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Study of Hydrolytic Non Methanogenic Bacteria from Substrates and Biogas Digester Effluent

Microbiology

KEYWORDS : Renewable energy, Biogas

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ABSTRACT

Biogas Production from weed and agro industrial wastes is a promising source for energy. It is a renewable and clean energy source. Biogas production takes place in three stages involving different types of microorganisms .The present work includes the study of different types of bacteria found on substrates and in digester effluent and their role in biogas production. The untreated weed biomass and distillery waste was used as sole substrate by step wise removal of dung. Experiments were carried out in 4-L digester and scale up was carried out in 25, 50 and 100-L digesters. Amount of biogas production was 467.30L/kg BOD/day with 62.32% methane in 100-L digester. SPC of bacteria was determined from substrates and 100-L digester effluent. Weed biomass, distillery waste and dung slurry shows 24, 14 and17 bacterial isolates respectively. They were identified up to species level by using standard methods. In all 13 types of bacteria were reported from substrates and out of them 10 types were found in effluent showing that all the bacteria present in digester were came from the substrates. They play important role in biogas production. Three types of bacteria present on substrates were not found in effluent this may be due to environmental conditions prevailing in digester may not be suitable for the growth of these bacteria.

1) INTRODUCTON

Energy needs of man have increased many folds in the past century due to growth of human population and Industrialization. In the years to come, it is feared that the crisis will reach to such an extent that no conventional fuel will be available anywhere in the world. Moreover, all the conventional sources of energy are non renewable and their use often cause a variety of pollutions. Biogas production from biomass and agro-based industrial wastes is an important way of renewable energy generation.

Biogas production involving three stages and different groups of bacteria such as hydrolyzing, acidifying, acetogenic and methanogenic bacteria they produce CO2 and CH4 (Demirel and Scherer, 2008). First two stages partly involve both aerobic and anaerobic microorganisms, while third stage is essentially carried out by anaerobic microorganisms.

First stage is hydrolysis which breakdown insoluble complex organic matter (Madigan et al, 2008) like cellulose, hemi cellulose, lignin, starch, proteins and fats to form soluble sugars , amino acids , glycerol and long chain carboxylic acid (Ralph and Dong 2010), Hydrolysis is first and overall rate limiting step for the mineralization of organic matter in anaerobic environment (Higuchi et al,2005).Hydrolytic bacteria were studied by various workers and shown that they play important role in biogas production.

Ipomoea is important weed available in plenty and it can be efficiently used for biogas production, among the weeds water hyacinth aquatic weed has been extensively researched for production of biogas. Most aquatic and terrestrial weeds do not possess a soft tissue and, therefore, require an acid or alkali treatment before they can be used for production of biogas alone or in combination with other organic substrates (Chellapandi, 2004).Co-digestion of municipal, agricultural and industrial organic wastes can also be exploited for biogas production on a sustainable level (Alatriste MF et al., 2006).

The present work has been undertaken to study hydrolytic bacteria present on different substrates and in digester effluent, the types of microorganisms their characteristics and biomethanation potential of different substrates.

2) MATERIAL AND METHODS

MATERIALS

1) Selection of plant weed.
The plant selected for the study was Ipomoea carnea Jacq.sub sp. fistulosa (Mart, ex Choisy) of the family Convolvulaceae. Plant was selected on the basis of availability and nuisance caused by this weed.

2) Collection of plant weed.
The weed was collected from Sadar bazaar, Satara area from the habitats like road side, agricultural land, and pond and river waters. This plant was authenticated by Professor Dr. V.P. Khendekar, Head; Post graduate Department of Botany Yashawantrao Chavan Institute of science, Satara (India) The plant specimen was deposited as voucher specimen in YCIS, Satara Herbarium, as Ipomoea I, voucher number 107 in 2006 for record and further reference.

2) Distillery waste - It was collected from Ajinkyatara Sahakari Sakhar Karkhana Ltd. Distillery Shendre, Satara (India). Waste was collected as fresh composite sample in disinfected plastic carboys of 5 – 50-L capacity and stored at refrigeration till further use.

3) Slurry of ongoing cattle dung based biogas plant – It was collected from a biogas plant situated at Degaon, M.I.D.C. region Satara (India).

4) 4-L, 25-L, 50-L and 100- L capacity Biogas digesters - They were fabricated locally having Inlet, out let, gas holder and digester.

5) Gas collection, storage and analysis system

6) Media for microbiological study.
Standard Plate Count Agar (SPC agar) was used for isolation and study of SPC. The media, reagents and apparatus used for identification of microbial isolates and other tests were as per Bergey's Manual of Systematic Bacteriology.

METHODS

1) Isolation of Bacteria from substrates and its Standard Plate Count (SPC)

a) Weed surface - Leaves and twigs of weed collected were cut into pieces of about 2 cm sizes aseptically with sterile knife. 10 g of this material was soaked in 100 mall sterile saline water in 500 mL capacity flask and kept at room temperature for 1 hour on a rotary shaker at 175 rpm to bring surface micro flora of weed material into water medium. This washed water from weed was subjected to serial dilutions (viz. $10^{2,}$ 10^{3} $\cdots$ 10^{8}) and standard plate count for bacteria was carried out in triplicates using SPC agar media. Plates were incubated at room temperature (30 – 35^{0}C) for 1-3 days for bacteria; representative isolates were preserved in triplicates on the slants of respective media.

b) Distillery waste and dung slurry -well mixed 1 mL of each of distillery waste and dung slurry were subjected to Standard Plate Count (SPC) for bacteria in triplicates on media described earlier and after serial dilutions (10^{1}, 10^{2}, ... 10^{10}). The plates were incubated at ambient temperature for 1 to 3 days for

bacteria to get practicable colonies and then counts of bacteria were recorded (Table No 6). The representative bacterial isolates were preserved in triplicates on respective media slants at refrigeration temperature for further studies.

2) Biogas production study.

It was studied by using 4-L capacity digester with working volume 2400 mL, pH of digester material was adjusted at 7.5. The ambient temperature was 30^0C and retention time was 25 days. Admixture pattern of weed, waste and dung slurry was as shown in Table No1.

Table No1; Admixture pattern of weed, waste and dung slurry

Sr. No.	Volume of daily loading (g)	Proportion of dung slurry admixed with untreated weed biomass in combination with distillery wastes at various stages of experiment (g)			
		Stage I 1-10 days : 25% (weed + waste)+ 75% Dung slurry	Stage II 11-20 days : 50% (weed + waste) + 50% dung slurry	Stage III 21-30 day : 75% (weed + waste) + 25% dung slurry	Stage IV 31-40 days: 100% weed + waste
1	160	40 + 120	80 + 80	120 + 80	160

Gas collection system, storage and analysis-

The gas collection system used for all the digesters was an assembly of water displacement method. Gas was collected in bottles and stored at room temperature Analysis of gas was done at Agharkar Research Institute (ARI), Pune by gas chromatography (Perkin Elmer Auto System – XL). Carrier gas used was H_2 with gas flow rate of 60mL/min. Column used was para pack Q, detector used was TCD (thermal conductivity detector), column temperature at injection port was 60^0C and at detector 70^0C).

The scale-up studies- were performed with 25, 50 and 100-L capacity digesters using the parameters of retention time (25 days) and pH (7.5) while temperatures at 25, 50 and 100-L digesters were 33, 35 and 38^0C, respectively. The admixture of weed biomass-distillery waste with pH 7.5 was added at daily loading amounts of 1,000, 2,000 and 4,000 kg for 25, 50 and 100-L capacity digesters, respectively.

The initial seeding material was that obtained from 4-L digesters, which contained exclusive fermenting admixture of weed biomass–distillery waste, which was used for 25-L digester from which it was later used as initial seeding for 50-L digester. Ultimately the fermenting slurry from 50-L digester was used as initial seeding for 100-L digester. The biogas volumes were recorded daily for 25-L, 50-L and 100-L digesters, and the bacterial study was carried out by using sample from 100-Ldigester effluent.

Isolation of bacteria from digester effluent-The fermenting slurry from 100-L capacity digester was collected from digester outlet in a sterile bottle and subjected to isolation of bacteria and SPC studies by using media described earlier.

3) The identification of Bacterial isolates.

Bacterial isolates up to the species level was carried out on the basis of morphological, cultural and biochemical characteristics (Table No 2 to Table No5) with reference to Bergey's Manual of Systematic Bacteriology.

3) RESULTS

Table No 2; Bacterial isolates from surface of weed Ipomoea carnea

Sr. No.	Isolate Number	Glucose	Trehalose	Xylose	Lactose	Starch	Mannitol	Egg yolk reaction	Sucrose	M.R.	V.P.	Citrate	Gelatine liquification	Nitrate reduction	H_2S	Catalase	Oxidase	Arginine hydrolysis	Spore	Gram character	Identity of isolates
1	SB1	+	–	–	–	–	+	+	–	+	+	+	+	–	–	+	+	+	N	G -ve	Pseudomonas alcaligenes
2	SB2	+	–	–	–	–	+	+	+	+	+	–	–	–	–	+	+		N	G +ve	Listeria monocytogenes
3	SB3	+	–	–	+	–	+	+	–	+	+	–	–	–	–	+	+		N	G +ve	Listeria grayi
4	SB4	+	–	–	–	–	–	+	+	–	–	–	–	–	–	+	–		N	G –ve	Flavobacterium aquatile
5	SB5	+	–	–	–	–	+	+	+	–	–	–	+	+	–	+	+		C	G +ve	Bacillus cereus
6	SB6	+	–	–	–	–	–	+	+	+	–	–	+	+	–	+	+		C	G +ve	Bacillus laterosporus
7	SB7	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	S	G +ve	Bacillus sphaericus
8	SB8	+	+	+	–	+	+	+		+	+	+	+	+	–	+	+		O	G +ve	Bacillus megaterum
9	SB9	+	–	–	–	+	+	+	+	+	+	–	–	–	–	+	+		N	G +ve	Listeria monocytogenes
10	SB10	+	+	–	+	–	–	–	–	+	–	–	–	+	–	–	–		N	G +ve	Lactobacillus spp.
11	SB11	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+		O	G +ve	Bacillus sphaericus
12	SB12	+	+	+	–	–	+	+	–	–	+	+	–	–	–	+	+		O	G +ve	Bacillus pumilus
13	SB13	+	–	–	–	–	–	+	+	+	–	–	+	+	–	+	+		O	G +ve	Bacillus laterosporus
14	SB14	+	–	–	–	–	+	+	+	–	–	–	+	+	–	+	+		O	G +ve	Bacillus cereus
15	SB15	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+		O	G +ve	Bacillus megaterium
16	SB16	+	+	+	–	+	+	+	–	–	+	–	+	+	–	+	+	+	O	G +ve	Bacillus amyloliquefaciens
17	SB17	+	+	+	–	+	+	+	–	–	+	+	–	+	–	+	+	+	O	G +ve	Bacillus Coagulans
18	SB18	+	–	–	–	–	–	–	+	+	+	–	–	+	+	–	+		O	G +ve	Bacillus laterosphorus
19	SB19	+	–	–	–	–	–	–	+	+	+	–	–	+	+	–	+		O	G +ve	Bacillus laterosporus
20	SB20	+	+	+	–	+	+	+	–	–	+	+	+	+	–	+	+		O	G +ve	Bacillus subtilis
21	SB21	+	–	–	–	–	+	+	–	+	–	+	–	+	–	+	+		O	G +ve	Bacillus brevis
22	SB22	+	+	+	–	–	+	+	+	+	–	+	+	+	–	+	+	+	O	G +ve	Bacillus licheniformis

C : Central spore S : Spherical O : Oval

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Table No 3; Bacteria isolated from distillery waste

Sr. No.	Isolate Number	Glucose	Trehalose	Xylose	Lactose	Starch	Mannitol	Egg yolk reaction	Sucrose	M.R.	V.P.	Citrate	Gelatine liquification	Nitrate reduction	H_2S	Catalase	Oxidase	Arginine hydrolysis	Spore	Gram character	Identity of Isolates
1	DB1	+	–	–	–	–	+	+	+	–	–	–	+	+	–	+	+	–	C	G +ve	Bacillus cereus
2	DB2	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	–	O	G +ve	Bacillus megaterium
3	DB3	+	+	+	–	+	+	+	+	–	+	+	+	–	+	+	+	+	T	G +ve	Bacillus circulans *
4	DB4	+	+	+	–	+	+	+	+	–	+	+	+	+	–	+	+	+	O	G +ve	Bacillus licheniformis
5	DB5	+	–	+	–	+	+	+	+	–	–	+	+	+	–	–	–	+	O	G +ve	Bacillus mascerans *
6	DB6	+	+	+	+	–	+	–	+	+	–	+	–	+	–	–	–	–	–	G +ve	Lactobacillus plantarum
7	DB7	+	+	–	+	–	+	–	+	+	–	+	+	+	–	–	–	–	–	G +ve	Bacillus sp.
8	DB8	+	+	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–	–	G +ve	Lactobacillus helveticus
9	DB9		+	–	–	+	–	+	–	–	–	–	+	–	–	+	+	–	–	G +ve	Micrococcus luteus
10	DB10	+	+	+	–	+	+	–	–	–	–	+	+	–	+	–	+	–	–	G –ve	Serratia marscence
11	DB11	+	+	+	+	–	–	–	–	–	+	+	+	+	–	–	–	+	–	G –ve	Klebsiella pneumonie
12	DB12	+	+	+	–	+	+	–	+	+		+	+	+	–	+	+	–	–	G +ve	Arthrobacter globiformis
13	DB13	–	–	–	–	–	+	+	–	+	+	+	+	–	–	+	+	+	–	G –ve	Pseudomonas sp. *

*** Cellulolytic organisms**

Table No 4 ; Bacterial isolates from dung slurry.

Sr. No.	Isolate Number	Glucose	Trehalose	Xylose	Lactose	Starch	Mannitol	Egg yolk reaction	Sucrose	M.R.	V.P.	Citrate	Gelatine liquification	Nitrate reduction	H_2S	Catalase	Oxidase	Arginine hydrolysis	Spore	Gram character	Nature of isolates
1	DSB1	–	–	–	–	+	+	+	–	+	+	+	+	–	–	+	+	+	–	G –ve	Pseudomonas fluoroscens *
2	DSB2	+	–	+	–	+	+	–	–	–	+	–	–	–	–	+	+	–	C	G +ve	Bacillus alvei
3	DSB3	+	+	+	–	+	+	+	–	–	+	–	+	+	–	+	+	+	C	G +ve	Bacillus amyloliquefaciens
4	DSB4	+	–	–	–	+	+	–	+	–	+	–	+	–	+	+	–	–	C	G +ve	Bacillus brevis
5	DSB5	+	+	+	–	+	+	+	–	–	+	+	–	+	–	+	+	+	C	G +ve	Bacillus coagulans
6	DSB6	+	+	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	C	G +ve	Bacillus circulans *
7	DSB7	+	+	+	+	–	+	–	+	+	–	+	–	+	–	–	–	–	T	G +ve	Lactobacillus plantarum
8	DSB8	+	+	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–	N	G +ve	Lactobacillus helveticus
9	DSB9	+	+	+	+	–	–	–	–	–	+	+	+	+	–	–	–	+	N	G –ve	Klebsiella sp.
10	DSB10	+	–	–	–	+	–	–	+	–	+	+	+	+	–	–	+	–	N	G –ve	Enterobacter aerogenes
11	DSB11	+	–	+	–	+	+	+	+	–	–	+	+	+	–	–	–	+	C	G +ve	Bacillus mascerans *
12	DSB12	+	+	+	–	+	–	–	–	–	–	–	–	+	–	–	+	–	–	G +ve	Micrococcus roseus
13	DSB13	+	+	+	–	+	+	–	+	+	–	+	+	+	–	+	+	–	N	G +ve	Arthrobacter sp.
14	DSB14	+	–	+	–	+	+	–	+	+	–	+	+	–	–	+	+	–	N	G –ve	Cellulomonas flavigera *
15	DSB15	+	–	+	–	–	–	+	+	–	–	–	–	–	–	+	–	–	N	G –ve	Flavobacterium sp.
16	DSB16	+	+	+	+	–	+	–	+	+	–	–	–	+	–	–	–	+	N	G –ve	Escherchia coli
17	DSB17	+	–	+	+	+	+	–	+	+	+	+	–	+	+	–	–	+	N	G –ve	Citrobacter sp.

Table No 5; Bacterial isolates from scale-up level (100-L) digester effluent.

Sr.No	Isolate Number	Glucose	Trehalose	Xylose	Lactose	Starch	Mannitol	Egg yolk reaction	Sucrose	M.R.	V.P.	Citrate	Gelatine liquification	Nitrate reduction	H_2S	Catalase	Oxidase	Arginine hydrolysis	Spore	Gram character	Identity of isolates
1	EB1	+	+	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	T	G +ve	Bacillus circulans *
2	EB2	+	+	+	–	+	+	+	–	–	–	+	–	+	–	+	+	–	C	G +ve	Bacillus firmus *
3	EB3	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	–	+	–	G – ve	Citrobacter freundii
4	EB4	+	+		+	–	–	–	–	+	–	–	–	+	–	–	–	–	–	G +ve	Lactobacillus helveticus
5	EB5	+	+	+	–	+	+	–	+	+	–	+	+	+	–	+	+	–	–	G – ve	Cellulomonas flavigera *
6	EB6	+	–	–	–	–	+	+	–	+	+	+	+	–	–	+	+	+	–	G – ve	Pseudomonas sp. *
7	EB7	+	–	–	–	–	–	+	+	–	–	–	–	–	–	+	–	–	–	G – ve	Flavobacterium sp.
8	EB8	+	+	+	–	+	+	–	+	+		+	+	+	–	+	+	–	–	G +ve	Arthrobacter sp.
9	EB9	+	+	+	–	–	+	–	+	+	+	+	+	+	+	–	–	–	–	G – ve	Proteus vulgaris
10	EB10	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	–	C	G +ve	Bacillus megaterium
11	EB11	+	–	–	–	+	+	+	–	+	+	+	+	–	–	+	+	+	–	G – ve	Pseudomonas aeruginosa
12	EB12	+	+	+	+	–	–	+	+	–	–	–	+	+	–	+	+	+	–	G +ve	Micrococcus varians
13	EB13	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	S	G +ve	Bacillus sphaericus
14	EB14	+	+	+	+	–	+	–	+	+	–	–	–	+	–	–	–	+	–	G – ve	Echerichia coli
15	EB15	+	–	–	–	–	–	+	+	+	–	–	–	+	+	–	+	+	c	G +ve	Bacillus cereus var mycoides

C – Central spore T – Terminal * Cellulolytic organism

Table No 6; SPC of different materials

Sr.No	Materials	Average SPC
1	Ipomoea weed surface	$8.13x10^4$
2	Distillery waste	$2.02x10^9$
3	Dung	$1.98x10^{10}$
4	Biogas digester effluent	$5.86x10^{10}$

Table No 7; Micro flora found common to digester effluent and to that of surface flora of Ipomoea material, distillery waste and dung slurry

Sr.No	Inoculum material	Micro flora common to anaerobic digester effluent
1	Ipomoea material	Bacillus cereus, Bacillus sphaericus, Bacillus megaterium
2	Distillery waste	Bacillus. Cereus, Bacillus megaterium, Bacillus circulans, Arthrobacter sp,. Pseudomonas sp.
3	Dung slurry	Pseudomonas sp., Bacillus circulans, Lactobacillus helveticus ,Arthrobacter sp,. Flavobacterium sp., Escherichia coli, Citrobacter sp.

Table No 8; Biogas production study in different digesters.

Sr.No	Digester capacity	Loading g BOD/day	Biogas production L/kg BOD/day	Methane %
1	4-L	13.84	243.40	54.67
2	25-L	86.53	325	56.32
3	50-L	173.10	384.10	59.61
4	100-L	346.10	467.30	62.32

4) DISCUSSION

The study was carried out to report different types of bacteria on substrates and in effluent. The bacterial isolates from Ipomoea biomass were 22 (Table No2) and majority of isolates were Bacillus (16) other includes Listeria, Flavobacterium, Lactobacillus and Pseudomonas spp. 13 isolates(Table No3) were reported from distillery waste six bacillus and two spps of Lactobacillus while Micrococcus, Serratia, Klebsiella, Arthrobacto and Pseudomonas. Dung slurry contains 17 isolates (Table No4) six spps of bacillus and others ten . The digester effluent shows 15 types of isolates (Table No5) 5 bacillus spps and 10 other spps.

From the different substrates 52 bacterial isolates were isolated and which includes 13 different types of bacterial genera. These were Bacillus, Pseudomonas, Lactobacillus. Klebsiella, Enterob acter,Micrococcus,Arthrobacter,Cellulomonas,Flavobacterium,E scherichia,Citrobacter, Serratia ,Listeria. Out of these 13 types

of bacteria 10 were detected in effluent and Seratia, klebshiella and Enterobactor were not detected they may be killed during biomethanation process.

The effluent from digester was subjected to Standard Plate Count (SPC) of aerobic and facultative anaerobic bacteria. It was evident that the average counts of bacteria, 5.86 x 10^{10} colonies/g. When the inoculum materials count was compared with that of anaerobic digester effluent, it was observed that bacterial count of the digester effluent was at much higher side (Table No 6). The increased bacterial count in the digester could be due to increase in ambient temperature and better acclimatization.

Micro flora common to anaerobic digester effluent and the substrates like Ipomoea, Distillery waste and dung slurry shown in (Table No 7) clearly indicate that the source of organisms in the digester was from the different substrates and these organisms also resist the conditions prevailed in the biogas digester.

Non methanogenic bacteria from effluent of digester run on cattle dung slurry was isolated by (Gore JA 1979) it mainly includes species of Arthrobacter, Listeria, Citrobacter, Pseudomonas, Escherichia, Bacillus, Flavobacterium, Micrococcus and Lactobacillus. In present study all these organisms were reported and additional Listeria spp, Proteus vulgaris and Cellulomonas flavigera were also reported. Bacteria in distillery waste lagoons Studied and reported by(Kale SC. 1986) shows similar types of bacteria.

Pathade GR, (1995) showed isolation of Citrobacter freundii, Bacillus mascerans, Lactobacillus agilis, from digester of distillery waste treatment. These organisms also reported in effluent and additional Echerichia coli, Staphylococcus arueus, Streptococcus faecalis, Enterobacter cloacae, Proteus vulgaris, Alkaligenes faecalis, Flavobacterium rigense, Arthrobacter simplex, Micrococcus, Pseudomonas aeruginosa it indicates that these organisms came from other substrates.

It was reported previously that other biomasses admixed with cattle dung produced significant biogas from amylase treated ground nut shells Chandrashekhar V et al.(2000) the biogas produced was 125 L/kg TVS, biogas produced from water hyacinth was 50 L/kg TVS Unni BG, (2004), while biogas produced using distillery waste was about 469L/kg TVS waste .The biogas production in different digesters is shown in (Table No.8) at 100-L capacity digester 467.30 L/kg BOD/day biogas with 62.32% methane was produced which is very significant amount.

Many of these bacterial isolates were hydrolytic in nature, which showed fermentation of sugars like glucose, trehalose, xylose, mannitol and sucrose. Many of them also showed production of enzymes like catalase, oxidase, nitrate reductase, and M.R., V.P., citrate utilization, and H_2S tests positive. Thus, bacterial isolates of inoculum materials showed a variety of hydrolytic activities which were important in first stage of biotransformation process. The associative action of the various groups of microorganisms in production of biogas was studied by Godbole SH, Gore J, Ranade DR (1981). The products which were formed in first stage were used by acetogenic and methanogenic organisms to produce biogas.

The main aim of the study was to report the different types of aerobic, facultative anaerobic, non methanogenic bacteria present in substrate and effluent and their correlation and their role in biogas production. It was fulfilled to some extent but for detail microbiological study require the study of anaerobic acetogenic and methanogenic bacteria also.

5) CONCLUSIONS

i) Bacterial count of the digester effluent was at much higher side it could be due to increase in ambient temperature and better acclimatization.
ii) 52 isolates were reported from the different substrates which contain 13 types of bacteria.
iii) 15 bacterial isolates obtained from the digester effluent having10 types of bacteria.
iv) All the 10 types of bacteria found in effluent were reported in the substrates indicating the source of organisms in digester is from its substrates and these organisms play important role in hydrolysis step.
v) Biochemical characteristics of the organisms show that they have hydrolytic activity which is essential for biomethanation process.
vi) Three types of bacteria were present in substrate but absent in digester effluent indicates conditions prevailing in digester were not suitable for its growth.
vii) 100-L biogas digester shows significant amount of biogas with high percentage of methane indicating conditions required for this process were optimum and having required microorganisms.

REFERENCE

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