International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 147-156
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved

Characterization of Biogas Slurry from Sadra, Gandhinagar District, Gujarat

[1]Priyanka A. Prajapati, [2]Noopur R. Goyal*, [3]Minal Trivedi and [4]Sumaiya A. Shaikh

[1]Department of Biotechnology Pacific University, Pacific Hills, Pratapnagar Extn, Airport Road, Debari, Udaipur, Rajasthan - 313003.

[2]M.G. Science Institute, Dada Saheb Mavlankar Campus, Opp. Gujarat University, Navrangpura, Ahmedabad, Gujarat - 380009.

[3]HVHP Institute of P.G. Studies and Research, S.V. Campus, Maniklal M Patel rd, Behind Railway Station, Ayodhya Nagar, Kadi, Gujarat - 382715.

[4]J. N. M. Patel Science College, D.C.Patel Navnirman Education Campus, New City Light Road, Bharthana, Vesu, Surat, Gujarat - 395017.

Received: 08 Jul 2019 / Accepted: 14 Aug 2019 / Published online: 1 Oct 2019
***Corresponding Author Email:** noopurgoyal@yahoo.com

Abstract

Biogas is a mixture of methane and carbon dioxide produced by anaerobic degradation of organic matter and used as a fuel. In the current study focusing on the characterization of biogas slurry from Sadra, Gandhinagar District, Gujarat. The physicochemical parameters like pH, BOD, COD, inorganic ion like Chlorine, heavy metal; residual sugar and protein concentration have been studied. Different bacteria and fungi were isolated using medium. The morphological and biochemical characteristic of isolates was performed. It was further confirmed with 16S rRNA sequencing & BLAST. Metagenomics of sample was also done.

Keywords

Biogas-Slurry, BLAST, Physiochemical, Protein, Sugar, Total viable count, 16S rRNA.

*****

INTRODUCTION

Human activities since civilization have resulted in huge amount of waste generation. In recent decades this has become hazardous for existence of humans on earth. Today a lot of emphasis is put on green technology. Biogas is on such method. Biogas technology uses cheap two benefits; it provides a mean for a sustainable waste management and fuel [1, 2].

By process of anaerobic digestion, both domestic and agricultural waste can be treated. This is the one of the oldest process [3]. In the anaerobic digestion methane and carbon dioxide are produced by degradation of organic matter [1, 4]. This process can be divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [5]. Anaerobic environmental ecosystems like sewage sludge, solid waste landfills and ruminant's stomach are natural habitat for Methanogens [6]. The slurry generated as end product that can be used as a fertilizer that in turn reduce the use chemical fertilizer which again abates pollution [7]. Anaerobic digestions are

influenced by many chemical and physical parameters like total contents of solids, the carbon & nitrogen contents and pH [8]. Also, the sludge digestion is dependent on microbial activity. Lots of concentration had more on methanogens but heterotrophs also play an important role in production of biogas. This study puts emphasis on heterotrophs present in slurry.

MATERIALS AND METHODS

Sample collection

Slurry sample was obtained from the Sadra, Gandhinagar District, Gujarat in the month of November.

Total Viable count

Total Viable Count was performed to assess no. of heterotrophs on Nutrient agar medium [9].

The Sugar Estimation

The sugar from slurry sample was estimated by Nelson-Somogyi procedure [10].

The Protein Estimation

The protein from slurry sample was estimated by Folin Lowery's procedure [10].

Isolation of bacterial colonies from biogas slurry

Biogas slurry sample was collected & homogenized. 20ml of sample was inoculated in 100ml of nutrient broth flask & incubate 24-48 hrs. Then streaking the Nutrient agar plate was streaking by using overnight culture flask & incubates at 37^{o}C for 24-48 hrs. After 48 hrs of incubation, colonies seen onto the medium were selected for microbial identification by using different mediums [4, 11].

Identification of bacterial isolates

(a) Morphological identification

Attempts were made to identify isolate using morphological & biochemical characteristics. Isolates were identified [12, 13].

Morphological characters include colony characters and Gram staining [4, 11, 14 and 15].

(b) Molecular identification

For the identification of bacterial isolates, 16S rRNA sequencing was done. 16S rRNA sequences of bacterial strains were deposited in the GenBank and analyzed using the BLAST program in GenBank at National Centre for Biotechnology Information https://www.ncbi.nlm.nih.gov/ [4].

Metagenomics

Metagenomics of slurry sample was done by standard methods [16].

RESULTS AND DISCUSSIONS

Total Viable Count

The total viable count for this sample was 85*10^{4} CFU/ml of sample.

The Estimation of Sugar& Protein

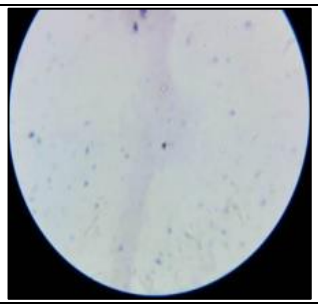
Sugar and Protein concentration was 1.21 mg/ml & 0.072 mg/ml respectively.

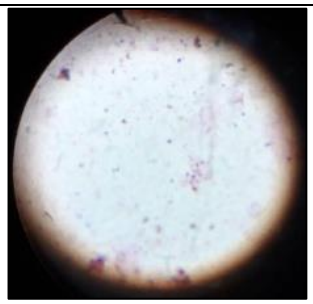
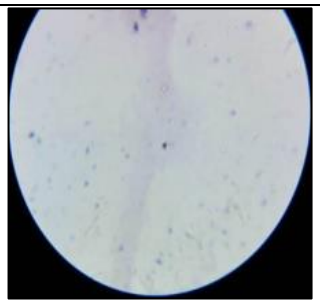
Identification of bacterial isolates

Morphological Identification

Total three isolates were isolated from the slurry sample [13]. Total two Gram negative and Gram-positive isolates & one Actinomycete were isolated from the slurry sample.

Table -1 depicts results of Gram staining & colony characters of isolates obtained on different media.

Sr. No.	Isolates	Colony Characteristics	Gram staining	Figure
1	Isolate 1	Large, circular, moist, flat, transculent, bluish-green pigment	Gram negative short rods.	

2	Isolate 2	Small, smooth, moist, regular, low convex	Gram positive coccii	
3	Isolate 3	Dry, powdery, chalky white colonies small in size and flat opaque.	Gram positive filamentous growth without spores but fragments	

Two bacterial isolates giving prominent growth & one Actinomycete were selected for further study.

Table - 2 Sugar utilization

Sr. No.	Isolates	Sugar fermentation					
		Glucose	Maltose	Mannitol	Sucrose	Lactose	Xylose
1	Isolate 1	+	+	+	+	+	-
2	Isolate 2	+	+	-	-	-	+

Isolates 1 shows Xylose negative and remaining all sugar should be positive while isolates 2 shows the Glucose, Maltose and Xylose should be positive & Mannitol, Sucrose and Lactose should be negative.

Table - 3 Biochemical test

Sr. No.	Isolates	Indole	MR	VP	Citrate	Urea	Nitrate	Gelatin	H_2S	TSI
1	Isolate 1	+	-	-	-	-	+	-	-	+
2	Isolate 2	+	+	-	-	+	+	-	-	-

Isolate 1 shows Indole, Nitrate and TSI should be positive while other test should be negative whereas in Isolate 2, it shows the Indole, MR, Urea & Nitrate should be positive while other test should be negative.

Molecular Identification

16s rRNA gene sequencing analysis revealed that bacterial isolates were *Pseudomonas aeruginosa*, *Staphylococcus succinus* & *Nocardiopsis terrae* obtained. Shown in Table - 4

Sr no	Accession no	Identified Organism
1	MH333221	*Pseudomonas aeruginosa*
2	MK045726	*Staphylococcus succinus*
3	MK045733	*Nocardiopsis terrae*

Metagenomics

Figure – 1 QC on Agarose gel

Table-5 Qualification using NanoDrop

Sr no	NanoDrop Readings (ng/µl)	NanoDrop OD $A_{260/280}$	NanoDrop OD $A_{260/230}$
1	12.3	1.98	0.97

Qualification of isolated metagenomic DNA sample on NanoDrop.
Nanodrop reading of DNA sample, Nanodrop OD $A_{260/280}$ and Nanodrop OD $A_{260/230}$ should be 12.3 ng/ µl, 1.98 and 0.97 respectively.

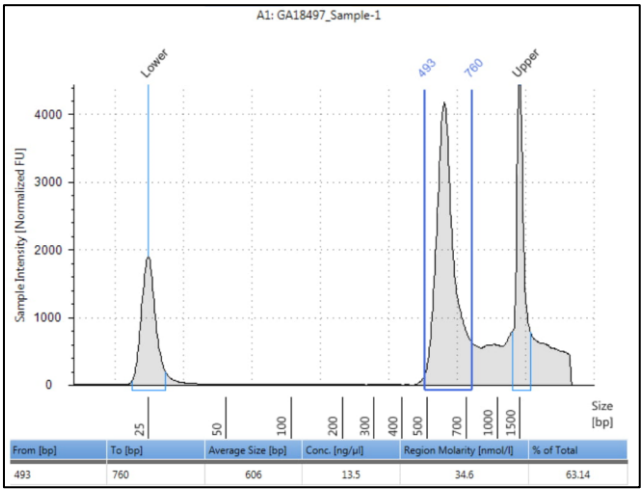


Figure: 2 Tape Station Profile Libraries

Library profile of sample on Agilent Tape Station using D1000 Screen Tape

The mean of library fragment size distribution was found to be 600bp

Bioinformatics

Results with different charts, figures at different taxonomical levels. These graphs are shows below.

1. High Quality FASTQ read statistics

Table- 6

#Reads	Total Bases	Data in MB
165,878	79,763,420	~80

2. Pie Chart

Pie Charts for the samples have been plotted at six taxonomic levels. These pie charts depict distribution of relative abundance profiles of Operational Taxonomic Units in sample with taxonomic assignments. In the pie charts, the top 19 categories at each of taxonomical levels have been plotted. The category "others" include the rests of the classification.

a. Taxonomic distribution of Sample at Phylum level

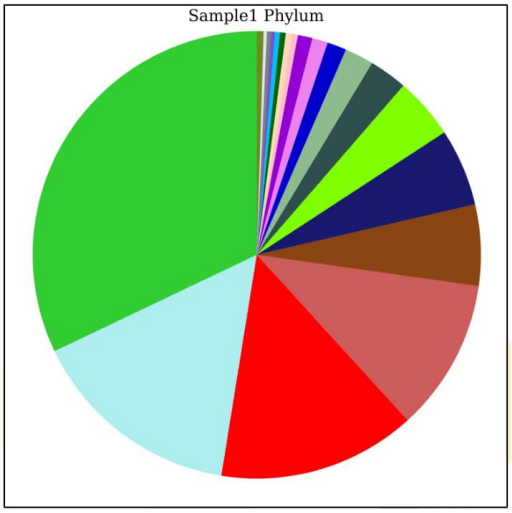


Figure – 3 Pie chart showing the absolute abundance of the sample at phylum level within each microbial community. From the figure it can be inferred that the most abundant phylum is Firmicutes

Phylum Legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Firmicutes	32.09%
	k__Bacteria;p__Bacteroidetes	15.38%
	k__Bacteria;p__Synergistetes	14.28%
	k__Archaea;p__Euryarchaeota	11.01%
	k__Bacteria;p__OP9	5.87%
	k__Bacteria;p__Verrucomicrobia	5.59%
	k__Bacteria;p__Chloroflexi	4.43%
	k__Bacteria;p__Thermotogae	2.75%
	k__Bacteria;p__Proteobacteria	2.09%
	k__Bacteria;p__Actinobacteria	1.39%
	k__Bacteria;p__Tenericutes	1.12%
	k__Bacteria;p__WS6	1.06%
	k__Bacteria;p__WWE1	0.44%
	k__Bacteria;p__Planctomycetes	0.43%
	k__Bacteria;p__SAR406	0.41%
	k__Bacteria;p__OD1	0.35%
	k__Bacteria;p__Lentisphaerae	0.3%
	k__Bacteria;p__Spirochaetes	0.29%
	k__Bacteria;p__WPS-2	0.23%
	Others	0.49%

Firmicutes, Bacteriods, Synergistetes and Euryarchaeota were the most abundant bacterial populations found at phylum level, accounting for 32.09%, 15.38%, 14.28% and 11.01% of all the bacteria reads, respectively [16, 17 and 18].

Various volatile fatty acids can be degraded by these syntrophic bacteria which belonged to the Firmicutes phylum [16].

b. Taxonomic distribution of Sample at class level

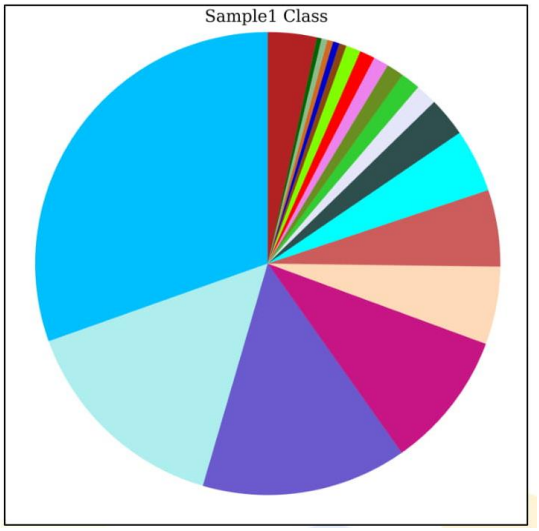


Figure - 4 Pie chart showing the absolute abundance of each class within each microbial community. From the figure, it can be inferred that the most abundant class is Clostridia

Class legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Firmicutes;c_Clostridia	30.44%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia	15.04%
	k_Bacteria;p_Synergistetes;c_Synergistia	14.28%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia	9.58%
	k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae]	5.42%
	k_Bacteria;p_OP9;c_JS1	5.36%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae	4.41%
	k_Bacteria;p_Thermotogae;c_Thermotogae	2.75%
	k_Bacteria;p_Firmicutes;c_Bacilli	1.49%
	k_Archaea;p_Euryarchaeota;c_Methanobacteria	1.36%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria	1.21%
	k_Bacteria;p_WS6;c_SC72	1.06%
	k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria	1.05%
	k_Bacteria;p_Tenericutes;c_Mollicutes	1.03%
	k_Bacteria;p_OP9;c_OPB46	0.51%
	k_Bacteria;p_WWE1;c_[Cloacamonae]	0.44%
	k_Bacteria;p_SAR406;c_AB16	0.41%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia	0.41%
	k_Bacteria;p_OD1;c_ABY1	0.32%
	Others	3.4%

Clostridia were associated with a high rate of hydrolysis in the anaerobic digestion sludge [16]. During the anaerobic digestion process, Bacteriodetes class was similar to the class Clostridia that play an important role in hydrolyzing and fermenting organic materials and producing low carbon organic acids, CO_2 and H_2. It is also well-known fermentative bacteria [16].

c. Taxonomic distribution of Sample at order level

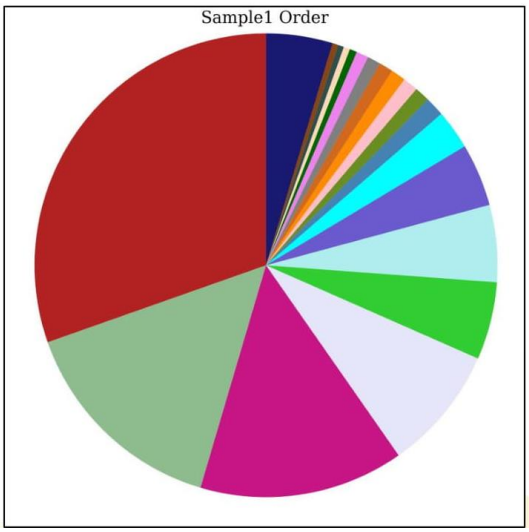


Figure - 5 Pie chart showing the absolute abundance of each order within each microbial community. From the figure, it can be inferred that the most abundant order is Clostridia

Order Legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	30.38%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	15.04%
	k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales	14.28%
	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales	8.7%
	k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales]	5.42%
	k__Bacteria;p__OP9;c__JS1;o__BA021	5.36%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	4.38%
	k__Bacteria;p__Thermotogae;c__Thermotogae;o__Thermotogales	2.75%
	k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales	1.36%
	k__Bacteria;p__WS6;c__SC72;o__WCHB1-15	1.06%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales	1.05%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales	1.04%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	0.98%
	k__Bacteria;p__Tenericutes;c__Mollicutes;o__Acholeplasmatales	0.92%
	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales	0.85%
	k__Bacteria;p__OP9;c__OPB46;o__SHA-1	0.51%
	k__Bacteria;p__WWE1;c__[Cloacamonae];o__[Cloacamonales]	0.44%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales	0.41%
	k__Bacteria;p__SAR406;c__AB16;o__Unclassified	0.41%
	Others	4.64%

Order level Clostridiales was followed by Bacteriodales and Synergistales [18].

d. Taxonomic distribution of Sample at Family level

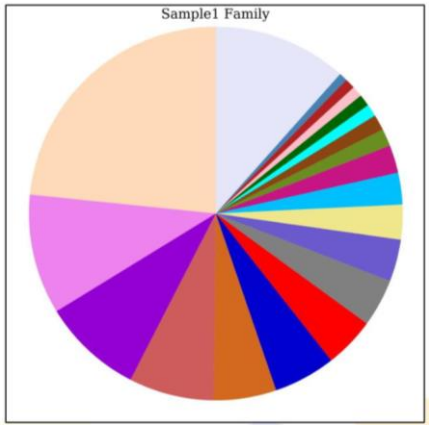


Figure - 6 Pie chart showing the absolute abundance of each family within each microbial community. From the figure, it can be inferred that the most abundant family is Clostridiaceae.

Family Legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae	23.38%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Unclassified	10.38%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_Methanosarcinales;f_Methanosaetaceae	8.69%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Thermovirgaceae	7.36%
	k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae];o_[Pedosphaerales];f_R4-41B	5.42%
	k_Bacteria;p_OP9;c_JS1;o_BA021;f_Unclassified	5.36%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae	4.38%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Mogibacteriaceae]	4.14%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae	3.64%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Dethiosulfovibrionaceae	3.01%
	k_Bacteria;p_Thermotogae;c_Thermotogae;o_Thermotogales;f_Thermotogaceae	2.75%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Synergistaceae	2.41%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Aminiphilaceae	1.5%
	k_Archaea;p_Euryarchaeota;c_Methanobacteria;o_Methanobacteriales;f_Methanobacteriaceae	1.36%
	k_Bacteria;p_WS6;c_SC72;o_WCHB1-15;f_Unclassified	1.06%
	k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Campylobacteraceae	1.03%
	k_Bacteria;p_Tenericutes;c_Mollicutes;o_Acholeplasmatales;f_Acholeplasmataceae	0.92%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Actinomycetaceae	0.89%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_Methanomicrobiales;f_Methanospirillaceae	0.73%
	Others	11.58%

Sample belongs to the Clostridiaceae family having 23.38% reads

e. Taxonomic distribution of Sample at Genus level

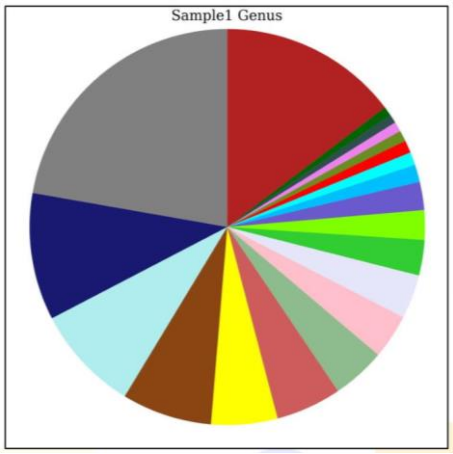


Figure - 7 Pie chart showing the absolute abundance of each genus within each microbial community. From the figure, it can be inferred that the most abundant genus is *Clostridium*.

Genus Legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Clostridium	22.25%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Unclassified;g_Unclassified	10.38%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_Methanosarcinales;f_Methanosaetaceae;g_Methanosaeta	8.69%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Thermovirgaceae;g_Unclassified	7.36%
	k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae];o_[Pedosphaerales];f_R4-41B;g_Unclassified	5.42%
	k_Bacteria;p_OP9;c_JS1;o_BA021;f_Unclassified;g_Unclassified	5.36%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_T78	4.37%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Mogibacteriaceae];g_Anaerovorax	3.64%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Unclassified	3.59%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Dethiosulfovibrionaceae;g_HA73	2.9%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Synergistaceae;g_vadinCA02	2.41%
	k_Bacteria;p_Thermotogae;c_Thermotogae;o_Thermotogales;f_Thermotogaceae;g_Kosmotoga	2.3%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Aminiphilaceae;Other	1.42%
	k_Bacteria;p_WS6;c_SC72;o_WCHB1-15;f_Unclassified;g_Unclassified	1.06%
	k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Campylobacteraceae;g_Unclassified	1.02%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Actinomycetaceae;g_N09	0.89%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Unclassified	0.8%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_Methanomicrobiales;f_Methanospirillaceae;g_Methanospirillum	0.73%
	k_Archaea;p_Euryarchaeota;c_Methanobacteria;o_Methanobacteriales;f_Methanobacteriaceae;g_Methanobrevibacter	0.72%
	Others	14.69%

Clostridium was the major genome while Methano brevibacter was the minor genome in sample [18]. *Clostridium* fermented a wide variety of carbon source and produced VFAs and alcohols that serve as a substrate for methanogenesis [18].

CONCLUSION

- Total count of bacteria by standard plate count method gives the $85*10^4$ cfu/ml.
- The concentration of sugar & protein was 1.21 mg/ml & 0.072 mg/ml respectively.
- The heterotrophic organism like *Pseudomonas aeruginosa*, *Staphylococcus succinus* & *Nocardiopsis terrae* were identified in biogas plant slurry by 16S rRNA homology. The accession numbers were MH333221, MK045726 & MK045733 respectively.
- 32.09% Firmicutes were identified with metagenomics.
- Among heterotrophs most prominent organism found was *Clostridium*.

Acknowledgement

I am very thankful to the head of the microbiology department and the entire staff of the J. M. N. Patel Science college, Bhartana, Surat, Gujarat.

REFERENCES

[1] N. Voća, T. Krička, T. Ćosić, V. Rupić, Ž. Jukić, S. Kalambura, Digested residue as a fertilizer after the mesophilic process of anaerobic digestion, *Plant Soil Environ., 51, 2005* (6): 262–266, (2005).

[2] Sokan-Adeaga Adewale Allen, Oseji Mathew Ejike, Ana Godson R.E.E., Evaluation of Biogas Yield and Microbial Species from Selected Multi-biomass Feedstocks in Nigeria, *London Journal of Research in Science: Natural and Formal, Volume 17, Issue 1*, (2018).

[3] Shikha Mehta, Kamla Malik, NishaVerma and Ramesh Chander Anand, Effect of Microbial Inoculum on Biogas Production from Cattle Dung under Anaerobic Batch Digestion, *International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 7 Number 02*,897-904, (2018).

[4] P. Merlin Christy, L.R. Gopinath and D. Divya, Microbial dynamics during anaerobic digestion of cow dung, *International Journal of Plant, Animal and Environmental Sciences, volume 4, Issue 4*, 86-94, (2014).

[5] Mashudu Mukhuba, Ashira Roopnarain, Rasheed Adeleke, Mokhele Moeletsiand Rosina Makofane, Comparative assessment of bio-fertiliser quality of cow dung and anaerobic digestion effluent, Mukhuba et al., *Cogent Food & Agriculture* 4: 1435019, (2018).

[6] Krzysztof Ziemiński, and Magdalena Frąc, Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms, *African Journal of Biotechnology Vol. 11*(18), pp. 4127-4139, DOI: 10.5897 / AJBX11.054, (2012).

[7] Amabelia del Pino, Omar Casanova, Mónica Barbazán, Victoria Mancassola, Laura Arló, Liliana Borzacconi, Mauricio Passeggi, Agronomic Use of Slurry from Anaerobic Digestion of Agroindustrial Residues: Effects on Crop and Soil, *Journal of Sustainable Bioenergy Systems, 4, 87-96*, (2014) .

[8] Francesco Fantozzi, CinziaBuratti, Anaerobic digestion of mechanically treated OFMSW: Experimental data on biogas/methane production and residues characterization, *Bioresource Technology 102*, 8885–8892, (2011).

[9] Amrita shah and Subhas Chandra santra, Isolation and Characterization of Bacteria Isolated from Municipal Solid Waste for Production of Industrial Enzymes and Waste Degradation, Journal *of Microbiology & Experimentation, Volume 1 Issue 1*, 1-8, (2014).

[10] Holme, D.J., and Peck, H., *Analytical Biochemistry*, Longman Group Limited. Essex, England, (1983).

[11] L.R. Gopinath, P. Merlin Christy, K. Mahesh, R. Bhuvaneswari, D. Divya, Identification and Evaluation of Effective Bacterial Consortia for Efficient Biogas Production, *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 8, Issue 3 Ver. 1*, PP 80-86, (2014).

[12] S. Monica, L. Karthik, S. Mythiii and A. Sathlavelu, Formulation of effective Microbial Consortia and its Application for sewage Treatment, *Microbial & Biochemical Technology, volume 3(3)*, 051-055, (2011).

[13] Mohammad Badrud Duza And S A Mastan, Isolation, Characterization And Screening Of Enzyme Producing Bacteria From Different Soil Samples, *International Journal of Pharma and Bio Sciences, 4(3)*: (B) 813 – 824, (2013).

[14] C Keffala, F Zouhir, K Ben Hadj Abdallah, S Kammoun, Use of Bacteria and Yeast Strains for Dairy Wastewater Treatment, *International Journal of Research in Engineering and Technology, volume 06, Issue 04*, 108-113, (2017).

[15] Sharda Dhadse, N C Kankal and Bharti Kumari, study of diverse methanogenic and Non-methanogenic bacteria used for the enhancement of biogas production, *International Journal of life Sciences Biotechnology and Pharma Research, Vol. 1, No. 2*, (2012).

[16] Jianhua Guo, Yongzhen Peng, Bing-Jie Ni, Xiaoyu Han, Lu Fan and Zhiguo Yuan, Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing, *Guo et al. Microbial Cell*

Factories ,14:33, DOI 10.1186/s12934-015-0218-4, (2015).

[17] Yongjun Wei, Haokui Zhou, Jun Zhang, Lei Zhang, Alei Geng, Fanghua Liu, Guoping Zhao, Shengyue Wang, Zhihua Zhou and Xing Yan, Insight into Dominant Cellulolytic Bacteria from Two Biogas Digesters and Their Glycoside Hydrolase Genes, *PLOS ONE*, *DOI: 10.1371/journal.pone.0129921*, (2015).

[18] Mingwei Cai, David Wilkins, Jiapeng Chen, Siu-Kin Ng, Hongyuan Lu, YangyangJia and Patrick K. H. Lee, Metagenomic Reconstruction of Key Anaerobic Digestion Pathways in Municipal Sludge and Industrial Wastewater Biogas-Producing Systems, *Frontiers in Microbiology*, *doi: 10.3389/ fmicb. 2016.00778*, (2016).