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Isolation and Characterization of Microbial Community in Biogas Production from Different Commercially Active Fermentors in Different Regions of Gujranwala

Aisha Khalid and Shagufta Naz

Department of Biotechnology and Microbiology,
Lahore College for Women University, Pakistan

Abstract: The anaerobic digestion of organic wastes is a sustainable waste management strategy that produces an agriculturally valuable sludge, as well as biogas, which can be used to generate electricity. Therefore, an experiment has been carried out to isolate aerobic and anaerobic bacteria from biogas digester. Different bacterial species have been isolated from the biogas slurry prepared from cow dung. The morphological and microscopic studies have been carried out to study its gram staining properties. The aim was to link microbial community structure to process parameters. Sludge samples from 5 biogas plants were collected in 2013 which are located at different regions of Gujranwala. The main objective of this work is analyzed different bio gas plants and study microbiological process involve in bio gas digestion and isolate microorganisms in biogas slurry and identify the microbes which are present in bio gas digester from API kit(analytical profile index). Physiochemical analysis e.g. Moisture content, Ash content, Organic matter content, Nitrogen content, Carbon content, Nitrogen to Carbon ratio, Total solid, Volatile solid and pH were measured in the laboratory soon after collection. In anaerobic isolates both methanogenic and non methanogenic bacteria present e.g *Methanobrevibacte rruminantium, Methanobacterium formicicum, Bacteroides fragilis, Peptostreptococcus, Methanothrix soehngenii, Methanothrixsoehngenii, Clostridium difficile, Methanosarcinafrisia.* In aerobic isolates different bacteria e.g. *E coli, micrococcus, Bacillus anthracis, Enterococcus, Burkholderia vietnamiensis,Bacillus cereus, Bacillus anthracis, Bacillus subtilis,Corynebacterium amycolatum*, *Pseudomonas borbori, Salmonella enteric, Streptococcus bovis, Enterococcus.*

Key words: Microbial Community · Biogas Digester · Fermentors · Gujranwala · Methanogenic Bacteria · LCWU · PCSIR

INTRODUCTION

Human beings are the sole animals with the capability to ignite and usage a fire. This benefit has been significant for the growth of humanity, particularly during the preceding few decades, when the very quick rate of change in industry be particularly facilitated by the infinite richness of oil [1]. Livestock waste, like cow manure in the absence of applicable disposal actions can cause unfavorable environmental and health issues such as: air borne ammonia, exhalation, pathogen contamination, greenhouse gases, etc [2]. Anaerobic analysis comprises of decay of organic matter in the absence of charge less oxygen and result of methane, ammonia, carbon dioxide and traces of some other gases and organic acids of low molecular weight [3].

Methanogens (methane producing bacteria) are the last component in a chain of microbes which degrade natural material and return the decay products to the environment. In this series of action biogas is generated, a beginning of renewable energy [4]. Low sludge generation and high cost influence compared with aerobic assimilation make anaerobic digestion a suitable process for waste and waste H_2O treatment [5-8]. A wide domain of organic waste types could be used as substrate for the generation of biogas from anaerobic degeneration, such an animal dung, agricultural residual and by products, discharge sludge, source-separated domestic waste and natural industrial waste [9,10].

In addition to threatening greenhouse gas emissions over substitute of fossil fuel with biogas, the indirect environmental advantage associated with the anaerobic

Corresponding Author: Aisha Khalid, Department of Biotechnology and Microbiology, Lahore College for Women University, Pakistan.

degeneration of wastes are of great significance, particularly due to the forceful; impact of methanogen worldwide warming. Such advantage include a degradation in the spontaneous emission of NH_3 (ammonia) and methane (CH_4) that otherwise appear during composting or storage of untreated animal droppings [11, 12]. Hydrogen and carbon dioxide or acetate is used by methanogenic archaea for the fructification of methane, (also termed methanation) [13]. Finally, the methane is reformed to electricity. To close the series, in most cases, the digestate of the biogas-forming procedure can be recycled as soil amendments [14]. Community structure is frequently used to describe abundance determinations which include assimilation of the distinguish taxons [15]. Although it is burdensome to detect attenuated organisms in very distinct communities acting as those in anaerobic digesters, defining community morphology can provide precious information concerning the functional potential of the community [15, 16].

Environmental factors which effect biological reaction, such as pH, temperature, nutrients and inhibitors compositions are amenable to the extrinsic control in the anaerobic action. Any forceful change in these parameters can adversely act on the biogas production. So these factors should be adjusted in the desirable range to operate the anaerobic digester efficiently [17, 18]. Bio-slurry is the side product of anaerobic fermentation. It has various terminologies such as slurry, bio-fertilizers, biogas mulch, effluent etc. all mean the same [19].Investigations of microbial communities present in biogas or in natural gas have generally relied on the use of lab culture methods [20]. Very little is known about the interaction of the microorganisms inside a biogas reactor. Therefore it is important to understand and describe the microbial communities and growth dynamics inside the bioreactor in order to further optimize the conditions of biogas production [13].

MATERIALS AND METHODS

Isolation and characterization of microbial community in bio gas production from different commercially active fermentors in different regions of Gujranwala was done in Lahore College for Women University and PCSIR (Pakistan Council of Scientific and Industrial Research) Lahore. Visits of different biogas production plants were done which are located in different regions of Gujranwala for the quality production of biogas. The type of animal feed was judged and composition of substrate was checked.

Physical parameters of biogas digester includes Condition of biogas digester, Temperature and pH. The chemicals/reagents used for the research were of good purity. The chemicals were obtained from the Lahore College for Women University and PCSIR (Pakistan council of scientific and industrial research) Lahore. Nutrient broth, used for bacterial growth were prepared according to the methods recommended by Harrigan and McCance (1976) and Harrigan (1998). The pH of media was adjusted by using 0.1N NaOH and 0.1N HCl. The nutrient agar was prepared by adding 1.5% agar in nutrient broth (prepared above) and was autoclaved. Sterilized the Petri plates by autoclaving at 121°C for 15 minutes, under 15lb pressure and dried at oven. Cleaned the work area Bunsen burner. Pour 2/3 of the nutrient agar in Petri plates near the burner and cover the plates with its lid. Place on a flat surface undisturbed for about 10 minutes to allow the agar to completely gel then invert the plates and stored in plastic bags at 4°C. By using micropipette 100µl of the 10^{-5} dilution was then spread plated on the nutrient agar plates and further incubated at 35°C for 24h aerobically and an aerobically. Following incubation, distinct pure colonies obtained on the plates were isolated and purified using streak plate method and transformed to nutrient agar slants as stock.

Table 1: Locations of Biogas Digesters.

No.	No. of biogas digester	Location of biogas digesters
1.	No. of biogas digester 1	Located at Bank e Cheema village near Gujranwala.
2.	No. of biogas digester 2	Located at Pier coat road near the Ghakkhar (Gujranwala).
3.	No. of biogas digester 3	Located at Adil ghar near the Ghakkhar (Gujranwala).
4.	No. of biogas digester 4	Located at Shairan mor near Ghakkhar.
5.	No. of biogas digester 5	Located at Waris coat rice mill Ghakkhar.

Bacterial isolates were characterized using different staining procedure e.g. gram staining, acid fast staining, endospore staining and API (analytical profile index). The physicochemical analysis included determination of moisture content of the substrates, determination of ash content of the substrates, determination of organic matter content of the substrates, determination of carbon content of the substrates, determination of nitrogen content of the substrates by KJELDAHL method, determination of carbon to nitrogen ratio of the substrates, determination of total solid contentand determination of volatile solid content.

RESULTS AND DISCUSSIONS

This study investigated the effectiveness of cow dung for biogas production and isolation of microorganisms form bio gas digester. Bio wastes contain different types of pathogenic microorganisms, the type and load of which depends on the type of waste studied [7]. The anaerobic digestion of organic wastes is a sustainable waste management strategy that produces an agriculturally valuable sludge, as well as biogas, which can be used to generate electricity. Therefore, an experiment has been carried out to isolate aerobic and anaerobic bacteria from biogas digester. Different bacterial species have been isolated from the biogas slurry prepared from cow dung.

The impact of the composition and variety of the microbial community on the stableness of the biogas-forming progression and on the biogas output is of great interest [13].So far, various studies centralized on the microbial diversity in anaerobic digester supplied with renewable primary outcome and liquid fertilizer as substrates [21-26]. In contrast, this work examines and determines the composition and the variety of the microbial community, as well as the stableness of the microbial community over the interval of a mesophilic, frequently operated, agricultural biogas plant, which is especially supplied with biowaste (mainly antiseptic food residues and decayed bread in changeable compositions).in this work also checked the percentage of ash content, organic content, total volatile solids, carbon content, nitrogen content, carbon to nitrogen ratio. The percentage of organic content is higher in biogas digester 3 which is 60.00. Carbon content is higher in biogas digester 5 which is 6.8229. Percentage of nitrogen is higher in biogas digester 1 and 3 which is 0.2268. Carbon to nitrogen ratio is higher in biogas digester 5 which is 30.844. Total solid content is higher in biogas digester 1 is 233.435.volatile solid is higher in biogas digester 1 is 39.98.

Table 2: Organic matter of biogas digester

Number of biogas digester	Moisture content	Ash content	Organic matter 100-(moisture + ash)
Number of biogas digester 1	67.2	25.93	58.73
Number of biogas digester 2	78.94	25.93	46.99
Number of biogas digester 3	67.20	27.20	60.00
Number of biogas digester 4	75.97	25.88	49.91
Number of biogas digester 5	77.27	25.98	48.6

Table 3: Carbon content of biogas digester:

Number of biogas digester	Mv	M”	$TFeSo_4$-TB=V	F	Carbon (%)
Number of biogas digester 1	10	0.5	7.6-5.9 = 1.7	1.33	6.44385
Number of biogas digester 2	10	0.5	6.98-5.9 = 1.6	1.33	4.09374
Number of biogas digester 3	10	0.5	7.5-5.9 = 1.6	1.33	6.0648
Number of biogas digester 4	10	0.5	7.6-5.9 = 1.7	1.33	6.44385
Number of biogas digester 5	10	0.5	7.7-5.9 = 1.8	1.33	6.8229

Table 4: Nitrogen content of biogas digester

Number of biogas digester	T	B	MHCl	$V1Cm^3$	$V2Cm^3$	T-B	N (%)
Number of biogas digester 1	8.4	5.7	0.01	60	10	2.7	0.2268
Number of biogas digester 2	8.1	5.7	0.01	60	10	2.4	0.2016
Number of biogas digester 3	8.4	5.7	0.01	60	10	2.7	0.2268
Number of biogas digester 4	8.266	5.7	0.01	60	10	2.5	0.21559
Number of biogas digester 5	8.33	5.7	0.01	60	10	2.633	0.2212

Table 5: Determination of Carbon to Nitrogen ratio

Number of biogas digester	Carbon (%)	Nitrogen (%)	C/N
Number of biogas digester 1	6.44385	0.2268	28.41
Number of biogas digester 2	4.09374	0.2016	20.306
Number of biogas digester 3	6.0648	0.2268	26.740
Number of biogas digester 4	6.44385	0.21559	29.889
Number of biogas digester 5	6.8229	0.2212	30.844

Table 6: Determination of Total Solid Content

Number of biogas digester	Weight of resdue	Weight of sample (g)	Total solid content
Number of biogas digester 1	4.6687	2.0	233.435
Number of biogas digester 2	4.6587	2.0	232.935
Number of biogas digester 3	4.6887	2.0	232.435
Number of biogas digester 4	4.5789	2.0	232.945
Number of biogas digester 5	4.6589	2.0	228.945

Table 7: Determination of Volatile Solid Content

Number of biogas digester	W (total)	W (volatile)	W (sample)	% volatile solid
Number of biogas digester 1	6.6587	6.2589	2.0	39.98
Number of biogas digester 2	6.6587	6.3568	2.0	30.19
Number of biogas digester 3	6.6587	6.3968	2.0	26.01
Number of biogas digester 4	6.6587	6.3569	2.0	30.18
Number of biogas digester 5	6.6587	6.3789	2.0	27.98

Table 8: Means of Ash content of biogas digester, Moisture content of biogas digester

Number of biogas digester	Means
Ash content of biogas digester 1	25.93
Ash content of biogas digester 2	25.93
Ash content of biogas digester 3	27.20
Ash content of biogas digester 4	25.88
Ash content of biogas digester 5	25.93
Moisture content of biogas digester 1	67.2
Moisture content of biogas digester 2	78.94
Moisture content of biogas digester 3	67.20
Moisture content of biogas digester 4	77.27
Moisture content of biogas digester 5	75.97

In anaerobic isolates both methanogenic and non methanogenic bacteria present e.g *Methanobrevibacte rruminantium, Methanobacterium formicicum, Bacteroides fragilis, Peptostreptococcus, Methanothrix soehngenii, Methanothrixsoehngenii, Clostridium difficile, Methanosarcinafrisia.* In aerobic isolates different bacteria e.g. *E coli, micrococcus, Bacillus anthracis, Enterococcus, Burkholderia vietnamiensis, Bacillus cereus, Bacillus anthracis, Bacillus subtilis, Corynebacterium amycolatum*, *Pseudomonas borbori, Salmonella enteric, Streptococcus bovis, Enterococcus.* Microbial conversion of organic matter to methane has become attractive as a method of waste treatment and resource recovery. This process is anaerobic and is carried out by action of various groups of anaerobic bacteria.

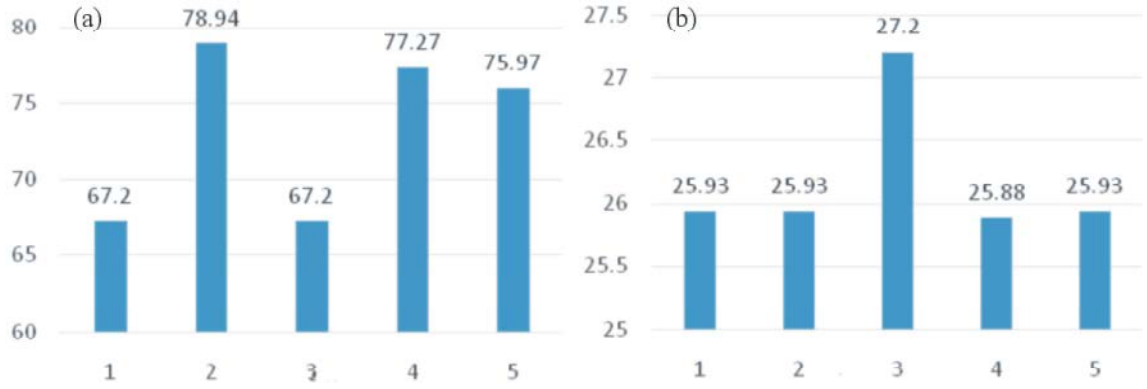

Fig. 1: Continued

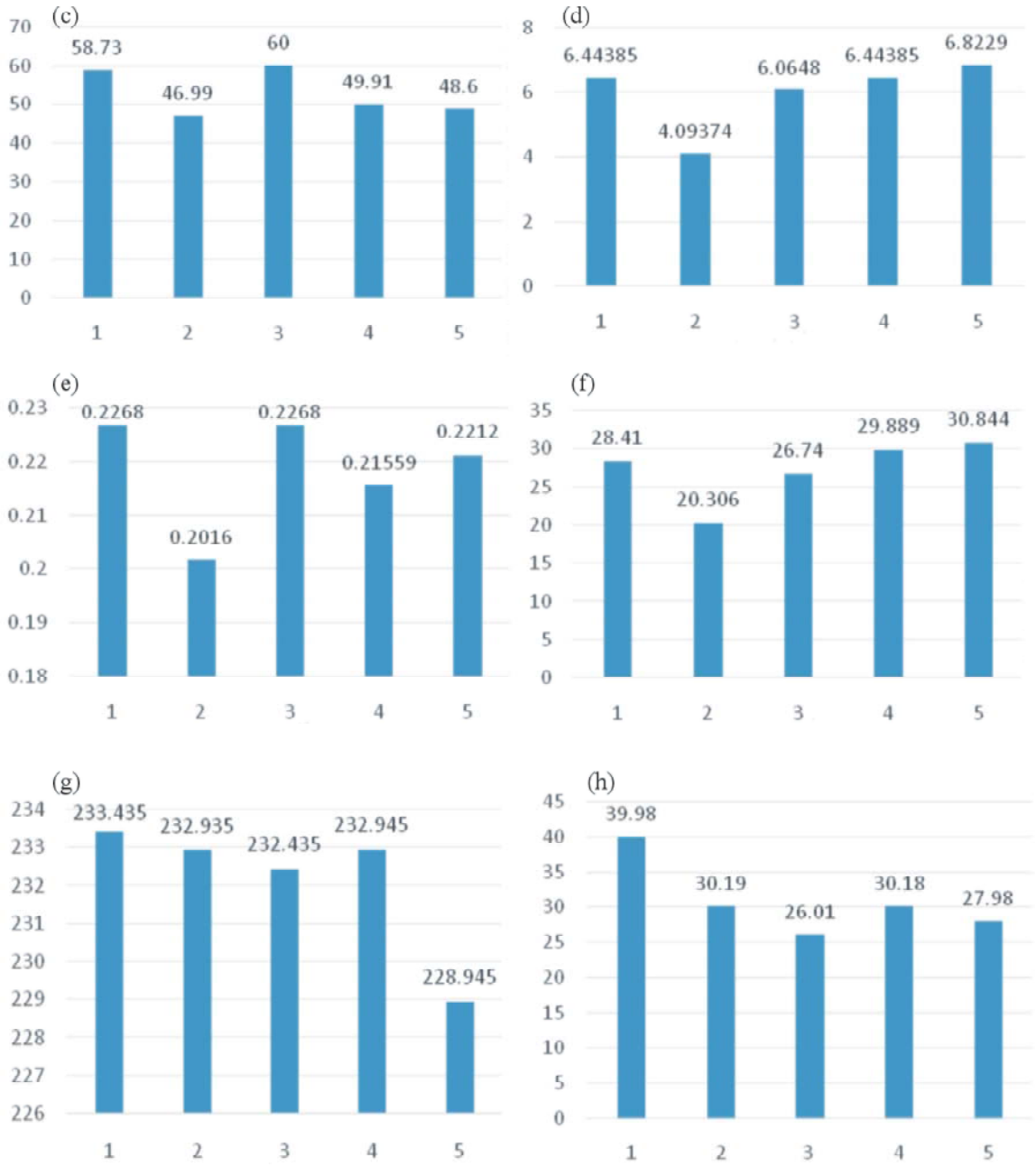

Fig. 5: shows the Graphical representation ofmoisture content (a), ash content (b), organic matter (c), carbon content (d), nitrogen content (e), carbon to nitrogen ratio (f), total solid content (g) and volatile solid content (h) of biogas digesters.

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