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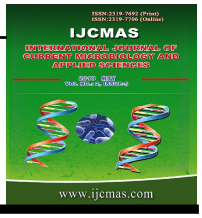

Original Research Article

Study on the influencial interplay of diverse factors in the course of sustainable biomethanation of urban waste

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Keywords

Anaerobic digestion; kitchen waste; VFA; COD; retention time; Biomethanation; urban waste.

A B S T R A C T

Biomethanation of urban waste was carried out in laboratory scale anaerobic biogas digesters at three different temperatures ranges of 20^0C, 35^0C and 55^0C. For the efficient biogas production, studies on the interplay and influential role of substrate, pH, temperature, C: N ratio, total solids on the sustainable anaerobic digestion process measured in terms of periodical evaluation of methane production, volatile fatty acids accumulation, chemical oxygen demand and load of fermentative, acetogenic and methanogenic bacteria etc., was undertaken. Stable performance of the digester over 3 different temperatures ranges of 37^oC to 35^oC and 55^oC) led to the observation that the mesophilic temperature shows better substrate utilization at a longer HRT in comparison to psychrophilic or thermophilic temperatures. The inhibitory effect of lower pH, accumulating VFA and ammonia on methanogenesis was observed. Isolation and enumeration of anaerobes by roll tube technique using Hungates mediums, Mah's medium, total anaerobic medium and anaerobic cellulolytic medium was carried out and their load correlates with and intrinsically related to influence of the other parameter on biogas digester stability. Development of a consortium of methanogens and other fermentative bacteria and testing of the consortium for biomethanation of urban waste has to be further assessed.

Introduction

Waste management is a topic of prime importance in every bustling cities of the world. Land filling has been a century old process that is being seriously reviewed now in wake of environmental pollution and space constraint. As the amount of solid waste increases in terms of hundreds disposal has to be addressed at the earliest. With space constrain the wastes tend to accumulate at the collection point and this tends to cause not only air and water pollution but also helps in breeding of vectors that supports spreading of diseases caused by harmful microorganisms.

Anaerobic digestion of urban waste offers an excellent opportunity in treatment of waste in a closed environment but also release of methane as bio fuel. Most of the important bacteria involved in biogas production process are anaerobes and are slow growing, a great degree of metabolic specialization is observed in these anaerobic microorganisms and most of the free energy present in the substrate is found in the terminal product methane, since less energy is available for the growth of organisms, less microbial biomass is produced and consequently, disposal of sludge after the digestion may not be a major problem.

The study was aimed to understand the factors that play important role in digestion of urban waste for efficient biogas production in comparison to the well established anaerobic digestion of cow dung. Microbial diversity in cow dung fed biogas digesters is as great as that of rumen (Nagamani *et al*., 1999) where, many bacterial species have been reported to play an important role in the production of biogas. Slurry collected from the outlet of the cow dung based working biogas plant can act as an ideal inoculum source for lab scale kitchen waste digesters. The four groups of microorganisms observed during a typical anaerobic digestion processes are fermentative acidogenic bacteria, organic acid oxidizing acetogenic bacteria, Hydrogen utilizing methanogens and aceticlastic methanogens (Balstone *et al.*, 2004). By participating in the terminal steps of organic material degradation, methanogenic archaea play a pivotal role in the anaerobic treatment of solid waste. The most critical and challenging task in practical operations is to fully use the total biodegrading potential of a whole microbial assemblages in –situ while preventing inhibition to the well organized cooperation among the interdependent metabolic groups of microorganisms (Robin Anderson *et al*., 2003). Though it is clearly evident that the nature of the substrate determines the type and extent of the different bacterial groups present in the digesters, the optimization of physical parameters and removal or neutralization of potential inhibitors will influence biogas production.

Materials and Methods

Feed materials collection and chemical analysis

Urban waste was collected from waste dumping sites around Bangalore, the waste comprised of market waste, waste paper, leftover food, cardboards, wood, fallen dry leaves, etc which were segregated from plastics, tyres, leather, textiles, soil, metals and glass to mention a few. The Organic fraction of the segregated waste was considered for anaerobic digester as the content was easily digestible and results in biogas production.

The organic fraction was finely macerated, oven dried and subjected for chemical analysis of Total solids, Volatile solids, Cellulose content, Hemicellulose, Starch, soluble sugar, lignin, fat and protein content (Ranade *et al*., 1987).

Chemical analysis

Laboratory scale batch digestion was conducted with glass reactors (1.0 L) containing 15% dry weight of urban waste. About 5% of the slurry from an active anaerobic digester was added as inoculum to the reactors. One set of reactors was incubated at 20^0C, the other at 37^0C and third set at 55°C. Triplicates were maintained for all treatments.

The anaerobic digestion was carried for 5

weeks; the gas produced from each reactor was collected and measured on daily basis by volume displacement method. Changes in pH with time were monitored by removing a small drop of the slurry form sealed bottles with a needle and syringe, the solution was spotted onto pH paper specific for a various pH ranges, the accuracy of this method was checked occasionally by determining slurry pH with a pH meter (Gary King *et al*., 1980). The volatile fatty acid was estimated by using distillation method by APHA (1989). The methane production was measured using Chemito Gas Chromotography fitted with Propak Q Column (6' X 1/8'' ss). The oven and injector/detector port temperatures were maintained at 70 Deg C and 100 deg C respectively. Nitrogen was used as carrier gas at a rate of 30 ml/min. Oxygen and hydrogen were used for the flame. The total solids content was determined as given in APHA (1989).

Isolation and cultivation technique

Dilution medium was prepared under nitrogen atmosphere. 9.0 ml of dilution medium was added in 30 ml serum vials. The vials were sealed; sterilized Cysteine hydrochloride was used as a reducing agent. The slurry samples were diluted by adding 1.0 ml sample to sterilized and cooled vials with 9.0 ml dilution medium under nitrogen atmosphere. Then the dilutions were used for the isolation of anaerobic organisms by Roll tube technique (Ramasamy *et al.*, 1990). About 0.5 ml of the diluted sample was taken in a sterile roll tube and appropriate medium was added with continuous nitrogen flushing. Then tubes were sealed quickly and rolled on a wet sponge for quick solidification of the medium. The tubes were incubated at 20°C, 37°C and 55°C in an inverted position to prevent disturbance of colonies by condensed waterlets during sub-culturing. Total anaerobes were enumerated by using the modified Hungate's medium. Total methanogens were enumerated by using Mah's media with modification from serially diluted samples. Enumeration of fermentative bacteria was recorded by using modified Hungate's medium with starch as substrate. The appropriate medium for acid formers was prepared and cultured by roll tube technique

Result and Discussion

Several temperature ranges exist for the methane fermentation process, a thermophilic zone (above 45°C), a mesophilic zone (25°C) and a psychrophilic (Below 20°C) temperature. Typically, anaerobic digesters are designed to operate in either the mesophilic or thermophilic temperature ranges to maintain a high rate and steady state digestion.

A sharp decrease in the rate of methanogenesis was observed when the urban waste was digested at 20°C. Microbial specific growth rates decreased with a decrease in temperature. As reported by Masse *et al*., (1999), methanogens were found to be more sensitive to temperature than other organisms in the anaerobic digester. But by increasing the retention time of methanogenic activity for a period of 5 weeks, did improve biogas generation under all 3 different conditions *viz*., 37°C, 55°C and 20°C. Never the less, low temperature methanogenesis in natural habitats is considered to be significant in global methane emission to the atmosphere.

The production of total gas in digester fed

with urban waste was at the rate of 83 ml/day and gradually reduced to an average of 5 ml on the final week. A steady fall in average gas production was recorded in digesters incubated at 20°C and 55°C while mesophilic temperatures showed greater variation with a rise in gas production only at the 5th week. An average drop in the biogas production in the later stages of retention time can be attributed to the accumulation of volatile fatty acids.

The methane production of the digesters was quantified periodically from the headspace gas. Higher methane concentration was observed in digesters incubated at 20°C than at mesophilic and thermophilic conditions. Average methane content of 65% was observed along with considerable amount of carbon dioxide and traces of hydrogen sulphide.

The pH of the digesters varied between 3.98 and 7.36, urban waste fed digesters recorded a lower pH range when compared to cow dung digesters. There was a general reduction in the pH of the digesters as the days advanced and every week the digesters were supplemented with sodium hydro oxide solution to overcome inhibition of methanogenesis at lower pH. However the pH of the digesters with urban waste is much lower when compared to the cow dung fed digesters. The fermentation process was found to be optimum at pH about 6.7 to 7.4 and pH values below 6.0 or above 8.0 was highly restrictive. Total solids estimation is important as it represents the substrate level for anaerobic digestion; Lane, (1982) reported gas yields of 0.429 – 0.568 ml/g of fruit and vegetable waste total solids fed. It has been observed that the reduction of total solid was slightly lower in the urban waste fed digester in comparison to cow dung based digester. The reduction in total solids was observed in all the three incubation temperatures.

The initial chemical oxygen demand was higher in digester fed with urban waste. Reduction in chemical oxygen was observed at all the temperatures with higher reduction recorded in digesters incubated at thermophilic conditions. A tenfold reduction in Chemical Oxygen Demand was observed in anaerobic digester incubated at 37°C and 55°C, while digesters at 20°C showed a less than twofold decrease.

The major volatile acids usually produced are acetate, propionate and butyrate while others are not present in detectable amounts. Fermentation of fatty acids does not occur, as none of the known fermentative anaerobic bacteria oxidize fatty acids. Accumulation of the fatty acids leads to the overall lowering of the pH of the digesters; this may affect the viability of most of the bacteria. The volatile fatty acid (VFA) concentration was recorded from the first week up to the end of the batch digestion. Digesters incubated in thermophilic condition showed a gradual decrease in volatile fatty acid content. Higher volatile fatty acid concentration was observed in urban waste fed digester. However the urban waste fed digesters maintained constant VFA concentrations in both 37°C and 55°C temperatures.

The total methanogens were enumerated in all the digesters maintained at three different temperatures and have not shown much change in methanogen population with an average of 10^4 CFU /ml. Total anaerobes population was found to be on an average 10^7 CFU /ml of the digester sample.

Figure.1 Performance of the anaerobic digester at 35°C

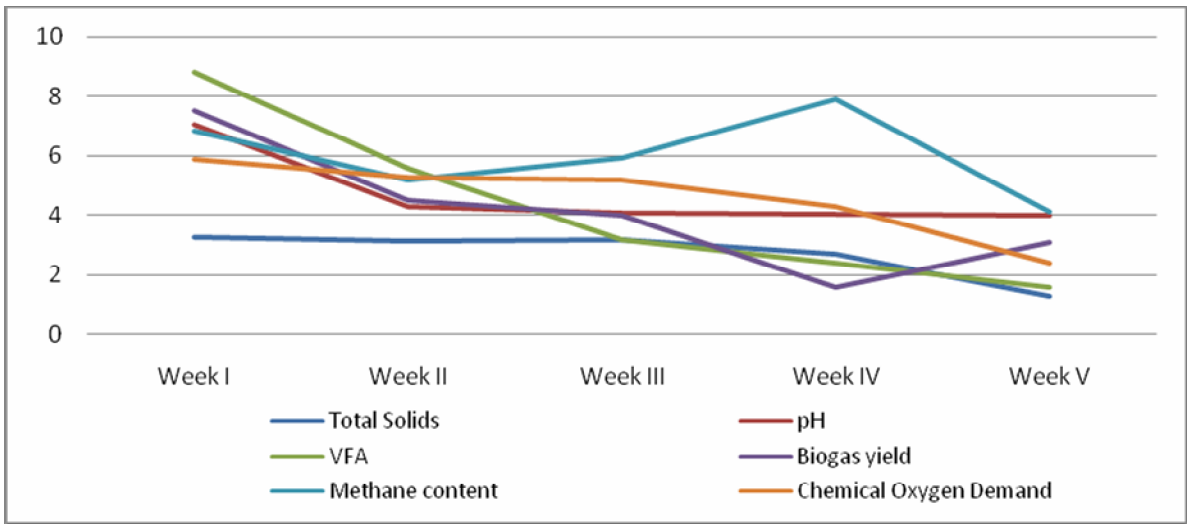


Figure.2 Performance of anaerobic digester at 55°C

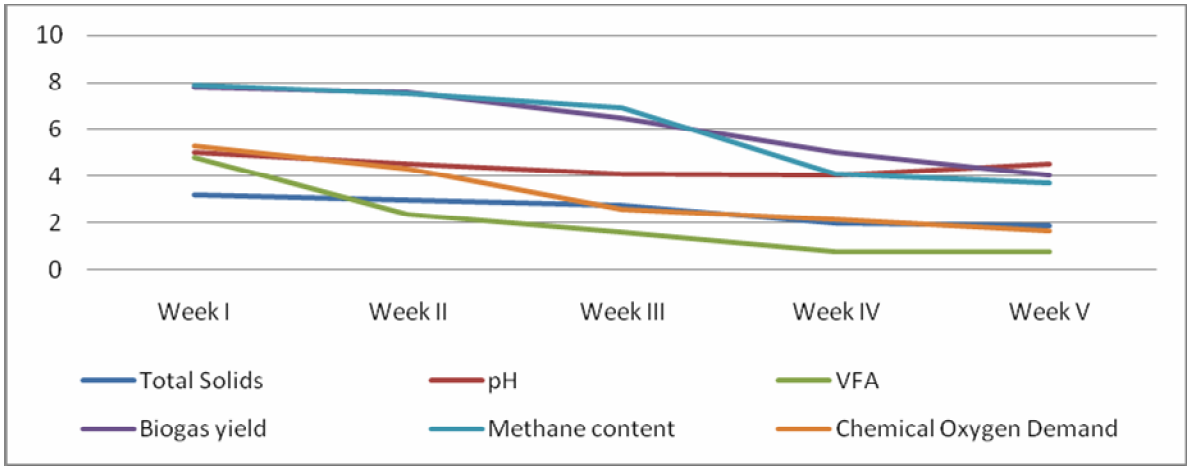


Figure.3 Performance of anaerobic digester at 20°C

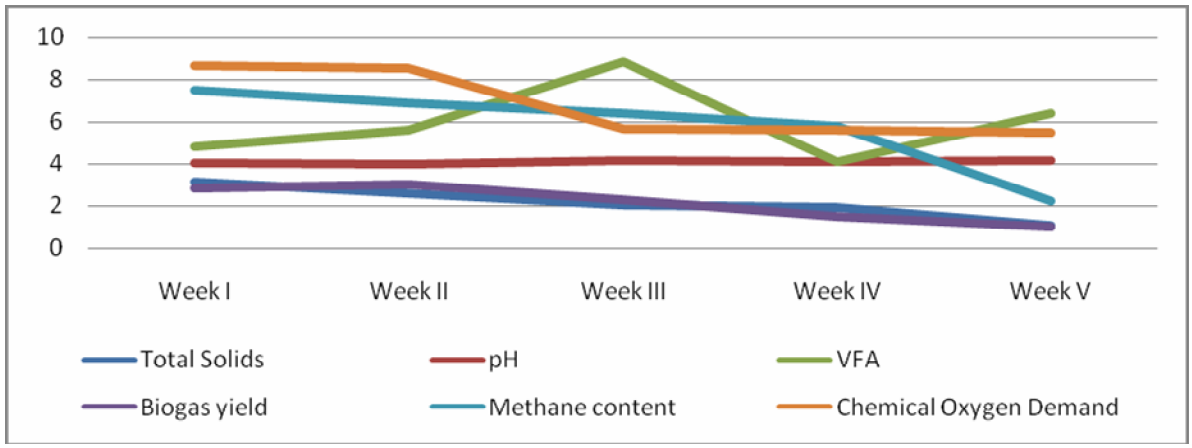


Figure.4 Load of Total anaerobes in the biogas digesters

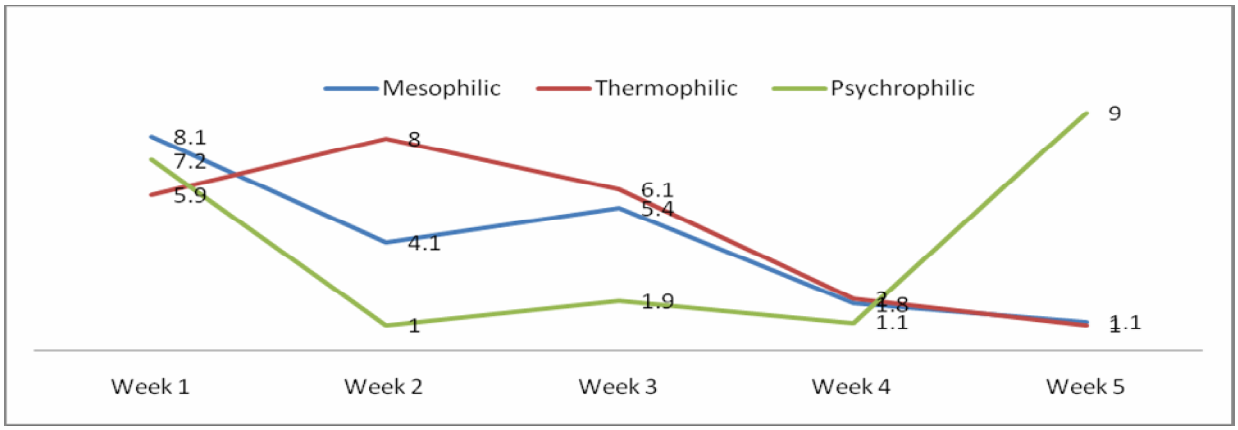
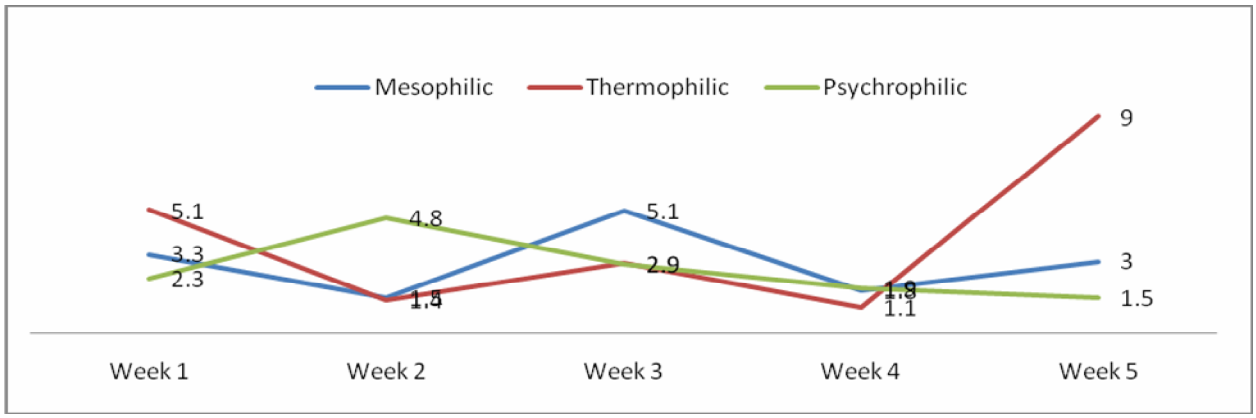


Figure.5 Load of total methanogens in biogas digesters



From the data the reactors maintained at different temperatures viz., 20^0C, 35^0C and 55^0C showed difference among performance factors were generally small. This could be attributed to the surface area and the microbial population. Methanogens span a wide range of growth temperatures, from moderate psychrophiles that grow optimally at temperatures around 20^0C to hyper-thermophiles that grow at temperatures up to 110^0C (Boone *et al*., 1993). By participating in the terminal steps of organic material degradation, methanogenic archaea play a pivotal role in the anaerobic treatment of solid waste (Robin Anderson *et al*. , 2003).

Thermophilic anaerobic process normally operates between 50^0C and 60^0C and because of the higher metabolic activities of thermophiles; the processes are capable of accommodating a very high loading rate at feasible removal efficiency. An alternative for treatment of middle and high strength wastes. But the processes are less stable and more sensitive to environmental changes than mesophilic processes (Hirayuki Imachi *et al*., 2000). Total methanogen load peaked during 5^{th} week of thermophilic digestion which was accompanied with drastic decrease in the load of total anaerobic eubacteria, and an exactly opposite effect was observed in 5^{th} week of digester incubated at 20^0C.

The peaking of methanogenic population in the 5^{th} week of thermophilic digesters

were accompanied with drastic reduction in COD of the digester sample, followed by reduced VFA accumulation and slight increase in pH. Conversely a peak of total anaerobic eubacteria lead to drastic increase in VFA leading to decrease in load of total methanogens which was evidently observed in least biogas production and low methane yield.

One possible way to improve anaerobic digestion of urban waste involves mixing equal ratio with cow dung as a substrate. Compared with structural carbohydrates (for example: cellulose), it was observed that the fermentation of non-structured carbohydrates (starch, sugars) results in less methane per unit of substrate fermented (Andrea macmuller *et al*., 2003). The gradual reduction in the gas production during the later period of incubation was due to the accumulation of volatile fatty acid, which inhibits methanogenic activity. Substrates with higher C:N ratio may reduce biogas production further aspects that affect biogas production is high level of readily degradable compounds (eg: more starch) or high level of recalcitrant compounds (lignocelluloses) Low C:N ratio based substrates based anaerobic digestion has to be operated at longer HRT (Panichmunsin *et al.,* 2006).

Hydrogen producers can increase methane production, while the inhibition of hydrogen producers leads to accumulation of propionate, at the expense of acetate, propionate may be useful inside ruminants, because propionate on oxidation releases more free energy for animals productive purpose, but in anaerobic digesters they are not productive. In competitive interactions among different species of bacteria, the capability for growth on the lowest concentration of a growth limiting substrate may be the major factor determining the population outcome.

Branched chain fatty acids have stimulatory effect on cellulose digestion. Addition of starch can produce enough branched chain fatty acids for the requirement of cellulolytic rumen bacteria, and high concentration of starch as inhibitory effect on the growth of cellulolytic bacteria (Nideki miura et al. 1980). Thus the problem of VFA as an inhibitor of methanogenesis can be overcome by supplementing the urban waste based digester with cowdung. The Supplementary of Branched chain fatty acids are effective only when the main components of cellulose and urea are more. The methane content of the biogas increases with increase in HRT, High pH can be achieved in the digesters with substrates rich in readily available carbohydrate and low in protein.

Operating condition and temperature for laboratory digesters has to be standardized to generate biogas from the urban waste. From the above data it is clearly evident that urban waste can be used as a potential source for biogas production. The use of urban waste can reduce the problem of pollution. Methane yield and microbial activity can be correlated for better gas production. Pure methane at standard temperature and pressure has a lower heating value of approximately 34,300 kJ $m^{3.}$ Biogas, however, is typically only 40-80% methane and therefore, has a heating value of approximately 13,720 – 27,440 kJ m^3.Methane can be used for all the purposes where natural gas of fuel in a gasified form is used for cooking, lighting, refrigerator, incubator, gasoline engine,etc. In a general consensus digesters incubated at 37^0C performed better in the 1^{st} and 4^{th}

week of anaerobic digestion and digesters at 20°C was showing optimum activity at 2nd and 3rd week of digestion. Overall the thermophilic digesters performed better than digesters incubated at 37°C and 20°C. The biogas yield and reduction in COD and VFA was comparatively better in thermophilic digesters. Though mesophilic digesters were most stable among the digester types on the basis of distribution of microorganisms, the digester at 200°C to 20°C showed the best reduction in Total solids content.

To summarize all the three digesters show properties of advantages than to single out one particular temperature, one possible solution will be to develop a consortium of microorganisms isolated from all the digester types that are less sensitive to inhibitors, more competitive and physiologically diverse.

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