Physicochemical and Microbial Characteristics of Slurry from Biogas Plant

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Abstract: *Biogas is a mixture of methane and carbon dioxide produced by anaerobic degradation of organic matter and used as a fuel. The physicochemical and microbial characteristics of slurry have been studied. Five slurry samples were collected from the different region across the North Gujarat. The pH of the samples were in the range of 7 to 9; BOD varied from 197 to 3950 mg/L; COD was in a range of 692 to 15462 mg/L, Chloride was in range of 1149 to 3948 mg/L while Copper was found in range from 0.190 to 0.700 m/L. Total viable counts of the samples were in a range from $85*10^4$ to $100*10^4$ CFU/mL. The sugar and protein were ranging from 0.645 to 0.835 mg/mL and 0.036 to 0.666 mg/mL respectively. Different microorganisms were isolated from the slurry samples.*

Key Words: *Biogas; Physicochemical parameters; Sugar; Protein.*

1. INTRODUCTION:

Renewable energy has remained one of the best alternatives for sustainable energy developed since the grid electricity has become too expensive. Biogas is one such source of renewable energy (S. T. Ubwa et al, 2013). It is a mixture of methane and carbon dioxide produced by bacterial degradation of organic matter and used as a fuel (N. Voća et al, 2005). Biogas slurry use as fertilizer that reduces the use of chemical fertilizer which leads to resource conservation (reduced consumption of fossil fuels and mineral resources), climate change mitigation and soil quality maintenance (Amabelia del Pino et al, 2014). After purification, biogas can be directly used for energy or filling liquefied gas or connect gas pipeline directly also generate electric power by biogas (Hui Feng et al, 2011). Biogas is developed from the biogas slurry and its processing remains a significant challenge (Xue Wang et al, 2017).

In the production of biogas much of our biodegradable waste is procured from kitchen, agriculture and animal are used (S. Dash et al, 2016). Biogas production technology is based on the phenomenon of biological decomposition of organic materials (ER Rahul Kadam et al, 2017). During the anaerobic digestion, biogas is produced by four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Ramansu Goswami et al, 2016 and Mashudu Mukhuba et al, 2018). Four stages of biogas production were catalyzed by different group of microorganisms (Ramansu Goswami et al, 2016). Anaerobic digestion and biogas production is affected by several parameters like temperature, pH and buffering capacity etc. (U. Udochukwu et al, 2016). Bacteria from the facultative anaerobes of genera like *Streptococcus* & *Enterobacterium* are involved in the process of hydrolysis. Hydrolysis is the main step where heterotrophus bacteria and fungi are involved. It is the first step and microbial consortia would decide fate of substrate. In the hydrolysis process, organic substrate is converted into liquefied monomers by different group of bacteria. In the acidogenesis, *Pseudomonas, Bacillus, Clostridium, Micrococcus or Flavobacterium* bacteria are involved. Acidogenesis products are converted into hydrogen, carbon dioxide and acetic acid by acetogenic bacteria in acetogenesis steps. In this step, genera of *Syntrophomonas* and *Syntrophobacter* are involved. Methane and water are produced during methanogenesis process (Krzysztof Ziemiński, et al, 2012 and B. E. Asikong et al, 2016).

2. MATERIALS& METHODS:

2.1 Physicochemical Analysis: The liquid slurry samples from the biogas plant were collected & analyzed for the physicochemical parameters such as total dissolved solids, total solids, chloride as Cl, copper as Cu, COD, BOD, sulphide as S, total organic carbon and phosphate as PO4 (Lal Chand Malav et at, 2015, Sharda Dhadse et al, 2012). Total solids were determined by using IS3025 [P-15] 84Re.03 method. Total dissolved solids were determined by using [APHA 22nd Ed.2012, 2540-C], IS 3025 [part 16] 1984 (A. U. Ofoefule et al, 2010). Total phosphate content of the slurry sample was estimated by APHA 4500-P-C method (D. Indumathi, 2017).

2.2 Total Viable Count: Total viable count was performed using standard plate count method (Cappuccino J.G. et al, 2005).

2.3 Isolation of microorganism: The slurries were inoculated into liquid media and incubated at room temperature for 24-48 hours. After the incubation period, growth was observed then it was transferred on solids medium (plate). Plates were incubated at room temperature for 24-48 hours (Cappuccino J.G. et al, 2005).

2.4 Morphological studies of isolates: A single colony was isolated from the mixture of colonies through isolation process. This process was done by using streaking method to obtain pure cultures (WMF Wan Ishak et al, 2011).

1. **Colony characteristics:** Colony characters were studied by visual observation**.** (Mohammad Badrud Duza et al, 2013).
2. **Gram stain:** The organisms were further characterized by Gram staining. Gram character, arrangement, size and shape were noted (Sharda Dhadse et al, 2012).
3. **Biochemical characteristic:** Biochemical characteristics of microorganisms were studied to identify the unknown bacteria (L.R. Gopinath et al, 2014).

2.5 The Sugar Estimation: The sugar from slurry samples were identified by Nelson-Somogyi's procedure (Holme D.J. at el, 1983).

2.6 The Protein Estimation: The protein from slurry samples were identified by Folin-Lowery's procedure (Holme D. J. at el, 1983).

3. RESULT:

3.1 Characteristics of Slurry Samples: The characteristics of some of the important physicochemical parameters are listed in Table: 1. The COD concentration was in between 692 to 15462 mg/L. The BOD concentration ranged from 197 to 3950 mg/L. Total solids ranged from 7020 to 16920 mg/L. Total dissolved solids ranged from 2100 to 303040 mg/L.

Table: 1

Sr. No.	Parameters	Samples					Methods
		1	2	3	4	5	
1	Total Dissolved solids	7000 mg/L	6060 mg/L	5390 mg/L	303040 mg/L	2100 mg/L	(APHA 22nd Ed.,2012,2540-C), IS 3025 (part 16) 1984
2	Total solids	10512 mg/L	16920 mg/L	15780 mg/L	9220 mg/L	7020 mg/L	IS3025 (P-15) 84 Re.03
3	Chloride as Cl	3498 mg/L	2249 mg/L	1649 mg/L	1249 mg/L	1149 mg/L	IS3025 (P-32) 88 Re.99 Argento metric method.
4	Copper as Cu	0.260 mg/L	0.640 mg/L	0.580 mg/L	0.700 mg/L	0.190 mg/L	AAS-APHA (22nd Edi) 3111 B
5	COD	692 mg/L	15462 mg/L	14315 mg/L	14596 mg/L	8040 mg/L	APHA (22nd Edi) 5220-B (OPEN REFLUX)/ Saline SOP
6	BOD (3 days @ 27^0c)	197 mg/L	3950 mg/L	3900 mg/L	3850 mg/L	2300 mg/L	IS 3025 (P-44) 1993
7	Sulphide as S	18.66 mg/L	85.53 mg/L	93.31 mg/L	54.43 mg/L	45.10 mg/L	APHA (22nd Edi) 4500-S
8	Total organic carbon (TOC)	0.100 %	0.220 %	0.211 %	0.390 %	0.140 %	FCO, 2006
9	Phosphate as PO_4	122 mg/L	168 mg/L	274 mg/L	138 mg/L	127 mg/L	APHA 4500-P-C

3.2 Enumeration of bacteria by TVC method: The total viable counts were carried out and results are shown in Table: 2.

Table: 2

Samples	CFU/ml
1	$85*10^4$
2	$64*10^4$
3	$100*10^4$

Sample 3 shows the highest count- $100*10^4$ CFU/ml.

3.3 Morphological & bacteriological characteristics of bacterial isolates: Totally eight organisms were isolated, of them four were bacteria, three were fungi and one was actinomycetes (P. Merlin Christy et al, 2014, Sharda Dhadse et al, 2012). The cultural and morphological characteristics of the isolates are summarised in Tables: 3, 4 & 5.

Table: 3

Sr. No.	Isolates	Colony Characteristics	Gram Staining
1	Isolate-1	Large, circular, moist, flat, opaque, bluish-green pigment	Gram negative short rods
2	Isolate-2	Slightly larger, soft, regular, low convex	Gram positive cocci
3	Isolate-3	Medium, circular, convex, undulate	Gram negative rods
4	Isolate-4	Large, slightly raised, opaque	Gram positive rods

Table: 4

Sr. No.	Fungal Isolates	Morphological characteristics
1	Isolate-1	Black, large, circular, flat, powdery growth
2	Isolate-2	Green, large, circular, flat, powdery growth
3	Isolate-3	White, loose, woolly growth

Table: 5

Sr. No.	Isolate	Characteristics
1	Isolate-1	Small, raised, opaque, dry, Powdery, chalky white growth

3.4 Biochemical tests: Sugar fermentation test and biochemical test were performed for bacterial isolates. Isolate-1 gives all sugar positive except xylose and also it gives indole & nitrate positive. Isolate-4 gives all sugar positive while Indole, MR, Urea, H_2S are negative.

Table: 6

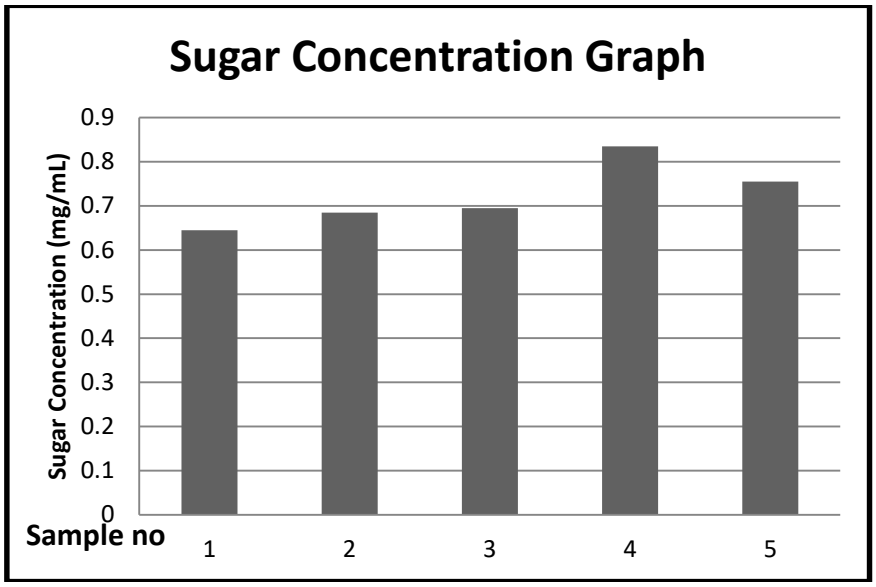
Sr. No.	Isolates	Sugar fermentation					
		Glucose	Maltose	Mannitol	Sucrose	Lactose	Xylose
1	Isolate-1	+	+	+	+	+	-
2	Isolate-2	+	+	-	-	-	+
3	Isolate-3	+	+	+	+	+	+
4	Isolate-4	+	+	+	+	Variable	+
Where: - = No Production, + = Gas & Acid Production, Variable = Present / absent							

Table: 7

Sr. No.	Isolates	Indole	MR	VP	Citrate	Urea	Nitrate	Gelatin	H_2S
1	Isolate-1	+	-	-	-	-	+	-	-
2	Isolate-2	+	+	-	-	+	+	-	-
3	Isolate-3	-	-	+	+	-	+	-	-
4	Isolate-4	-	-	+	+	-	+	+	-
Where: - = Negative results, + = Positive results									

3.5 The Sugar Estimation: The sugar estimation was carried out by Nelson-Somogyi's method and results are shown in Graph: 1.

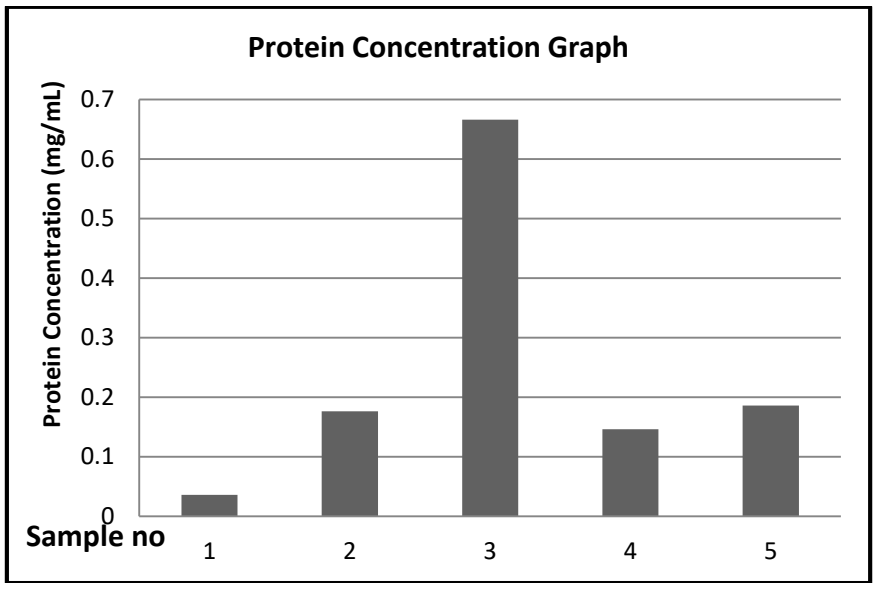
Graph: 1



Sample 4 gives highest sugar concentration.

3.6 The Protein Estimation: The protein estimation was carried out by Folin-Lowery's method and results are shown in Graph: 2.

Graph: 2



Sample 3 gives highest protein concentration.

4. DICUSSION :

Various physicochemical parameters were determined by using standard methods from the slurry samples. Cu concentration as well as sugar concentration increases in sample 4 (Table: 1 and Graph: 1). Total solids were found highest concentration in sample 2 where as it gave lowest CFU/ml (Table: 1 & 2). Sample 1 has the lowest concentration of COD, BOD, Organic carbon, Sulphide as S, phosphate as PO_4 and also sugar & protein concentration (Table: 1 and Graph: 1 & 2). Sample 3 has the highest protein concentration and also gave highest CFU/ml (Graph: 2 & Table: 2). Hidayati has also reported enumeration of bacteria using minimal medium from biogas slurry (Y. A. Hidayati et al, 2017). Biochemical characterizations of bacteria are shown in Table: 3, 4 & 5 (C Keffala et al, 2017 and B. E. Asikong et al, 2016).

5. CONCLUSION:

- Sample 4 has the highest total dissolved solids concentration where as sample 5 has the lowest concentration.
- Total eight isolates were found to be significant.
- Sample 2 has the highest BOD and COD value where sample 1 has the lowest BOD and COD value.
- Sample 4 has the highest Cu concentration.
- Sample 4 has the highest sugar concentration where sample 1 has lowest.
- Protein concentration was high in sample 3 and low in sample 1.
- Sample 3 shows the highest protein concentration along with highest CFU/ml.
- From the above study, it can be concluded that sample 3 gave more effective results than other samples.

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