production. In most of the cases, kitchen wastes from hostels, hotels, parties, etc. were disposed into landfills which may cause health hazards by promoting rats, mosquitoes and other disease bearing vectors (Suyog, 2011). The inadequate management of wastes not only pollutes the surface and also underground water through leachate. In addition, they also release unpleasant odour and greenhouse gases contributing to global warming. Methane is one of the gases which can be used as biofuel by employing proper anaerobic digestion and harvesting techniques (Luna et al., 2011).

In the biogas production, the role of methanogenic and acid producing bacteria acts in a symbiotic way. Acid producing bacteria creates anaerobic environment which favored the methanogenic bacteria for their growth and methanogenesis process. The methanogenic bacteria in turn utilize the toxic intermediates that inhibit the growth of acid producing bacteria (Plugge et al., 2010; St-Pierre and Wright, 2013). Hence, the knowledge of these bacteria and their association is essential for the successful biogas production. Based on these backdrops, the present study aimed at the utilization of food waste for the biogas production. The isolation and identification of bacteria responsible for the biogas production was also assessed.

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

MATERIALS AND METHDS

Collection and processing of food waste

The solid food wastes containing mixture of cooked rice and vegetable waste were collected from canteens of Srimad Andavan Arts and Science College, Tiruchirapalli. Everyday approximately 75 kg of food waste was accumulating in the college canteen. The collected wastes were grounded using mixer grinder and feed into digester in the form of slurry. A part of the sample was withdrawn for physicochemical and microbial analysis.

Inoculum Preparation

About 3 kg of one day old cow dung was collected in a plastic can and diluted with 3 L of water and homogenized to make dilution factor of 1:1. The resultant inoculum was fed into digester and the inlet chamber was closed and kept airtight.

Biogas production

Biogas production was carried out in 20 litre water container which was used as digester. Different concentration and combination of food waste and cow dung were used for biogas production: R1A0 (50% cow dung alone), R1A1 (40% Cow dung; 10% food waste), R1A2 (30% Cow dung; 20% food waste), R1A3 (20% Cow dung; 30% food waste), R1A4 (10% Cow dung; 40% food waste). After incubation, syringe method was used for the measurement of amount of methane and carbon dioxide produced. pH and Total solid concentration were also measured further (Suyog, 2011).

Isolation of methanogenic bacteria from cow dung

In order to identify the methanogenic bacteria responsible for the biogas production, bacteria present in it was isolated from cow dung using enriched medium. For the purpose, the cow dung sample was serially diluted and plated on enriched medium consisting (g/L): Sodium benzoate 0.2, Ammonium chloride 0.075, Di-potassium hydrogen phosphate 0.04, Magnesium chloride 0.01, Resazurin 0.0001. After inoculation, the plates were inoculated for 24 h and the bacteria were sub-cultured into pure cultures. The bacterial samples

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

were biochemically characterized (Mohammad Dadook et al., 2014).

Isolation of bacteria from the slurry

The presence of different bacteria in the food waste may attribute to either positive or negative regulation of the biogas production. Hence, the microorganism present in the slurry was also isolated. The slurry sample was serially diluted and plated on nutrient agar medium. After inoculation, the plates were inoculated for 24 h and the bacteria were sub-cultured into pure cultures. The bacterial samples were biochemically characterized (Mohammad Dadook et al., 2014).

Gas Chromatography

Gas Chromatography was used to detect the components based on the selective affinity of components towards the adsorbent materials. The biogas sample is introduced with the help of GC syringe into the injection port, it gets vaporized at injection port then passes through column using continuous flowing carrier stream (mobile phase). The peaks were visualized with the help of computer system in the form of peaks.

RESULTS AND DISCUSSION

Biogas serves as a low cost fuel replacement for the existing fossil fuels. The biogas comprised different gases such as methane (50%), CO_2 (30), nitrogen (5%) and trace amount of some volatile gases and water vapour. The accumulation of municipal solid waste causes various problems such as land, water and air pollution which can be resolved by microbial utilization of food waste contain rich amount of organic residues for their conversion into biogas and organic manure in the usable form. The food waste was degraded by anaerobic bacteria from 20th day of inoculation by the production of gas.

The production of biogas was observed maximum (210 mg/ml) in R1A4 group when compared with other groups R1A0 (161.25%) and R1A2 (143.75%). The results revealed that the maximum amount of kitchen waste produce more gas than cow dung slurry. In all the 4 groups, the gas produced burned with blue flame. The

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

amount of methane produced was depending upon the feed and time intervals of digestion process. The variation of gas production started at the first day of ignition to the end of the experiment (45 days) depending upon the anaerobic digestion process (Table 1).

From the results it is observed that the biogas production is time dependent and takes a few days for its incubation. The changes in temperature and PH have not much impact on biogas production. It is found that the temperature 24-25°C are favorable for the reaction to continue. The generation of bio progresses fast and attains a maximum at 25°C on the 22nd day and thereafter it slows down progressively. This is evident because of the fact that the generation of methanogenesis bacteria is progressively retarded with the prevalence of acid genesis bacteria because of absence of fresh feed from outside. Therefore, to continue the process, continuous feed supply is necessary (Geo Joy *et al*., 2014**)**

The physico chemical parameter such as pH and temperature changes was recorded and displayed in Table 2. The pH was reduced during the process of biogas production, due to the conversion of the waste material into fatty acid by the bacteria. The methanogenic bacteria utilize the fatty acid slowly, so the reaction was very slow when compared with other step, also the pH was reduced slowly. In group R1A3, which contain large amount of kitchen waste, pH reduced rapidly as the fatty acid conversion and utilization rate was high in methanogenic bacteria. The percentage of protein and carbohydrate level was also decreased in the anaerobic digestion process, because the gas producing bacteria utilize maximum amount of carbohydrate and protein as a nutrients (Minde et al., 2013).

From our study it is evident that food waste can become a good feedstock for the biogas production. The food waste contains more biodegradable solids (9.3%), with higher volatile solids (94.9%) than cow dung. There is an increase in moisture content of the digestate up to 3.56%. 12% decrease in volatile solids was observed after 60 days. Since food waste contains 93.7% volatile solids, it thus has a great potential of biogas production and can be

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

used as potential raw material for biogas production (Somashekar *et al*., 2014) .

The result of the microbial total viable count revealed the progression of the microbes that converted the wastes to biogas. At the time of gas production the number of microbes has increased under complete anaerobic process. Further, due to the stabilization of the conditions inside the digester the number of microbes reached to maximum which can be seen in the results. At the same time gas production was highest and flame was strong with blue color (Godfrey, 2004). Sewage water sample inoculated into enriched medium showed lemon yellow colonies. Gram staining of acetogenic bacteria showed gram positive, oval shaped, short rods and appeared in purple color and methanogenic bacteria showed gram negative, spiral shaped and pink coloured colonies. Biochemical test revealed that both the micro-organisms were positive for methyl red, citrate and triple sugar ion tests and negative for catalase, lipid and starch hydrolysis. Differences were found in indole test where acetogenic bacterium is negative and methanogenic bacterium is positive .The morphological and biochemical parameters revealed that acetogenic bacterium was *Acetobacterium woodii* and the methanogenic bacterium was *Methanospirillum hungatei*.

Non methanogenic bacteria was also isolated from effluent of digester and were identified to belong to the species of the genera *Pseudomonas, Staphylococcus, Streptococcus* and *E.coli.* The similar results were observed by Deshmukh (2014) clearly showed that biogas can be produced from cow dung through fermentation using fresh cow rumen as source of methanogens. The result also revealed the applicability of the locally made bio-digesters for the biogas production. The remaining slurry in the bio-digester after biogas production was also found to be enriched with compost which can be used as biofertilizer to improve agricultural soil nutrient and productivity (Deshmukh, 2014). Studies have also found that biogas can also be produced from plant wastes as a substitute for fossil fuels. The process also creates an excellent residue that retains the fertilizer value of the original waste products. The increasing cost of

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

conventional fuel in urban areas necessitates the exploration of other energy sources. Moreover, the search for alternative energy sources such as biogas should be intensified so that ecological disasters like deforestation, desertification, and erosion can be arrested in our rural areas (Nijaguna, 2006). The performance of the plastic synthetic bio-digester employed in this study was found to be very satisfactory in the provision of clean fuel and good quality fertilizer.

CONCLUSION

In the present study, anaerobic digestion of food waste was achieved from different types and composition of food waste resulting in varying degrees of methane yields. Biogas is produced by bacteria through the biodegradation of organic material under anaerobic conditions. Biogas production is so many advantages in transformation of organic food waste into high quality fertilizer improve the hygienic condition through the reduction of pathogen and protect the environment control the waste through waste management system of anaerobic digestion process. The process also gives job opportunity for most of the rural people in waste management system.

REFERENCES

1. Al Seadi, T., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., Janssen, R., 2008. Biogas Handbook. University of Southern Denmark, Esbjerg, 125pp.

2. Dadook, M., Mehrabian, S., Salehi, M., & Irian, S. (2014). Morphological, Biochemical and Molecular Characterization of Twelve Nitrogen-Fixing Bacteria and Their Response to Various Zinc Concentration. *Jundishapur Journal of Microbiology*, *7*(4), 1-5.

3. Deshmukh HV. production of biogas from agro-industrial waste and *Typha angustata* weed and its eco-friendly Disposal. International Journal of Biology, Pharmacy and Allied Sciences 2014, 3(4): 593-600.

4. Geo Joy1 , Juhina Das2 , Pranab Das. Generation of Bio Gas From Food Waste Using An Anaerobic

Reactor Under Laboratory Conditions. International Journal Of Scientific Research And Education 2014, 2(7): 1453-1465

5. Insam, H., Franke-Whittle, I., Goberna, M., 2010. Microbes in Aerobic and Anaerobic Waste Treatment. In: H. Insam, I. Franke-Whittle, M. Goberna (Editors), Microbes at Work, From Waste to Resources. Springer-Verlag, Berlin, 1–34.
6. Luna del Risco, M.A., Normak, A., Orupõld, K., 2011. Biochemical methane potential of different organic wastes and energy crops from Estonia. Agronomy Research 9 (1-2), 331– 342.
7. Luna del Risco, M.A., Normak, A., Orupõld, K., 2011. Biochemical methane potential of different organic wastes and energy crops from Estonia. Agronomy Research 9 (1-2), 331– 342.
8. Minde, G. P., Magdum, S. S. and Kalyanraman, V. (2013). Biogas as a sustainable alternative for current energy need of India. Journal of Sustainable Energy & Environment, 4, 121-132.
9. Nijaguna B. T. (2006). Biogas technology. New Delhi: New Age International Pvt Ltd.
10. Plugge, C.M., van Lier, J.B., Stams, A.J.M., 2010. Syntrophic Communities in Methane Formation from High Strength Wastewaters. In: H. Insam, I. Franke-Whittle, M. Goberna (Editors), Microbes at Work, From Waste to Resources. Springer-Verlag, Berlin, 59–77.
11. Plugge, C.M., van Lier, J.B., Stams, A.J.M., 2010. Syntrophic Communities in Methane Formation from High Strength Wastewaters. In: H. Insam, I. Franke-Whittle, M. Goberna (Editors), Microbes at Work, From Waste to Resources. Springer-Verlag, Berlin, 59–77.
12. Somashekar, R. K., Verma, R. and Naik, M. A. (2014). Potential of biogas production from food waste

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

in a uniquely designed reactor under lab conditions. International Journal of Geology, Agriculture and Environmental Sciences, 2(2), 1-7.

13. St-Pierre, B., Wright, A.-D.G., 2013. Metagenomic analysis of methanogen populations in three full-scale mesophilic anaerobic manure digesters operated on dairy farms in Vermont, USA. Bioresource Technology 138, 277–284.

14. St-Pierre, B., Wright, A.-D.G., 2013. Metagenomic analysis of methanogen populations in three full-scale mesophilic anaerobic manure digesters operated on dairy farms in Vermont, USA. Bioresource Technology 138, 277–284.

15. Suyog Vij, 2011. Biogas production from kitchen waste. A Seminar Report submitted to National Institute of Technology, Rourkela.

16. Weiland, P., 2010. Biogas production: current state and perspectives. Applied Microbiology and Biotechnology 85, 849–860.

Table 1: Biogas production monitored by Syringe method

Group	Day 1	15th day	30th day	45th day
R1A0	10	35	40	20
R1A1	50	120	150	80
R1A2	65	140	165	110
R1A3	80	170	190	135
R1A3	95	180	210	160

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 07
June 2017

Table 2: Changes in pH Temperature and total solid percentage during biogas production

	R1A0			R1A1			R1A2			R1A3			R1A4		
	pH	**T (°C)**	**TS (%)**	**pH**	**T (°C)**	**TS (%)**	**pH**	**T (°C)**	**TS (%)**	**pH**	**T (°C)**	**TS (%)**	**pH**	**T (°C)**	**TS (%)**
Day 1	7.25	37	8	7.2	37	6	7.25	37	8	7.2	37	9	7.35	37	7.6
Day 15	6.7	40	7.6	5.8	42	5.4	6.6	43	7.5	6.6	40	7.3	7.25	40	7.4
Day 30	6.85	45	7.6	6.45	45	5.4	6.9	47	7.5	6.75	45	7.5	7.0	47	7.4
Day 45	6.65	50	7	4.92	48	4.7	6.5	49	7	6.4	48	7.1	6.9	50	7.2

T – Temperature

TS – Total Solids