



Biogas Production by Anaerobic Digestion of Cow Dung using Floating Type Fermenter

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Abstract

Biogas is a clean conventional source of energy for the rural community. The anaerobic digestion is a technology used for recycling organic materials. In the present work, a floating type biogas digester is constructed and used to evaluate the quantity of produced gas, in addition, to determine its chemical composition. To examine Microbiological analysis, different samples are obtained from the floating type fermenter unite. Total solids, nitrogen carbon content and pH is determined from analysis of a cow dung samples. The obtained results show that, the total produced gas is found to be about 101.7 liters and the initial pH in fresh cow manure sample was 8.1, this value decreased during fermentation period until reached to 4.8 inside digester. The experiment is conducted at low temperatures range of ambient between 15°C and 23°C in a batch mode. Microbial load is determined also from the digested. Poured plate method, was used for counting total bacterial count, spore former count (aerobes or anaerobes), fungal count, Salmonella and Shigella count, Proteolytic count, amylolytic count and lipolytic count. The most probable number (MPN) technique was used for counting Coliform, anaerobic spore formers producing H₂S count and cellulolytic microorganisms. The highest value was 97x10⁸ cfu/ml in total bacterial count in fresh samples and lowest number was in the case of Salmonella and Shigella count in samples A, M and B was a same value being ND.

Keywords: Biomass, Biogas, Microbiology science, Anaerobic fermentation

1 Introduction

Recently, Biomass is important resources used as a renewable energy for many applications. Biogas is one of the biomass products, and it can be produced by the anaerobic digestion of the organic materials. Biogas is a gaseous fuel that is collected from the microbial disintegration of the organic materials under anaerobic situations. Biogas is consisted of methane (CH₄) and carbon dioxide (CO₂) in addition to some of trace gases depends on the types and quantities of the supplied feedstock [1]. There are many types substrate suitable for fermentation inside biogas stations and various technologies for handling these types. Moreover, there are many digester designs and arrangements of operation. The different types of biogas plants can be summarized as follows [2]:

- i. According to feed type (continuous type and non-continuous type (batch type)).
- ii. According to scale (large scale and small scale).
- iii. Wet and dry digesters.

Rihan and Jacinthe [3] studied the effect of using straw rice in biogas production in an Egyptian village called Kafr El-AMIR. The results show the advantages of biogas units are safe additional source of fuel, save effort and time. The disadvantages of using biogas units as indicated from kafr El-Amir are the weak availability of spare parts, weak availability of consistent maintenance staff and weak gas pressure. Daniel et al. [4] used many types of wastes in one digester, co-digestion, to improve the productivity of biogas. The mixing of wastes or other feedstocks for anaerobic digestion is an economical method for renewable energy production. To

prevent the low nitrogen content, which forms ammonia NH₃ that kills the Methanogenic bacteria, mixing the different wastes must be done in identified accurate ratios.

Algae is an effective source to produce a clean renewable fuel. Algae can be used to produce biodiesels and biogas, Nerlam and Singh [5]. There are two different algae groups (Macroalgae and Microalgae). A biogas is produced from microalgae by anaerobic digestion. The productivity of biogas can be increased by mixing the microalgae with sewage sludge or waste paper and therefore the C/N ratio of the digester feeding will increase. A Chinese study on mixing the Chinese food waste with tall fescue had been done by Ting et al. [6]. They discussed the results of mixing food waste with tall fescue in different ratios and the effect of that on the biogas production and the organic loading rate (OLR). Anaerobic digestion of Chinese food waste with tall fescue increased the methane yield and degradability efficiency compared to the anaerobic digestion of single substrate. Miah et al. [7] produced biogas from the poultry litter with co-substrate cow dung and poultry dropping. Poultry litter is a mixture of chicken excreta of broilers, saw dust and rice hulls. The researchers established four digesters with different ratio of poultry litter and cow dung. The all digesters were batch type and had the same size and the experiments had been done in the same conditions. The researchers found that the poultry litter has low C/N ratio, so ammonia could be formed and it is harmful gas because it is toxic and bacteria would die. Also, they found cow dung has high C/N ratio, so mixing the poultry litters with cow dung is much effective. The researchers found that cow dung at 25%

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and poultry litter at 75% was the optimum composition for biogas production.

Luter et al [8] investigated the biogas productivity from digesting maize cobs and from co-digesting the maize cobs with other wastes like cow manure or poultry dropping of goat faeces. The results indicated that the lowest biogas production was from the digester, which contained maize cobs only and the highest biogas production from the digester, which contained maize cobs with cow manure and poultry dropping. Youssef and El-bakhashwan [9] used the solar energy to increase the biogas production by controlling the temperature inside the digester. They built and installed a solar water heater at a farm scale operated digester of dairy cows' wastes in November and December at Alexandria, Egypt, in the winter season. The solar heater was connected to a heat exchanger inside the digester to maintain the temperature of wastes at the mesophilic temperature range (37 ± 2). The total biogas production rate and net energy production were increased by using the solar heating system. El-Bakshwan et al [10] studied the influence of mechanical stirring on biogas production. Stirring of the materials inside biogas digester is recommended to ensure good and warm contact between the microorganisms and particle organic material. Moreover, stirring increases the degradation of organic compounds and the biogas production. Stirring speed at 60 RPM gave the high values of biogas production rate, the stirring period of 15 Min/2Hr gave the maximum biogas production rate. Nino-Navarro et al. [11] discussed the effect of stirring by using two different types of impellers (pitched blade PB4 and radial flow (Rushton)). The results showed that axial pitched blade PB4 impeller is better than radial Rushton impeller in biohydrogen productivity because it improves the hydrodynamics inside the reactor and the hydrodynamic has great influence on mass transfer phenomena, especially the transfer of biohydrogen dissolved in the liquid state.

Zhang et al. [12] discussed the effect of thermophilic treatment process on the gas production from the anaerobic digestion of sewage sludge. The solar pond is used to increase the temperature of the digester to 53°C for improving the gas productivity. The experiments were conducted in batch mode. Zhang et al. [13] examined the impact of low temperature on the biogas production in cold rural areas in china and the impact of using a hybrid heating system of solar and biogas to improve the biogas production from the digestion process. The solar energy was used to heat the digesters at morning and a part of produced biogas was used to heat the digesters at night. Aliyu and Zahangir [14] discussed the different strategies for upgrading biogas. Upgrading and purifying the biogas is important process to enhance the methane content.

In this paper the suitable steps to establish a small biogas unit (floating type) with its components is presented. Moreover, the amount of biogas generated is evaluated. Furthermore, the gas components are analyzed. In addition, the microbial load is determined.

2 Materials and methods

2.1 Cow manure source

The biogas unit was established to produce biogas which is used as a heat source and examine the produced slurry which is used as a bio fertilizer. Experimental tests were conducted on the cow manure which was brought from a farm in Gamasa city near Port Said University and used to evaluate the different TS, C/N content, PH and volatile solids. Many experimental runs were held to examine the slurry produced from the biogas unit. While other experiments were done to evaluate the components

and the characteristics of the produced gas. Experimental test was done on a sample of a fresh cow manure to identify the percentage of total solids TS and is found to be 16 %. A 100 gm of manure was burnt in an oven at 105°C for 24 hours and the weight of sample after burning is 16 gm, the moisture content is 84% [7]. The nitrogen carbon content was determined with a C-H-N elemental analyzer and the C/N ratio is found to be 24. The samples PH were measured by using a digital PH meter, the initial pH in fresh sample was 8.1, this value decreased during fermentation period until reached to 4.9, 4.8 and 4.8 for samples A, M and B, respectively.

3 Materials and methods

3.1 Cow manure source

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3.2 Microbiological analysis

3.2.1 The collection and preparation of Samples

Four samples (fresh cow manure, the above (A), the middle (M) and the bottom (B) of the floating type fermenter unite at the end of the experiment) were collected. All samples were transferred into icebox to the laboratory of Agricultural microbiology Department, Faculty of Agriculture, Damietta University, Egypt, for determining general and specific microbial groups. Decimal serial dilutions were prepared and only one ml of each the last three dilutions were used for the following examinations. Specific methods and media were used for enumeration of the microbial counts of different groups of microbial flora such as nutrient agar medium [15] for both total bacterial count [16] and spore former count [17]. The detection of aerobic spore forming bacteria was done by pasteurization the dilutions at 80°C for twenty minutes in water bath, then the dilutions were transferred on nutrient agar medium and under aseptic conditions and the plats were incubated at 30°C for three days [18]. The detection of anaerobic spore forming bacteria was determined by using Cooked Meat Medium (CMM) [15]. The tubes were inoculated sealed with sterile mixture of Paraffin oil and Vaseline and in 1:1 ratio and incubated at $35 \pm 2^\circ\text{C}$ for up to seven days. The presence of *Clostridium* sp. was detected at the end of the incubation by measuring the gas layer which pushing the vaspar layer up [19]. Potato dextrose agar medium [15] for fungal count, MacconKey broth medium [17] for Coliform count [20], Staph medium No. 110 [21] plates for detection of *Staphylococcus aureus* [20]. Detection of anaerobic spore formers producing H_2S : Determine of anaerobic spore forming bacteria producing H_2S , using peptone iron agar (PIA) [15], in

five tubes for each dilution. The inoculated tubes were also, sealed with Vaseline and Paraffin oil and incubated at 55 °C for about three to five days. After incubation period the number of black tubes was counted [21]. SS agar medium [15] was used for *Salmonella* and *Shigella* determination [21]. Proteolytic, amylolytic and lipolytic microorganisms were determined on nutrient agar medium supplemented with skim milk, starch or oil, respectively [22]. Basal salt media containing filter paper for cellulolytic microorganisms [23].

3.2.2 Cultivation methods

Poured plate method [24] was used for total bacterial count, spore former count (aerobes or anaerobes), fungal count, *Salmonella* and *Shigella* count, Proteolytic count, amylolytic count and lipolytic count. From all the samples after preparing suitable serial dilutions, one ml was plated in triplicates into sterilized glass Petri dish. About fifteen ml of melted suitable medium at about 45°C was aseptically poured in each sterilized glass Petri plate, then mixed well and left the pates for solidification. All plates and tubes were incubated at a suitable temperature for a suitable time according the microbes in a digital incubator (Switc, MPM Instruments s.r.l., Bernareggio/Made in Italy). The developed colonies were counted per each plate, after the incubation period. The mean values of colonies were calculated as follows: The bacterial or fungal count (colony forming unite, cfu) (cfu/ml or cfu/g) = the mean number of three replicates of the same dilution x reciprocal of the dilution which used [17, 25, 26]. The most probable number (MPN) technique [17] was used for counting Coliform, anaerobic spore formers producing H₂S count and cellulolytic microorganisms. Three decimal dilutions of each sample in the last three replicates were used. 1 ml of each suitable dilution of samples were added to test tube containing suitable cultivation media, then incubated at suitable temperature for a suitable time according the microbes. The number of positive tubes were recorded. The most probable number of microbes per gram or ml of sample was calculated from standard Tables [27, 28,29].

3.3 Experimental set up and procedure

Experimental setup was established to investigate the biogas production from the anaerobic digestion of cow dung. The current biogas unit is classified as a floating type as shown in Figure 1. It consists of a 205 liter as a fermenter tank (2) and a 137 liter as a gas holder (3). The unit is provided with especially designed manual stirrer (6) to mix the slurry inside the tank (75.52 Cow dung + 75.085 water). This stirrer is 100 cm length; each blade is 7cm x 4cm x 4cm. Each stage consists of 3 blades and each blade is welded at angle 45°. The distance between each two stages of blades is 15 cm. A gas storage (5) is used to store the produced gas from the unit.

The following equations for the biogas unit will help to determine the size of this unit [30, 31, 32].

$$\text{Totally daily weight produced by N animals} = \text{manure from one animal} * \text{number of animals. (Kg/day)} \quad (1)$$

$$\text{Dry matter (DM)} = \text{Total daily weight} * \text{TS \%}. \text{ (Kg/day)} \quad (2)$$

$$\text{Total weight of slurry} = \frac{\text{dry matter (Kg/day)}}{\text{dry matter concentration of slurry}} \quad (3)$$

$$\text{Total volume of slurry} = \frac{\text{Total weight of slurry}}{\text{density of slurry}}. \text{ (m}^3\text{/day)}. \quad (4)$$

$$\text{Volume of digester} = \text{Total volume of slurry} * \text{hydraulic} \quad (5)$$

$$\text{Retention time (m}^3\text{)} \quad (5)$$

$$\text{Daily loading rate of volatile solids} = \frac{\text{Dry matter} * \text{VS\%}}{\text{digester volume}}. \quad (6)$$

$$\text{The volume of holder} = 10 \text{ to } 20 \% \text{ of the digester volume. (m}^3\text{)} \quad (7)$$

The experiment was held in a closed room at ambient average temperature of 15°C. During the experiment the volume of the produced gas was measured by measuring the distance or height of the floating gas holder which moves upward as shown in Figure 2 and Figure 3. As the gas holder moves upwards, we measure this distance starting from the white sign, then we multiple this distance with the cross-sectional area of gas holder to get the produced volume of generated biogas. The digester was operated in batch mode and fed manually and the stirrer was worked at different times manually.

- 1- Fermenter inlet.
- 2- Fermenter.
- 3- Gas holder with gas outlet.
- 4- Fermenter outlet.
- 5- Gas storage.
- 6- Stirrer

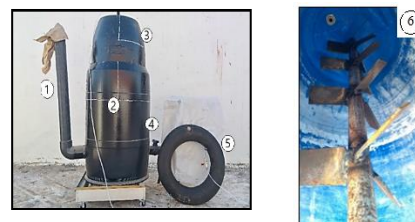


Figure 1: A photograph of floating type biogas unit



Figure 2: Measuring method of produced gas.

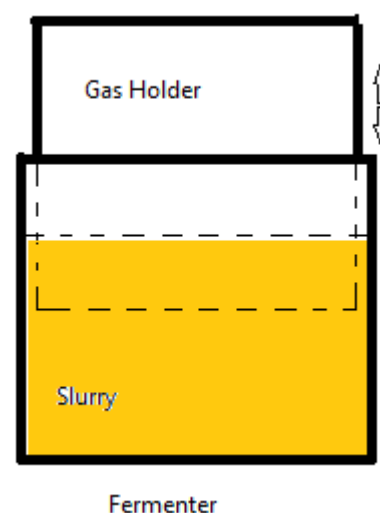


Figure 3: Sketch of measuring method of produced gas.

4 Uncertainty analysis

Normal uncertainties for instrumentation employed in the considered experimental setup are calculated using the below relation [33, 34, 35]:

$$u = a/\sqrt{3} \quad (8)$$

where x denotes to instrument accuracy and standard uncertainty is u (see Table 1).

Table 1: Instruments accurateness and standard uncertainties

Device	Accuracy	Range	Standard Uncertainty
Gas meter	0.05 L/min	5 L/min	0.02 L/min
Thermocouple	0.18 °C	0-150 °C	10.40x10 ⁻² °C
Rotameter	0.1 L/min	10 L/min	0.05 L/min
Pressure transducer	0.0125 bar	0-2.5 bar	7.2x10 ⁻³ bar
Balance	0.5 g	1 to 25000 g	0.289 g

5 Experimental results and discussion

5.1 Biogas production and composition

Experimental study held to study the biogas production from anaerobic digestion of cow dung at average temperature between 15 °C to 23 °C. The experiment was held at a batch mode and it took 84 days. The weekly gas production is shown in Fig. 3, where the maximum gas production was obtained at the 4th week as a result of high C/N ratio that reducing the hydraulic retention time. The total gas production is 101.7 liters.

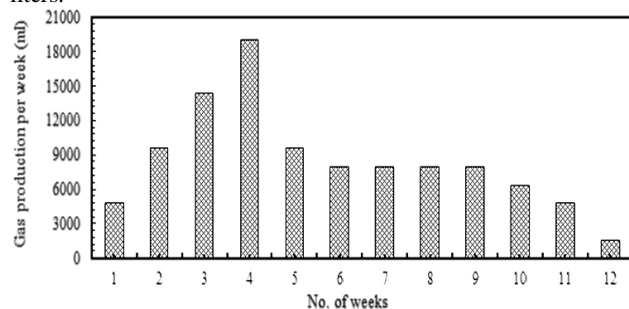


Figure 3: Gas productivity

The stirrer was used to mix the components of fermenter to enhance the quality of microbial load and to break the scum layer which was formed on the surface of sludge. The scum layer is harmful because it decreases the strength of microbes and so on the gas productivity is decreased. The total gas production from anaerobic digestion of cow dung from earlier work [36] where ambient temperatures varied between 18 and 28°C is a little similar to total gas production from anaerobic digestion of cow dung from the current work. The total gas production is low due to the low temperature of weather. The composition of biogas produced from the fermenter was determined through using a gas chromatograph. Table 2 shows the different composition of produced biogas. While Table 3 shows the summary of the result for the cow dung wastes for the 84 days period.

Table 2: Produced biogas composition by % volume

Waste Stock	Carbon Dioxide	Hydrogen Sulfide	Carbon Monoxide	Methane
Cow dung	32.2	0.1	6.7	60.9

Table 3: Summary of Results

Item	Cow Dung
Mass of Waste Used(kg)	75.52
Mass of Water Used (kg)	75.085
Total Mass of slurry(kg)	150.605
No of Days of Digestion	84
Total Volume of Gas Generated (L)	101.7
Maximum Ambient Temp. (°C)	23
Maximum Slurry Temp. (°C)	26
Peak volume of Gas. (L / week)	19.083

5.2 Microbiological analysis of the samples obtained from the floating type fermenter unit

The highest number of total bacterial count in water samples was in fresh sample being 97x10⁸ cfu/ml and lowest number was in sample No. A being 42x10⁸ cfu/ml. On the other hand, total bacterial count in samples M and B was a same value being 43 x10⁸ cfu/g. The maximum value of Total fungal count in water samples was in fresh sample being 65x10³ cfu/ml and lowest value was in sample No. B being 10x10³ cfu/ml. On the other hand, total fungal count in samples A and M were being 13 x10³ cfu/g and 12 x10³ cfu/g respectively. The maximum value of Spore formers count in water samples was in fresh sample being 75x10⁵ cfu/ml and lowest value was in sample No. M being 53x10⁵ cfu/ml. Moreover, Spore formers count in samples A and B were being 55 x10⁵ cfu/g and 61 x10⁵ cfu/g respectively. The maximum value of Coliform count in water samples was in fresh sample being 37x10⁵ cfu/ml. Coliform count in samples A, M and B was a same value being 36 x10⁵ cfu/g. The highest value of Staphylococci count in water samples was in fresh sample being 21x10³ cfu/ml and lowest value was in sample No. M being 18x10³ cfu/ml. On the other hand, Staphylococci count in samples A and B was a same value being 19 x10³ cfu/g.

The maximum value of Anaerobic spore forming bacteria count in water samples was in sample No. M being 72x10⁵ cfu/ml and lowest value was in fresh sample being 64x10⁵ cfu/ml. In addition, Anaerobic spore forming bacteria count in samples A and B were being 70 x10⁵ cfu/g and 69 x10⁵ cfu/g respectively. The highest value of Anaerobic spore formers producing H₂S count in water samples were in samples No. A and M being 72x10³ cfu/ml and lowest value was in fresh sample being 44x10³ cfu/ml. Anaerobic spore formers producing H₂S count in sample B was being 48 x10³ cfu/g. The highest value of Proteolytic microorganisms count in water samples were in samples No. A and fresh sample being 50x10⁴ cfu/ml and lowest value was in sample No. B being 46x10⁴ cfu/ml. Proteolytic microorganisms count in sample M was being 47 x10⁴ cfu/g.

The maximum value of Lipolytic microorganisms count in water samples was in fresh sample being 24x10⁴ cfu/ml and lowest value were in samples No. M and B being 16x10⁴ cfu/ml. Moreover, Lipolytic microorganisms count in sample A was being 17 x10⁴ cfu/g. The maximum value of Amylolytic microorganisms count in water samples was in fresh sample being 22x10⁴ cfu/ml and lowest value was in sample No. B being 13x10⁴ cfu/ml. Moreover, Amylolytic microorganisms count in samples A and M were being 18 x10⁴ cfu/g and 15 x10⁴ cfu/g respectively. The maximum value of Cellulolytic microorganisms count in water samples was in fresh sample being 89x10⁴ cfu/ml and lowest value was in sample No. B being 53x10⁴ cfu/ml. Moreover, Cellulolytic microorganisms

count in samples A and M were being 76×10^4 cfu/g and 73×10^4 cfu/g respectively. Finally, the maximum value of Salmonella and Shigella count in water samples was in fresh sample being 7×10^2 cfu/ml, while Salmonella and Shigella count in samples A, M and B was a same value being ND. Table 4 illustrates a summary of microbial analysis of the samples obtained from the floating type fermenter unit.

Table 4: Microbiological analysis of the samples obtained from the floating type fermenter unit

Microbiological analysis	Fresh	A	M	B
Total bacterial count (cfux 10^8)	97	42	43	43
Total fungal count (cfux 10^3)	65	13	12	10
Spore formers count (cfux 10^5)	75	55	53	61
Coliform count (cfux 10^5)	37	36	36	36
Staphylococci count (cfux 10^3)	21	19	18	19
Anaerobic spore forming bacteria count (cfux 10^5)	64	70	72	69
Anaerobic spore formers producing H ₂ S count (cfux 10^3)	44	51	51	48
Salmonella and Shigella count (cfux 10^2)	7	ND	ND	ND
Proteolytic microorganisms count (cfu $\times 10^4$)	50	50	47	46
Amylolytic microorganisms count (cfu $\times 10^4$)	22	18	15	13
Lipolytic microorganisms count (cfu $\times 10^4$)	24	17	16	16
Cellulolytic microorganisms count (cfu $\times 10^4$)	89	76	73	53

These results were similar to [37, 38] who reported that, during the anaerobic condition of biogas production twelve microbial groups included bacteria and fungi was observed. The microbial count in the first stage (fresh substrate) in most microbial groups was higher than the later stages. These changes in microbial counts were appeared in the final stage of biogas production. The present study took the same trend of [39,40, 41] who reported that, the degradation of cellulose by specific microorganisms shown to be importance and the presence of this bacterium was negatively correlated to the ammonia accumulation, this idea might have a negative impact on the degradation of cellulosic material. The period of fermentation time (12 week) was similar to [42] who reported that, the detection of methanogens and non-methanogens bacteria in the digester was 14 weeks. It is important to note that this treatment of this wastes caused a decrease in the number of pathogenic bacteria such as Salmonella, Shigella and Staphylococcus.

6 Conclusions

The experimental results show that, the produced biogas from fermenter is 101.7 liters. The fermenter stopped producing gas after 84th day. The produced biogas has methane yield of 60.9% by volume. The results indicate that, the low temperatures (cold weather) are not effective in producing biogas, so it is recommended to provide the unit with heat exchanger to increase the temperature of fermenter. During the anaerobic condition of biogas production twelve microbial groups included bacteria and fungi was observed. The microbial count in the first stage in most microbial groups was higher than the later stages. The changes in microbial counts were appeared in the final stage of biogas production. This experiment also, had an environmental dimension in terms of disposal of waste, produced an environmentally friendly materials, and disposal of pathogenic microbes.

Nomenclature

C/N	Carbon Nitrogen content
cfu	Colony forming unit
DM	Dry matter

HRT	Hydraulic retention time
OLR	Organic loading rate
VS	Volatile Solid
TS	Total Solid

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Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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