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Role of microbial Inoculum on sustainable biomethanation from Urban Waste based laboratory scale biogas digesters

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Abstract: Sustainable biomethanation of urban waste is the need of the hour as the anaerobic technology can lead to zero carbon accumulation. The anaerobic digestion is a complete process with safe manure for organic farming and energy generation in terms of biogas and finally disposal of municipal waste and its alternative to landfill and prevention of environmental air pollution. In the present study a laboratory scale digester was initiated with urban waste as substrate and inoculated with microbes collected from diverse environment as landfill, waste treatments plants like municipal waste treatment plant, fruit waste biogas digesters, kitchen waste based digesters, dairy waste digesters and regular cow dung based biogas digesters. The load of methanogen varied among digesters and was found to directly influence biogas production.

Keywords: *methanogens, biogas, urban waste, eubacteria, landfill, inoculum.*

INTRODUCTION

Microbial population in biogas digesters plays a predominant role in deciding the overall conversion of organic matter into bio fuel. The rate at which conversion of organic matter takes place is referred as hydraulic retention time (HRT) and it is a critical aspect in continuous digestion of organic matter as in case of anaerobic digestion of sewage sludge in waste water treatment plant. The HRT is interrelated to the nature of substrate, loading rate and the microbial diversity in the digesters at any given point of time of digestion. The substrate quality should be uniformly maintained during the entire course of digestion especially the concentration of carbohydrates, proteins and fats as these three principle substrates are said to have overall influence on the digester performance. The microbial capability lies in the uniformity of the substrate composition and a balanced content would lead to better digestion and consistent biogas production. Microbial load plays a significant role in case of urban/municipal waste as it is uncharacteristic heterogeneous waste from diverse source of inconsistent quality[1,2]. It requires robust and specially developed microbial inoculums for biogas production from urban waste which is special in terms of complex and substrate heterogeneity[3,4]. The fate of the digesters is directly related to ration of eubacterial and archaeabacterial load which in turn depends on operational parameters like pH, temperature, total and volatile acidity, concentration of ammonia, presence of inhibitors etc[5,6]. The microbial population of the biogas digesters are said to be most diverse and it is highly impossible to completely characterize the entire population of *Archaeabacteria* and *Eubacteria* but it has been noted that the ratio of microbial population holds key to sustainable biogas

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production. Although molecular techniques like polymerase chain reaction (PCR), Fluorescence In-Situ Hybridization (FISH), Denaturant Gradient Gel Electrophoresis (DGGE) are preferred over cultural methods for analysis of microbial population of biogas digesters still laboratory cultivation of total anaerobes could give a glimpse of diversity and load of total methanogens, obligate anaerobic hydrolytic bacteria, fermentative, acidogenic and acetogenic miroflora of the digesters.

In the present study an effort has been taken in this regard to check the load of microbial population in batch digesters run for 35 days HRT over the different temperatures ranges of thermophilic, mesophilic and psychrophilic ranges using urban waste as substrate with inoculums sourced from diverse environmental samples.

METHODS

Cultivation of obligate anaerobes requires strict anaerobic conditions and thus was carried out by Roll tube technique with maintenance of anaerobic environment using periodical flushing with oxygen free hydrogen (H_2) and carbon dioxide CO_2) gases. Most of the microflora and especially methonogens are fastidious by nature with no standard method for cultivation and detection in normal lab conditions are laborious and time consuming. Most of the *methanogens* sp. is very slow growers and sensitive to a broad range of inhibitors which are commonly found in growth media.

Substrate collection:

Municipal solid waste was collected from ten different locations of landfill site from urban areas of south India. The waste was segregated for organic fraction which constituted a mixture of domestic house hold waste, agricultural and restaurant waste, paper and cardboard wastes. The waste was collected from different point of time for analysis over different seasons and the work was carried out over a period of 2 years for monitoring microbial load. The substrate was also subjected to characterization before feeding into the biogas digesters.

Inoculums collection:

The inoculums were collected from preexisting digesters viz. waste water treatment plants, fruit waste processing plant slurry, kitchen waste treatment plant, dairy waste treatment plant, cow dung based biogas plant and also from environments rich in anaerobic microflora like landfill leachate.

Inoculum Characterization:

The inoculums was initially enriched by inoculation into basal medium containing 0.3% yeast extract in 100ml of sterile digestion fluid with intermittent H_2 and CO_2 flushing. The leachate sample was collected from multiple locations from landfill site at depths ranging from 5cm, 15cm, 20cm and 25cm etc. 10gm of each inoculums was enriched in one liter of methanogenic basal medium supplemented with 1% acetic acid and incubated at 35 °C. The quality of the inoculums was evaluated by measuring the daily average gas production.

Bioreactor startup:

Batch digesters for experimentation was loaded with 20% dry weight of urban waste, about 15% of the inoculums was added to the reactors. The digesters were prepared in triplicates and incubated at three different temperatures of 20 °C, 37°C and 55°C. The digestion was carried out for 5 weeks and

monitored on a daily basis for biogas production and reduction on chemical oxygen demand.

Sample processing:

Samples were collected on a weekly basis from digesters for analyzing total microbial load. The digester samples would be rich in anaerobic micro flora and hence pre-isolation protocol includes serial dilution of digester samples. Dilution medium was prepared and 9 ml of the medium was dispensed into 30ml sterile serum vials and sealed. To the sterile dilution medium 1 ml of sample collected digesters are added under nitrogen (N_2) atmosphere. Subsequent dilutions were made as per the requirements.

Roll tube Technique

Role tube technique as prescribed by Ramasamy *et al*., 1990 was followed for isolation of total obligate anaerobes. Total eubacterial microflora was isolated and enumerated by using anaerobic medium Na_2CO_3- 4.0g/L; trypticase 2.0g/L; yeast extract-0.5g/L; K_2HPO_4-0.3g/L; $C_{34}H_{32}CIFeN_4O_4$- 0.001g/L; $C_{12}H_6NNaO_4$ - 0.001g/L; Supplements 1 (NaCl, KH_2PO_4, $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2.2H_20$); Supplements 2 (Glucose, Cellubiose, Glycerol, Maltose & starch soluble); $HSCH_2CH(NH_2)COOH.HCl.H_2O$ -0.25g/L; Na_2S-0.25g/L; volatile fatty acids mixture-3.1ml/L.

Total methanogens were enumerated using Modified methanogen media with a composition of NH_4Cl-0.1g/L; K_2HPO_4-0.4g/L; $MgSO_4$-0.1g/L; yeast extract-2.0g/L; sodium formate /sodium acetate-10.0g/L; tryptone-2.1g/L; Supplements 3 (KH_2PO_4, $N(CH_2COOH)_3$, $CaCl_2$. $2H_2O$, $FeCl_3$, $CoCl_2$, $MnCl_2$, $ZnCl_2$, H_3BO_3, Na_2MoO_4, Na_2SeO_3); multivitamins as Supplement 4; $NaHCO_3$-0.5g; $C_{12}H_6NNaO_4$ – 0.001g/L; $HSCH_2CH(NH_2)COOH.HCl.H_2O$-0.5g/L; pH 7.0 at 25 °C. *Hydrogenotroph*s and acetate utilizing methanogens were cultured separately in specific media, *hydrogenotrophs* were specially supplied with H_2/CO_2 sparging over pressurized to 0.5-1 bar with a gas mixture of 80% H_2 and 20% CO_2. Media was prepared under 80:20 N_2 - CO_2 flushing, vitamin solutions and trace element solution were prepared, filter sterilized and then added aseptically to the sterile medium.

RESULTS AND DISCUSSION

The landfill leachate on enrichment in methanogenic media yielded to growth, evaluation of population showed a definitive pattern of increase in methanogen load with increase in depth of leachate samples collected. the samples collected at about five centimeter depth yielded the lowest methanogen load of 3×10^4 while a leachate samples from the depth of 25 centimeters resulted in higher numbers of 1×10^9.

The load of methanogens was found varying in enriched leachate samples and it was in found to be directly related to depth from which the sample was collected. Leachate samples collected from deeper landfill yielded higher load of methanogens in comparison to samples collected from surface of the landfill. Highest load was found in a depth of 20 cm but almost same but lesser load was observed at 25 cm deeper pile of landfill, load of methanogens drastically reduced upto 5 cm of the depth of landfill. This was in direct correlation with

methane yield obtained on seeding the inoculums in enrichment medium.

The differentially sourced inoculums based digesters on evaluation yielded varied concentrations of biogas which was used as a means to evaluate the quality of the inoculum. Further the digesters were started with urban waste as substrate inoculated with differently sourced inoculum such as dairy waste treatment plant slurry (DWTP); Food waste treatment plant slurry (FWTP); Cow dung digester slurry (CDS); Land fill slurry (LFS); Kitchen waste treatment plant (KWTP); Municipal waste water treatment plant slurry (MWWTP); etc. for a period of 35 days HRT. On analysis of eubacterial load of individual digesters (Fig1) the initial eubacterial load was highest in MMWTP inoculum based digesters, and lowest load of eubacterial in KWTP based inoculum. There was a slight decrease in total eubacterial load over a period of five weeks and lowest load of eubacterial in 5^{th} week of digester performance.

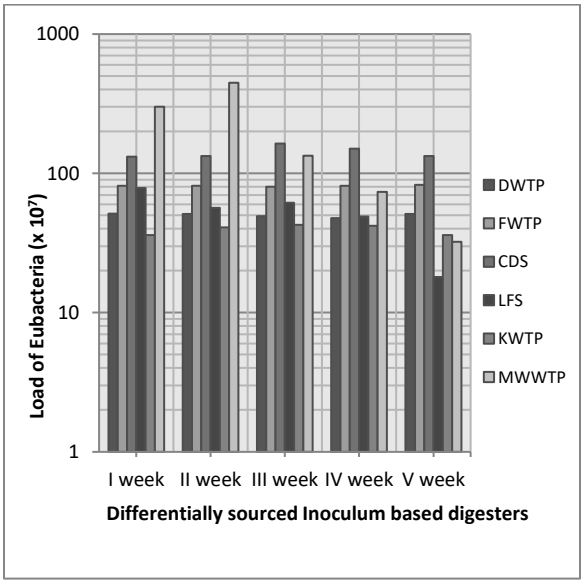

Figure 1: Eubacterial Load in Anaerobic digesters

The entire process was maintained at 55 deg C as it was found to increase biogas yield. Drastic reduction in eubacterial load was observed in LFS based digesters while MWWTP digesters also showed decreasing trend in eubacterial load over the period of 35 days HRT. Overall the eubacterial load of CDS digesters was found to be more stable in 5 weeks of digesters performance. The overall load of eubacteria were in a range of 108 to 109 and was found to be satisfactory in dealing with diverse nature of urban waste substrate.

Methanogen load was highest in the first week of digestion and gradually reduced over the period of 5 weeks (Fig 2). The decrease in methanogen load was more pronounced in MWWTP inoculum based digester and the highest methanogen load was also observed in the same digester type, there was even a slight increase in methanogen load on the second week. The second best digester was CDS based digester and was one among the best digester as the methanogen load was constantly high and was by far the most successful digester. The first four weeks of the LFS as inoculums based digesters exhibited higher methanogen load and only in the final week the load was lower.

All the digesters showed a decrease in methanogen load which could be attributed to accumulation of inhibitors in the form of pH changes due to volatile fatty acids and ammonia accumulation. The lowest methanogen load was observed in DWTP inoculum based digesters wherein the numbers were as low as 10^2 cells/ ml. CDS based digesters were also not performing well in terms of variable number of methanogen load. KWTP inoculum based digester carried a constant load of methanogens but was comparatively lesser

load to that of LFS and MWWTP inoculum based digester. FWTP based digesters were better in terms of methanogen load in comparison to DWTP based digesters.

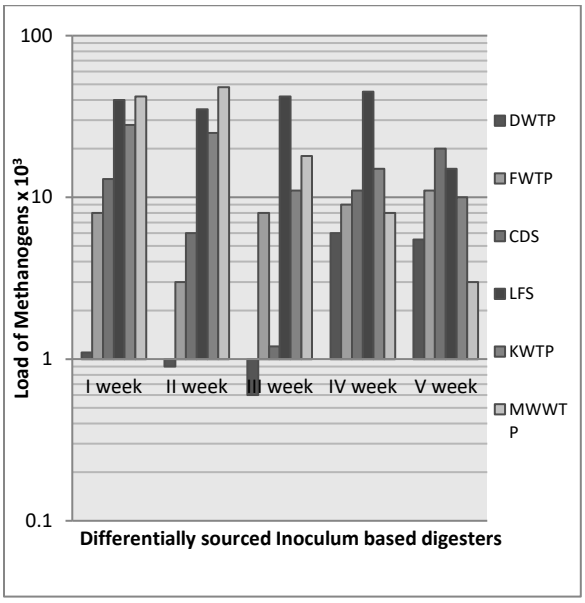

Figure 1: Methanogen Load in Anaerobic digesters

Conclusion

In the present study, performance of anaerobic digester was judged based on proper utilization of organic substrates and its conversion into biogas. The activity of the digester was correlated with load of microbes in the digesters especially methanogens. There was direct relationship between digester performance and methanogen load, eubacterial load was constant in almost all the digesters based on different inoculum. Thus methanogenic population tends to influence the overall digester activity. The decrease in methanogen load over the 35 days HRT could be attributed to accumulation of metabolites that negatively influence methanogen activity. Different inoculum was found to have differential influence on digester performance, none of the inoculum was exceptionally good for biogas production from urban waste but performed well in different periods of HRT and thus a consortium of inoculum could help in developing a good quality inoculum for sustainable biomethanation.

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