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FACULTATIVE AND NON METHANOGENIC MICRO FLORA FROM BIOGAS DIGESTER RUNS ON DISTILLERY WASTE AND IPOMOEA WEED BIOMASS.

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ABSTRACT
Production of biogas from agricultural waste is very promising alternative for energy generation. The present paper includes the study of different types of microorganisms involved in biogas production process .The untreated Ipomoea weed biomass and distillery waste were used as substrates instead of conventional substrate like cow dung. Experiments were carried out in 100-L digester. In general facultative and non methanogenic micro flora mainly bacteria, yeasts and molds were isolated by using standard Medias, SPC of bacteria, yeasts and mold were 5.86 x 10^{10}, 1.62 x 10^{3} and 1.36 x 10^{2} respectively . By using standard procedures15 bacteria, 4 yeasts and 5 mold isolates were identified from digester effluent and sludge.

KEY WORDS: Agricultural wastes , Biogas, SPC, Micro flora, Weeds

INTRODUCTION

In India almost 70% population lives in villages, where the plant and animal biomass in the term of cattle dung, dry leaves, agricultural residues and plant weeds is available in plenty, which can easily be converted into biomass. The generation of biogas in villages can be a boon for development of rural area Biogas is mixture of methane (65 – 70%) and CO_2 (30 – 35%) together with prior gases like NH_3, H_2S, H_2 and N_2 etc. in trace quantities produced from organic matter under anaerobic conditions

Biogas production involving three stages first two stages partly involve both aerobic and anaerobic conditions, while third stage is essentially anaerobic viz. stage of biogas formation. These three stages, in fact, do not exist in isolation but overlap with each other, with the final result of production of methane, carbon dioxide and traces of hydrogen, ammonia, nitrogen and hydrogen sulfide etc. The mixture of these gases with methane content of 60 – 70%, which comes out from biogas plant, is highly combustible and called 'biogas'.

The first stage hydrolyzes the complex biopolymers like cellulose, hemi cellulose, lignin, starch, proteins and fats etc. to form their simpler monomers by extracellular enzymes of microorganisms. These complex polymers are, in fact, first hydrolyzed mainly to oligomers, which along with monomeric carbohydrates, amino acids and some organic acids such as lactic acids are later degraded by the fermentation reaction to form various simpler organic acids and acetate (in IInd stage). After the hydrolysis and liquefaction, the degradative products are utilized by the microorganisms, provided that they are able to pass into the cell through the cytoplasmic membrane. This membrane selectively regulates the flux of nutrients, ions and waste products in and out of the cell. Membrane is composed of protein and lipids, where proteins act as carrier for specific substrates or type of molecules. Substrates accumulate in cells by the phenomenon of active transport. The membrane fluidity is determined by relative amount of saturated and unsaturated fatty acids in the lipid portion.

The low molecular weight acids produced in the acid production stage are further degraded to methane and CO_2 by highly specialized group of bacteria commonly referred to as the methane producing bacteria. These organisms have unique ability to couple organic oxidation to reduction of CO_2. In this process CO_2 is the terminal hydrogen acceptor and is analogous to oxygen in aerobic respiration. Non methanogenic microorganisms Cellulolytic (aerobic / facultative and anaerobic*)* These mainly include Cellulolytic organisms, which are capable of digesting cellulose. Moreover these same organisms are also capable of digesting hemicelluloses and to some extent lignin. The proteins and fats are degraded by other organisms. Non-Cellulolytic (aerobic / facultative and anaerobic) The non-Cellulolytic organisms other than methanogenic bacteria include many aerobic / facultative and anaerobic organisms involved in many subsidiary biochemical reactions like degradation of lipids, proteins and other minor compounds found in the source. The products formed by the action of these organisms on fats, proteins and other compounds are CO_2, H_2, H_2S, N_2, various hydrocarbons and also many simple and complex fatty acids, alcohols etc.

MATERIALS AND METHODS

1) Weed material - *Ipomoea carnea*
2) Agrobased organic waste - Distillery waste
3) Slurry of ongoing cattle dung based biogas plant
4) Biogas digester- 100 lit capacity digester

5)Media for microbiological study

Different media used for microbiological studies are enlisted in Table 1. The media, reagents and apparatus used for identification of microbial isolates and other tests were as per Bergey's Manual of Systematic Bacteriology, 9th edition (Krieg *et al.*, 1984), Godbole, *et al.* (1981) and Bergey's Manual of Determinative Bacteriology (8th edition) (Buchana, *et al.*, 1974).

Table 4.3: Media used for microbiological studies.

Sr. No.	Group of organisms under study	Medium used
1	Bacteria	Standard Plate Count Agar (SPC agar)
2	Yeast	Yeast Extract, Malt Extract, Glucose Agar (YEMEGA)
3	Molds	Martin's Rose Bengal Agar (MRBA)
4	MPN of methanogenes	Touzel and Albagnac medium (T-A Medium)

MICROBIOLOGICAL STUDIES

The bacterial, yeast and mold isolates from digester efluent were identified to the species level as follows.

1) Bacteria The identification of bacterial isolates up to the species level was carried out on the basis of morphological, cultural and biochemical characteristics and with reference to Krieg *et al.* (1984), Buchanan *et al.* (1974), Gibbs *et al.*, (1966, 1968) and Cruickshank *et al.*, (1965, 1975).

2)Yeasts – The yeast isolates were identified to species level on the basis of following morphological, cultural and biochemical characteristics as described by Kreger-Van-Rij (1984); Barnett et al., (1990); Gibb's *et al.*, (1986); Campbell *et al.*, (1988) and Rose *et al.*, (1969).

3) Molds -The identification of the mold isolates was done on the basis of colonial, morphological and microscopic observation of the wet mounts with reference to Barnett and Hunter (1972) and Domach *et al.* (1980).

RESULTS AND DISCUSSION

SPC of aerobic and facultative anaerobic bacteria, yeasts and molds were studied From anaerobic digester effluent (100 – L capacity) on SPC, YEMEG and MRB agar media incubated at room temperature (38^0C) for 48 – 96hrs.

Table 1. Average counts of bacteria, yeasts and molds

Sr. No.	Week No.	SPC (Colony count / g)		
		Bacteria	**Yeasts**	**Molds**
1	Ist	7.1×10^{10}	1.9×10^{3}	2.1×10^{2}
2	IInd	4.5×10^{10}	3.1×10^{2}	1.6×10^{2}
3	IIIrd	8.3×10^{9}	4.1×10^{3}	1.5×10^{2}
4	IVth	1.1×10^{11}	1.7×10^{2}	2.4×10^{1}
5	**Average of four weeks**	**5.86×10^{10}**	**1.62×10^{3}**	**1.36×10^{2}**

It was evident that the average counts of bacteria, yeasts and molds were 5.86×10^{10}, 1.62×10^{3} and 1.36×10^{2} colonies/g respectively (Table 1).

Bacteria - Out of the total sixteen bacterial isolates obtained, nine were (56.25%) Gram positive, and seven Gram negative (43.75%) Amongst Gram positive, out of nine, five were species of *Bacillus* i.e., *Bacillus circulans, Bacillus firmus, Bacillus megaterium, Bacillus sphaericus* and *Bacillus cereus,* Var. mycoides and remaining were *Lactobacillus helveticus, Micrococcus varians* and a *Streptomyces* sp. Gram negative members were found to be *Citrobacter freundii, Pseudomonas* sp., *Cellulomonas flavigera, Flavobacterium* sp., *Proteus vulgaris, Pseudomonas aerogenosa* and *Escherichia coli.*

Gore (1979), who studied nonmethanogenic bacteria from effluent of digester run on cattle dung slurry, isolated species of *Arthrobacter, Listeria, Citrobacter, Pseudomonas, Escherichia, Bacillus, Flavobacterium, Micrococcus* **and** *Lactobacillus*. Kale (1986) reported isolates of *Staphylococcus arueus, Streptococcus faecalis, Escherichia coli, Enterobacter cloacae, Citrobacter freundii, Proteus vulgaris, Proteus mirabilis, Alkaligenes faecalis, Flavobacterium rigense, Arthrobacter simplex, Bacillus licheniformis, Bacillus subtilis, Bacillus megaterium, Bacillus firmus, Bacillus polymixa, Bacillus cereus, Bacillus pumilus, Lactobacillus fermentatum, Micrococcus, Pseudomonas* sp., *Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhozopus, Candida, Hansenulla, Saccharomyces, Schizosaccharomyces,*

and *Torulopsis* sp. from anaerobic lagoons of distillery waste. Pathade (1995) showed isolation of *Citrobacter freundii, Bacillus mascerans, Lactobacillus agilis, Schizosaccharomyces pombe* and *Leucosporidium scottii* from digester of distillery waste treatment. It was found that bacterial isolates showed different kinds of hydrolytic activities like fermentation of glucose, lactose, trehalose, xylose, mannitol and sucrose, and other activities such as production of lecithinase, gelatinase, nitrate reductase, catalase, oxidase, arginine dihydrolase and tests like M.R., V.P., citrate utilization and H_2S production positive. The cellulolytic activity was shown by *Bacillus circulans, Bacillus firmus, Cellulomonas flavigera, Pseudomonas and Streptomyces* sp.

Yeasts - The yeast isolates from digester effluent included *Leucosporidium scottii, Geotrichum candidum, Lipomyces lipoferus* and *Candida pseudotropicalis*. All of these showed gelatinase and caseinase activities. In addition *Leucosporidium scottii* also showed lipase and amylase activities and *Geotrichum candidum* and *Candida pseudotropicalis* diastase activity. None of them showed cellulase and nitrate reductase activities. The five molds obtained in the present investigation were *Penicillium, Aspergillus, Tricoderma, Neurospora* and *Fusarium*, all of which showed amylase activity. All except *Neurospora* also showed cellulase and lipase activities. All except *Fusarium* also showed gelatinase, while all also showed caseinase activity except *Neurospora* and *Fusarium* indicating hydrolytic and cellulolytic natures of mold isolates.

CONCLUSION

SPC of bacteria, yeasts and molds in the effluent from 100-L capacity anaerobic digesters, run on admixture of *Ipomoea* biomass and distillery waste, averaged to 5.86×10^{10} colony count/g, 1.62×10^{3} colony count/g and 1.36×10^{2} colony/g for bacteria, yeasts and molds, respectively.

The aerobic and facultative anaerobic, non methanogenic, cellulolytic and hydrolytic bacteria isolated from this effluent included :

i) Bacteria: *Arthrobacter* sp., *Bacillus cereus, Bacillus circulans, Bacillus firmus, Bacillus megaterium, Bacillus sphaericus, Cellulomonas flavigera, Citrobacter freundii, Escherichia coli, Flavobacterium* sp., *Lactobacillus helveticus, Micrococcus varians, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas* sp., *Streptomyces* sp.

ii) Yeasts: *Candida pseudotropicalis, Geotrichum candidum, Lipomyces lipoferus, Leucosporidium scottii.*

iii) Molds**:** *Aspergillus* sp., *Fusarium* sp., *Neurospora* sp., *Penicillum* sp., *Tricoderma* sp.

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