Int J Pharm Bio Sci 2013 July; 4(3): (B) 1168 - 1177

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EXPLICIT ROLE OF METHANOGENIC CONSORTIUM DEVELOPED FOR IMPROVING THERMOPHILIC BIOMETHANATION OF URBAN WASTE

B.J.YOGESH*[1] AND A.MICHAEL[2]

1Department of Microbiology, The Oxford College of Science, Bangalore, India.
2Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

ABSTRACT

Land-filling option is drying out with rapid pile up of organic waste in urban areas; the problem can be circumvented with accelerated anaerobic decomposition which can also be a source of valuable bio-fuel. But many municipal anaerobic waste treatment systems fail due to the lack of adequate microbial inoculum and also hampered by the doubts on many aspects of its operation and stability. In this regard biomethanation of the organic fraction of urban waste was carried out in laboratory scale reactors with different inoculum. The interdependence among the anaerobic microbial community which leads to stability in operation was assessed with a set of diverse factors. Influential interplay of various physiochemical factors which includes Volatile fatty acid content, reduction in Chemical oxygen demand, average biogas production, methane content of the bio-fuel, variation in pH, influence of temperature and reduction in total solids content was assessed. The operation instability can be solely attributed to three factors, inadequate methanogenic diversity, differential nature of substrate and variation in temperature. Methanogens were isolated from different inoculum based stable digesters and an consortium was developed. The consortium not only circumvented the loopholes of the individual stable digesters but also demonstrated an enhanced stability, greater biogas yield, more methane content and higher reduction in chemical oxygen demand. To confirm the efficacy of the consortium, a direct equal mixture of various inoculum (bypassing the isolation step) was used for biomethanation process. Though it improved the performance of the digesters, the efficiency exhibited by the explicit role of the consortium was higher. Methanogen cell number was estimated both by direct count using fluorescence microscope and by roll tube technique on enriched media. Tracking individual methanogenic species of the consortium in the due course of organic waste digestion will be further carried out using FISH and immune - molecular techniques for further understanding of complex anaerobic process.

KEYWORDS: Urban waste, biomethanation, VFA, COD, methanogens, consortium.

B.J.YOGESH
Department of Microbiology, The Oxford College of Science, Bangalore, India.

**Corresponding author*

INTRODUCTION

Rapid Urbanization all over the world has created problem for the disposal of organic waste. Cities contribute much to the accumulation of wastes which is simply dumped or spread on the land. The methane generated by landfills account for 12% of global methane emissions and makes up about 25% of anthropogenic contribution to global warming. Accelerating the decomposition process can be helpful in preventing local air and water pollution, in this regard; aerobic and anaerobic process of treatments is available. Anaerobic processes are preferred based on the speed of digestion, pathogen free manure and as a potential source of bio fuel. Basically four groups of bacteria are known to be involved in anaerobic digestion of organic waste which culminates with methane production, the first two groups are eubacteria (fermentative bacteria and organic acid oxidizers) and last two are Archaebacteria (Acetoclastics methanogens and Hydrogenotrophs methanogens). For application of anaerobic processes to the treatment of solid waste, understanding the structures and metabolic functions of these microbial communities responsible for degradation is important[1]. Although Methanogenic Achaea make up only a small part of the microbial biomass[2], their population changes might impact the entire community by triggering an imbalance that is reflected in the bioreactor performance via accumulation of intermediates such as VFA, pH changes or reduced efficiency[3]. Temperature governs microbial population dynamics[4]. Thermophilic anaerobic process normally operates between 50^{o}C and 60^{o}C, because of the higher metabolic activities of thermophiles, the process are capable of accommodating a very high loading rate at feasible removal efficiency, but the processes are less stable and more sensitive to environmental changes than mesophilic processes[5]. The present study was carried out to understand the role of different inoculum on thermophilic anaerobic digestion of urban waste and to evaluate the performance of developed consortium in comparison to natural mixed inoculum.

MATERIALS AND METHODS

Substrate collection and processing

About 10 kg of Municipal Solid waste (MSW) was collected from ten points from outside and inside of the solid waste heap collected from Mavallipura landfill near Bangalore. The total quantity of collected waste is thoroughly mixed and subjected to shredding to a size as small as 2 – 5 mm pieces. The pieces were subjected to physical and chemical analysis. The organic fraction was finely macerated, oven dried and subjected for chemical analysis of Total solids, Volatile solids, Cellulose and starch content, fat and protein content[6], moisture content, Total carbon and Nitrogen content (C/N ratio).

Bioreactor operation

Laboratory scale digesters of 1 liter capacity were fed with 15% of finely macerated substrate and pasteurized. 10% of inoculum (of the working volume of the reactor) from three different inoculum sources was added to the pasteurized digesters and labeled appropriately as IL1 (Waste Water Treatment Plant based inoculum; WWTP) Vrishabhavathi valley treatment plant, Bangalore; IL2 (Cattle-dung based bio gas plant slurry; CDS) from Anekal taluk, Bangalore; and IL3 (Landfill slurry inoculum; LFS) etc. the digesters were incubated in an inverted position at two different temperatures of 35^{o}C and 55^{o}C respectively in quadruplets. Digesters were maintained for 5 weeks of digestion, the slow growth rate of methanogens normally involve a retention time greater than 10 days for efficient and stable operation[7].

Assessment of influencing parameters

Periodical assessment of pH, Volatile fatty acids content, Chemical oxygen demand, Total solids content, average biogas production and methane content was carried out. 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[8]. The volatile fatty acid content and chemical oxygen demand and Total solids content was determined as described[6]. Gas production from each reactor was collected and measured daily by volume displacement method. The methane production was measured using Chemito Gas Chromatography fitted with Propak Q Column (6 X 1/8 ss). The oven and injector/detector port temperatures were maintained at 70^{o}C and 100^{o}C respectively. Nitrogen was used as carrier gas at a rate of 30 ml/min. Oxygen and hydrogen were used for the flame[8].

Cultivation of methanogens

Samples were periodically collected form digester under constant Nitrogen flushing, dilution medium of Sodium Bicarbonate – 0.5%; Sodium carbonate – 1% was prepared under nitrogen atmosphere, 9 mL of dilution medium was added in 30 mL serum vials. The vials were sealed; sterilized Cysteine hydrochloride was used as an 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. The dilutions were then used for isolation of anaerobic organisms by Roll tube technique[9]. Total anaerobes were enumerated by using Hungate's Habitat-Simulating medium, Modified Hungate's medium for cellulolytic bacteria, Total methanogens were enumerated by using Mah's medium, methanogen medium for acetate utilizers, *Methanobacterium* mass culturing medium, *Methanococcus* medium and a appropriate medium for acid formers[10]. The random isolation of methanogens in small scale cultivation based studies is useful for obtaining isolates for detailed investigation and also indicates the presence of a species[2].

Microscopy

Methanogens were checked with phase contrast and fluorescence microscope - AXIOSCOPE, Ziess, Research grade Model equipped with high pressure mercury vapor lamp with coolcube 1, metasystem ccd camera). Cell number was estimated by a direct count using fluorescence microscopy[7].

RESULTS AND DISCUSSION

The composition and characteristics of Municipal Solid Waste (MSW) vary throughout the world. Even in a same country it changes from pace to place as it depends on a number of factors such as social customs, standard of living, geographic location, climate etc. In India, the biodegradable fraction is quite high, essentially due to extensive use of fresh vegetables. The mean chemical composition is given in Table 1.

Table 1
Chemical composition of urban waste

S No.	Parameter	Weight fraction (%) ; g per liter ; in ratio
1	Moisture content	60%
2	TS %	18.00
3	VFA (% of TS)	80.00
4	Total Carbon	25.87%
5	Total Nitrogen	1.45
6	C/N ratio	17.83
7	COD	95,000 mg/L
8	Cellulose	19.2
9	Starch	11.0
10	Total protein	14.5
11	Fat content	8.0

Anaerobic digester performance

Biomethanation of urban waste was carried out in laboratory scale digesters with three different inoculum sources at 2 different temperatures for a period of five weeks. The digesters were constantly monitored for their activity which was directly correlated with biogas production collected from the head space. The collected biogas was simultaneously quantified and methane content was evaluated by means of gas chromatography. The net biogas content was comparatively higher for IL2 (CDS) digesters and lowest for IL3 (LFS), average gas production for thermophilic digesters (irrespective of the inoculum) was comparatively higher (Table 2; Table 3) but Methane content of the biogas was more in digesters incubated at 35^0C. Lesser methane content may be attributed to lower methanogenic activity or accumulation of unreduced carbon dioxide or hydrogen sulphide production. Thermophilic digesters recorded higher Volatile fatty acid content and lower pH, which can be related to the higher metabolic activity. IL 3 digesters had the highest VFA accumulation which was more prominently visible in the poor performance of the digester in comparison to IL1 and IL2 based digesters, the VFA content of IL2 digesters was 41.7% higher at thermophilic conditions. (Table 2, Table 3).Thermophilic digesters performed better in terms of reduction in chemical oxygen demand and total solids content of the manure, the reason can be attributed to higher metabolic activity of bacterial population, the same could be the reason for the higher VFA hence lower pH, and higher biogas production could be due to accumulation of CO_2 which was evident from gas chromatography analysis. The thermophilic digesters holds a potential scope for methanogens growth and diversification, this can be envisioned based on the availability of high concentration of acetate (principle VFA in anaerobic digesters) and CO_2 and if methanogens tends to keep pace with eubacterial population the problem of high VFA, low pH and lower methane content of the biogas can be averted.

Table 2
Biomethanation of Urban waste at 35^0C

Inoculum source → / Performance of Various factors ↓	Landfill based slurry	WWTP* based slurry	CDS# based inoculum
Biogas Production (mL/day)	8.2	27.7	27.9
VFA content (mg/L)	6371	796	1113
Total solids (reduction in %)	13.05	14.12	14.89
Average deviation from pH 7.0	4.3	6.74	6.38
Methane Content (%)	61.6	69.4	72.0
COD (reduction in %)	42.6	72	50.0
Total bacterial count (CFU/mL)	1.92×10^5	3.3×10^7	1.8×10^7
Total methanogens count (CFU/mL)	1.95×10^7	1×10^7	1.0×10^7

**WWTP – Inoculum from Waste Water Treatment Plant;*
#CDS – Cattle dung based Biogas Plant Slurry.

Table 3
Biomethanation of Urban waste at 55^0C

Inoculum source → / Performance of Various factors ↓	Landfill based slurry	WWTP* based slurry	CDS# based inoculum
Biogas Production (mL/day)	17	35	40.2
VFA content (mg/L)	7320	1345	1909
Total solids (reduction in %)	14.5	14.89	15.0
Average pH	4.64	6.28	6.5
Methane Content (%)	60	65.4	69
COD (reduction in %)	51.4	76.4	67.0
Total bacterial count (CFU/mL)	3.7×10^7	3.4×10^7	2.9×10^5
Total methanogens count (CFU/mL)	2.2×10^7	0.96×10^7	2.5×10^7

**WWTP – Inoculum from Waste Water Treatment Plant*
#CDS – Cattle dung based Biogas Plant Slurry.

Microbial community dynamics

Periodical sampling of the digesters were carried out and the samples were subjected to microscopic observation and cultivation by roll tube technique on enrichment media. Instead of community analysis of inoculum, direct assay of performing digesters can help in identifying potential isolates. The values on phase contrast and fluorescence microscopic count was taken as enumerated total load of bacterial communities and cultural techniques were considered for identification of the species. Average eubacterial population load was highest in IL2 (CDS) based digesters (Table 3). This correlates with higher bacterial diversity in cattle dung based slurry (more than 40 species) and lowest archaeal diversity of less than 8 species have been reported[11]. This could be the possible reason for trying out different inoculum for improving biomethanation of urban waste. Lowest methanogenic load was observed in thermophilic digesters based on waste water treatment plant based slurry (Table 3). Fermenters employed in waste water purification typically have low acetate concentrations, enabling domination by acetoclastic Methanosaeta due to high substrate affinity[12]. Interestingly the higher load of methanogens was reported in thermophilic digesters, higher concentrations of VFA are expected to temporarily block methanogenesis, but the metabolic activity would resume immediately with neutralization of the inhibitors.

Cultivation of methanogens were precluded with serially dilution of digester samples up to 10^{-7} and 10^{-8}, this was done to maximize the chances of isolating a better strain. It was presumed that any methanogenic strain that adapts well to the complex environment could possibly be resistant to inhibitors, grow at lower threshold of substrate concentration, versatile in nutrition requirements and probably have an higher growth rate. Uncultivable forms of bacteria was not taken into account for the reason that their complex nutrient requirements itself may make them less competitive in the diverse environment. But their unknown influential role cannot be denied, in this regard, consortium was developed with the intention of using them as a supplementary to the usual environmental inoculum. It is rather difficult to establish a total species census for an environmental sample, as there are always undiscovered species left[13]. Samples were plated after serial dilution and appropriate conditions were provided for growth, the colonies were observed under fluorescence microscope and colony morphology was noted down. The isolates were carefully chosen based on the performance of the digester at the time of sampling but also by following up of their performance for the next few weeks (Table 4).

Table 4
Properties of the isolates (Methanogenic Consortia)

Methanogenic Isolates	Nutritional requirements	Morphological attributes	Source of the isolate
MI 1	Acetate	Rod shaped.	IL2
MI 2	$H_2 + CO_2$, formate	Rod shaped.	IL2
MI 3	$H_2 + CO_2$, formate	Spiral shaped	IL3
MI 4	$H_2 + CO_2$, formate	Rod shaped	IL1
MI 5	Acetate	Methanosaeta sp.	IL1

The thermophilic digesters showed significant changes in the methanogenic population over the period of 35 days HRT. Hydrogen utilizing methanogens were constantly present at highest dilutions and acetate utilization rod shaped bacteria were isolated from stable anaerobic digesters. The dominance of Hydrogen oxidizing *Methanobacteriales* organisms might be due to their higher specific growth rate than that of acetate – utilizing methanogens[14]. Thermophilic conditions might favor hydrogenotrops, as these are known to be more capable of adapting to a higher temperature than acetoclastic methanogens[15]. Spiral shaped methanogens growing on hydrogen and carbon dioxide mixture and rod

shaped acetate utilizing archaea were isolated repeatedly from WWTP based stable digesters (Table 4). Aceticlastic methanogens in the rumen face shortage of substrates as the acetate absorbed by rumen intestine, so instead of *Methansarcina sp*., the *Methanothrix* sp. may predominate in the cattle dung based biogas digesters because they have lower threshold for acetate as substrate.

Acidogens are more sensitive to Hydrogen and their removal pave way for growth and production of more VFA. Efficient Hydrogen consumption by hydrogenotrophic methanogens can result in elimination of inhibitory effect on hydrogen, leading to a nutritionally more favorable pattern for VFA formation by acidogens, thereby increasing the rate of fermentation[16]. Aceticlastic methanogens are also dominant in many biogas fermenters used for anaerobic wastewater treatment and sewage sludge digestion[17]. Population dynamics of both acetotrophic and hydrogenotrophic methanogens during the anaerobic digestion of particulate solid biomass for biogas production are rather scarce[4]. Sarcina shaped methanogens were not considered for the consortium, they were found to be present in all the initial inoculum and their pertinent presence in the latter stage of the digesters were taken into account. The presence of sarcina shaped methanogens varied widely in comparison to other methanogen species. Majority of rumen Archaea were identified as hydrogenotroph[18]. Even in the absence of acetotrophic methanogens, the anaerobic oxidation of acetate to hydrogen and carbon dioxide by syntrophic acetate oxidizing bacterium can be the major pathway enabling dominance of *Methanobacteriales* and *Methanomicrobiales* over *Methanosarcinales*[19].

Performance of the consortium

Each of the isolates were sub-cultured in serum bottles appropriately (H_2 and CO_2 in a ratio of 80:20 for hydrogen utilizers and N_2 and CO_2 of 80:20 for acetate utilizing methanogens). The digesters were supplemented with 1% of each isolate with equal amount of cattle dung based inoculum total amount to 10% of the total working volume of the digesters. A mixed inoculum (IL4**)** of 1/3rd of CDS, 1/3rd of WWTP slurry and 1/3rd of LF slurry (without consortium) was also tested for biomethanation of urban waste. Methanogenic Consortium based digesters were monitored for a period of 5 weeks, biogas and methane content was measured on daily basis; pH, VFA, COD, total solids, anaerobic bacteria load and Total methanogen load were measured on weekly basis. The pH of the digesters (IL5) remained uniformly around 6.6 -7.1 (figure 1), the digesters were never supplemented with sodium hydroxide over the course of digestion, unlike the individual inoculum based digesters which required constant neutralization of pH on weekly basis. Volatile fatty acids accumulation was not observed during the five week of digestion process, but further reduction in the inherent VFA of the digesters was observed and it was observed only during 4th week of digestion. On the contrary, the IL4 digesters (mixed inoculum) started with a pH of 5.86 during the first week of digestion and attained a pH of 4.76 at the 5th week period (figure 2). VFA accumulation in IL4 digesters for the 1st week of digestion amounted to 5770 mg/L which was at the same period 2306 mg/L for IL5 digester. Reduction in Chemical oxygen demand for IL4 was 68.4% while methanogenic consortium based digesters achieved 78.1% reduction of chemical oxygen demand. Total solids reduction for IL4 was 16.15 which were comparatively higher than the individual inoculum based digesters, while IL5 exhibited 17.11% reduction in Total solids content. Comparative reduction in total solids content in IL5 digesters was 5.61% more than that of IL4 (fig 1; fig2).

Figure 1
Performance of digesters at 55°C with methanogenic consortium

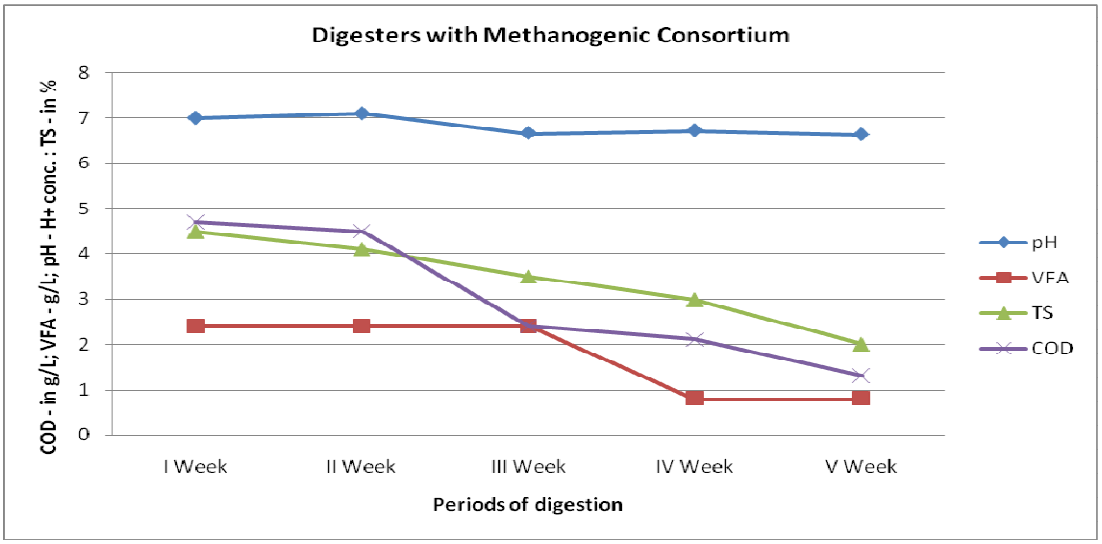
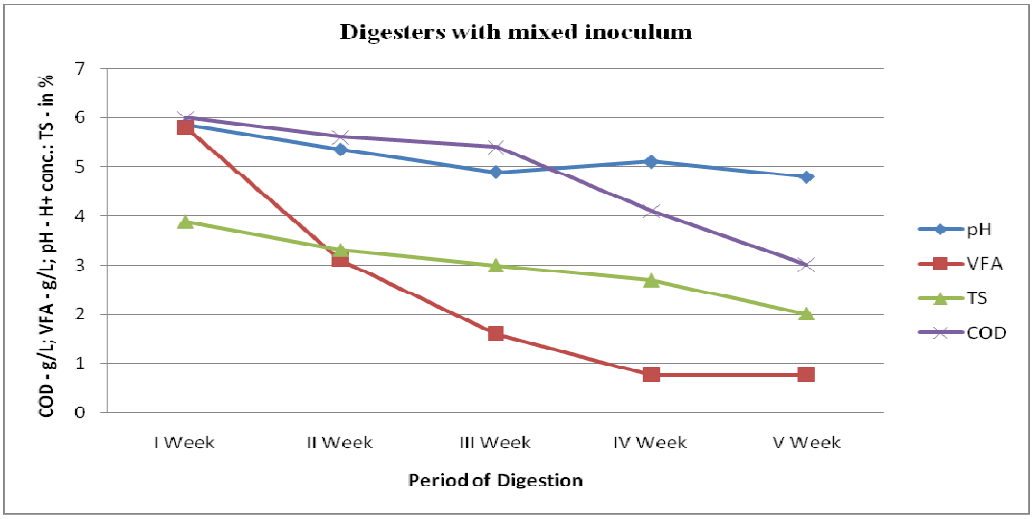
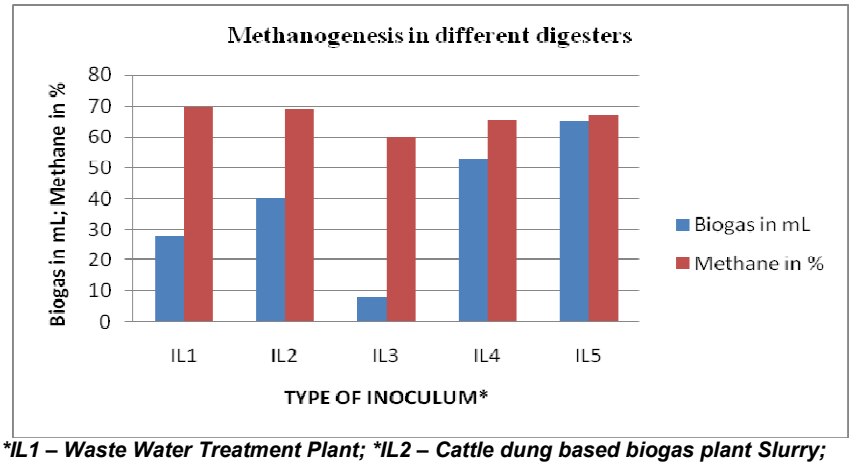


Figure 2
Performance of digesters at 55°C with mixed inoculums



Efficiency of anaerobic digesters was evaluated based on average biogas production and methane content of the bio fuel. These two parameters are quite helpful in assessing the stability of the digesters at a given point of time, any fall in biogas production can be further investigated by checking for VFA accumulation and changes in pH. A comparative account of methanogenic activity has been thus evaluated for all the five different anaerobic digesters that have been used for the experiment (Figure 3). It can be noted that the least performing digester was IL3 which had operated with landfill slurry as inoculum, while IL4 and IL5 digesters exhibited better higher biogas production, again asserting the role of inoculum in improving digester performance. The methane content of the digesters showed only slight variation and it was between 60 to 70% on an average (Figure 3).

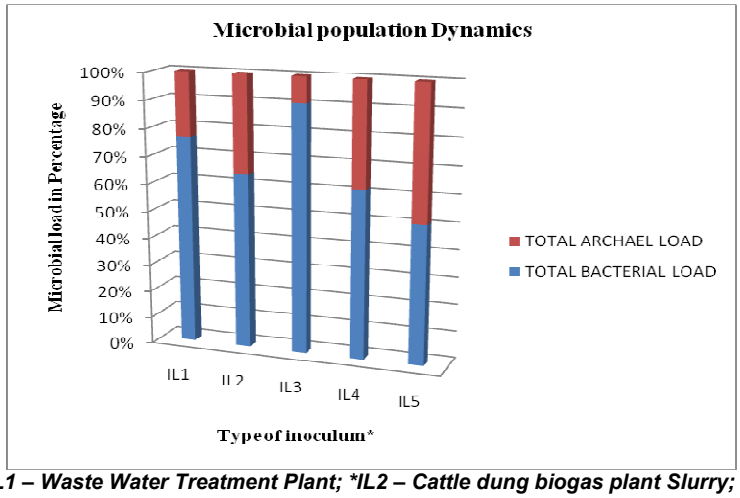
Figure 3
Comparative account of Methanogenic activity



**IL1 – Waste Water Treatment Plant; *IL2 – Cattle dung based biogas plant Slurry; *IL3 – Land fill slurry; *IL4 – Mixed inoculum; *IL5 – Consortium*

Lipids add up to the higher carbon content in the urban waste, the principal source of lipids are garbage, cooking oil and fats. Since lipids in the solid state become liquid at temperatures slightly above 25^0C, they add to the liquid content during waste decomposition. Aceticlastic methanogens are sensitive to long chain fatty acid which slightly inhibits gas production from acetate[20]. Long Chain fatty acids oxidation can only proceed via the syntrophic association of fatty acid oxidizing bacteria with hydrogen utilizing microorganisms such as hydrogenotrophic methanogens[3], but their optimal microbiological condition varies, the rate of fatty acid utilization by methanogens is an inherent property of the organism and environmental conditions in the digesters[21]. Heavy metals present in the waste may inhibit microbial activity. If the digester performance fails, the inhibitor concentrations have to be assessed for confirmation, neutralization of inhibitors may help in improving microbial processes. In urban waste the presence of starch based food may influence acid formation which may be a potential source of acetate for methane production.

Figure 4
Effect of inoculum on microbial population distribution in Urban waste digesters



**IL1 – Waste Water Treatment Plant; *IL2 – Cattle dung biogas plant Slurry; *IL3 – Land fill slurry; *IL4 – Mixed inoculum; *IL5 – Consortium*

The methanogenic population in relation to bacterial was assessed for all the five different inoculum based batch digesters, IL3 digesters had the highest bacterial load of 90.7, the digesters poor performance can be attributed to lower load of methanogenic population that lead to higher VFA accumulation (Figure 4). Though nature has equipped various bacteria and fungi (aerobic or anaerobic and mesophilic or thermophilic) to produce a set of complex enzymes that can completely hydrolyze the organic fraction of the urban waste[22], 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[23]. It is clearly evident from the population dynamics that the consortium's better performance can be attributed to the healthy ratio of methanogen and anaerobic bacteria in the digester.

CONCLUSION

The inoculum used for start up of anaerobic digesters is an immeasurable and infinite source of anaerobic microorganism. It is impossible to fathom their diversity, in this regard the molecular techniques like Fluorescence in situ hybridization (FISH); phylogenetic analysis by 16S rRNA gene amplification and Real time quantitative PCR can help to unravel the presence of undiscovered species. But without the ability to culture them under laboratory conditions, it is impossible to understand their true potential. Moreover, techniques have to be developed to keep track on the role of each species and their fate in the due course of anaerobic digestion. The behavior of each isolate in the methanogenic consortium has to be assessed for better understanding of their interaction and interdependence in stable operation of biogas digesters. Antibodies generated against methanogenic isolates of the consortium can help immensely to track the distribution of each isolate in a working digester. Further work will be done to develop immuno-molecular technique for understanding the complex anaerobic process. As such the consortium has to be tested by scale up process and its adaptability to variation in the substrate composition have to be assessed.

ACKNOWLEDGEMENT

We thank Dr.Bharathi.S, Professor and Head, Department of Microbiology, The Oxford College of Science, Bangalore for her constant encouragement and support.

REFERENCES

1. Shigematsu Toru, Yueqin Tang, Yuko Mizuno, Hirome Kawaguchi, Shigeru Morimura, and Kenji Kida. Microbial Diversity of Mesophilic Methanogenic Consortium That Can Degrade Long-Chain Fatty Acids in Chemostat Cultivation. Journal of Biosciences and Bioengineering, Vol. 102: 535 -544, (2006)
2. Peter H. Janssen and Marek Kirs.. Structure of the Archaeal community of the rumen. Applied and Environmental Microbiology, 74: 3619 -3625, (2008)
3. Schink, B. Principles and limits of anaerobic degradation: Environmental and Technological aspects. In A.J.B. Zehnder (ed.), Biology of Anaerobic Microorganisms. J.Wiley and Sons, Inc., New York, NY. 771 – 846, (1998)
4. Niclas Krakat, A. Westphal, S. Schmidt, and P.Scherer. Anaerobic Digestion of Renewable Biomass: Thermophilic Temperature Governs Methanogen Population Dynamics. Applied and Environmental Microbiology, 76(6); 1842 -1850, (2010)
5. Hiroyuki Imachi, Yuji Sekiguchi, Yoichi Kamagata, Akiyoshi Ohashi, and Hideki Harada. Cultivation and In Situ Detection of a Thermophilic Bacterium Capable of Oxidizing Propionate in Syntrophic Association with Hydrogenotrophic Methanogens in a

Thermophilic Methanogenic Granular Sludge, 66 (8): 3608 -3615. (2000)
6. APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington, DC. (2005)
7. Olga. Y. Ako, Y.Kitamura, K.Intabon, T. Satake. Steady state characteristics of acclimated hydrogenotrophic methanogens on inorganic substrate in continuous chemostat reactors. Bioresource Technology. 99: 6305 -6310, (2008)
8. Yogesh. B. J. Michael. A, Bharathi. S and K.V. Sekar. Study on the influential interplay of diverse factors in the course of sustainable biomethanation of urban waste. International Journal of Current Microbiology and Applied Sciences, 2(5): 357-365, (2013)
9. Ramasamy, K., B. Nagamani and Sahul Hameed, M. Tech. Bulletin. Fermentation Laboratory, Tamil Nadu Agricultural University, Coimbatore. pp.91. (1990)
10. Ronald M. Atlas. Handbook of Microbiological Media, 3rd Edn, CRC press: 1043-1083, (2004).
11. Mizhou, Emma Hernandez – Sanabria, and Le luo Guan. Assessment of the Microbial Ecology of ruminal methanogens in cattle with different feed Efficiencies. Applied and Environmental Microbiology. 75: 6524 -6533, (2009)
12. Jetten, M.S.M., A.J.M. Stams, and A.J.B. Zehnder. Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. FEMS Microbiology Letter, 88: 181 – 198, (1992)
13. Rastogi, G., D.R.Ranade, T.Y.Yeole, A.K.Gupta, M.S. Patole, and Y.S. Shouche. Molecular analyses of methanogen Diversity associated with Cattle dung. World Journal of Microbiology and Biotechnology, 24: 2973 - 2979, (2008)
14. Peu, P., H. Brugese, A.M. Poucher, M. Kerouredan, J.J. Godon, J.P. Delgenes, and P.Dabert. Dynamics of a pig slurry microbial community during anaerobic storage and management. Applied and Environmental Microbiology, 72: 3578 – 3585, (2006)
15. Chen,M. Adaptation of mesophilic anaerobic sewage fermentation populations to thermophilic temperatures. Applied and Environmental Microbiology, 45: 1271 -1276, (1983)
16. McAllister, T.A., and C.J. Newbold. Redirecting rumen fermentation to reduce methanogenesis. Australian Journal of Experimental Agriculture. 48: 7 -13, (2008)
17. Demirel, B., and P.Scherer. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Review of Environmental Sciences, Biotechnology and Bioengineering, 7: 173 -190, (2008)
18. Jansen, D.H. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology, 74: 3619 -1625, (2008)
19. Karakasher, D., D.J.Batstone, E. Trably, and I. Angelideki, Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of *Methanosaetaceae*. Applied and Environmental Microbiology 72: 5138 – 5141, (2006)
20. Hanaki, k., Matsuo, T., and Nagase, M, Mechanism of inhibition caused by Long Chain fatty acids in anaerobic digestion process. Biotechnology and Bioengineering, 2:3 1560 -1591, (1981)
21. Carolina Casserby, Leonardo Erijman. Molecular Monitoring of Microbial diversity in an UASB reactor. International Journal of biodeterioration and Biodegradation. 52: 7 - 12, (2003)
22. S.Pathak and H.S. Chaudhary. Perspective of microbial species used in lignocelluloses Bioconversion. International Journal of Pharma and BioSciences. 5(2): 1138-1153, (2013)
23. Li-Han Huang, Hui Zhou, Yeu-qin Chen, Shuo Luo, Chong-Yu Lan, Liang-Hu qu Diversity and structure of the archaeal community in the leachate of a full-scale recirculating landfill as examined by direct 16S rRNA gene sequence retrieval. FEMS Microbiology Letters 214: 235-240, (2002).