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RESEARCH ARTICLE

BIOGAS PRODUCTION FROM COW DUNG AND ISOLATION OF METHANOGENIC AND NON- METHANOGENIC BACTERIA IN YEMEN

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Abstract

Cow dung is a major source of biogas production and microorganisms play a vital role in production. this study aims to production of biogas by fermentation of cow dung. The production process has a batch digester (small size model); the biogas formed were measured by liquid displacement method.

The results of this study showed that the output of biogas production during summer and winter seasons were 2880 mL and 377 mL, respectively The pH value of cow dung was 7.32 while the pH value of biogas slurry was 7.91. temperature inside digester was higher than outside digester due to biogas production.

Different bacterial species had been isolated from the biogas slurry , which prepared from Cow dung after biogas production . Morphological and microscopic studies have been carried out to identify isolated bacteria. In anaerobic condition, both methanogenic and non-methanogenic bacteria were isolated e.g. Pseudomonas putida, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Proteus mirabilis, Proteus vulgaris, Enterobacterium cloacae, Streptococcus bovi, Methanobrevibacter smithii, Methanospirillum hungatii and Methanobacterium formicicum.

Keywords: Biogas, Cow dung, Biogas-slurry, Methanogenic bacteria.

1. Introduction

One of the most important challenges that our world will face in the Coming centuries will be continuing to meet the ever increasing energy needs of its citizen, especially With deep decrease of energy. **[1]**, on the other hand; animal wastes like cow dung with the absence of appropriate disposal methods can cause adverse environmental and health problems such as pathogen contamination, odor and air borne ammonia **[2]**.

The side effects and pollution caused by this animal waste can be eliminated in the production of biogas and be used as a fuel substitute **[1]**.

Biogas production is a complex biochemical process that takes place in the absence of oxygen **[3]**; it is also called swamp gas, sewer gas, digester gas and natural gas **[4]**.

The most used types of substrate in biogas technology are represented by: manure, residues or by-products from agriculture, energetic crops, organic waste from food industry, organic fraction of municipal solid wastes, sludge from wastewater treatment plants and food waste **[5]**.

There are four key biological and chemical stages of anaerobic digestion, hydrolysis, acidogenesis, acetogenesis and methanogenesis **[6-7].** Production of biogas in summer and winter season was studied by Almoustapha et al., **[8]** and Dhadse et al., **[9]**; They found that biogas production in summer was higher than winter season.

Cow dung (CD), coming from a rumen animal is known to contain the native microbial flora that aids in faster biogas production **[1]**. In nature, methanogens participate in the degradation of many organic compounds **[7-10]**.

Khalid and Naz, **[2]** in Pakistan, isolated both methanogenic and non-methanogenic bacteria from biogas slurry e.g *Methanobrevibacter ruminantium, Methanobacterium formicicum, Peptostreptococcus sp, Clostridium difficile, Escherichia coli, Micrococcus sp, Bacillus subtilis, and Streptococcus bovis.*

In another study in India; *Methanospirillum hungatei, Methanobrevibacter smithii, Pseudomonas putida, Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus bovis*, were isolated from cow dung **[11]**.

This study focuses on the following:

- Production of biogas from cow dung in two period summer and winter season
- Isolation of the methanogenic\ non methanogenic bacteria from biogas slurry which produce after the biogas production

2. Experimental Section

2.1 *Materials and methods*

Fresh cow dung were collected randomly from Sana'a city 1 Kg of cow dung was taken in clean bag, and homogenized, with 1 liter of distilled water in the ratio 1:1 then pouring to digester.

The main experiment apparatus consists of digester and biogas measurement. Anaerobic digester used for the biogas production from cow dung was a fixing batch prototype. The biogas formed was measured by liquid displacement method **[9-12]**.

The temperature was measured daily both outside and inside the digester by means of long thermometers as shown in fig 1. The fermentation was started up by providing the mixture in the reactor, and allowed to ferment until finished the production of biogas in an anaerobic condition.

The biogas production was checked daily. The effect experiments were carried out during the winter season and the summer season during (2015-2016)

Cow dung sample and biogas slurry were measured by pH meter (JENWAY Company, UK).

Figure 1: biogas digester.

Isolation of Methanogenic and Non-Methgenic bacteria:

Biogas slurry bacteria were isolated using the dilution plate method as described by Radhakrishnan & Ananthasubramanian **[13]**; with some modification. One mL of biogas slurry sample was added to 9 mL sterile water in a sterile conical flask of 250 ml capacity, which makes 10^{-1} dilution and subsequently diluted up to 10^{-5}. The culture medium used for the isolation of methanogenic bacteria was phosphate buffered basal (PBBM) medium as selective medium, which contained as mentioned in Zeikus et al., **[14]**.

The Methanogenic and Non- Methanogenic Bacteria were isolated from the biogas slurry and identified to genus and species level based on their morphological characteristics, Gram staining and biochemical test characteristics according to Holt **[15]**.

The following characteristics were studied: cell shape, Gram staining, IMViC Test (indol, motility, methyl red, voges proskauer, citrate test) and acid production, utilizing glucose and lactose, oxidase and catalase **[16-15-9]**

3. Results

Production of biogas from cow dung during winter and summer season

Biogas production started on the 3th day in the winter and on the 3th day in summer, with 35 ml and 120 ml, respectively. It was observed that biogas production was actually slow at the beginning and the ending of the experiment Fig.2. The highest biogas production was measured on the 7th day in summer season (420 ml), whereas the highest biogas production was measured on 6th Day in winter (110 ml).

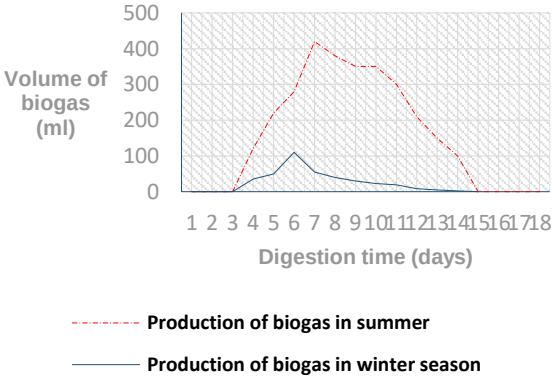

Figure 2: The cumulative biogas production during winter and summer season.

The pH value of cow dung was 7.32 while the pH value of biogas slurry was 7.91. also temperature inside digester was higher than outside digester.

When entering the cow dung sample to the digester it was quite similar to the dough. While it becomes more liquid when taken out of the digester as shown in the fig 3 ; which called. Which isolated from it the bacteria.

Figure 3: biogas slurry

In a preliminary study of biogas production and it has been tested of the ignition, lightening of the blue color flame was indicated for presence of methane as shown fig 4

Figure 4: biogas

Isolation and identification of methanogenic and non-methanogenic bacteria:

Eleven bacterial species have been isolated from biogas slurry prepared from cow dung.

In this study, 36 % of bacterial isolates were gram positive while the other 64 % were gram negative. 73 % of bacterial isolates were rod, 9 % cocci, 9 % cocci bacilli in shape as shown in Table (1). It is worthy to mention that only on isolate was of spiral shape.

Indole test which is the indicator for nitrogen metabolism; the ability that has role in biogas production was performed, and the results presented in Table (1), showed that 27% of isolated were positive and 73% were negative. As show in Table (1), 82% of bacterial isolates were positive to methyl red (MR) test, whereas 18% were negative; on the other hand, 27% were positive to voges proskauer (VP) test, while most of the isolates (73%) were negative.

Triple sugar iron test showed different results, as 55% of bacterial isolates were able to utilized both glucose and lactose , which 27% were able to utilize glucose only. The other 27% of bacterial isolates able to use neither glucose nor lactose (Table, 1). Further confirming tests were conducted, namely: citrate test, catalase and oxidase test. It was obvious that 73% were positive for citrate test, where the rest 27% were negative.

Catalase test showed positive results for 64% of the total isolates, whereas the other 36% were negative. In contrast, 36% and 64% of isolates were positive and negative for oxidase test, respectively (Table,1). Also test of gas production showed positive results for 64% of the total isolated whereas the other 36% were negative.

According to the previous observations, it was easy to categories the bacterial isolates obtained into two groups: Non-methanogenic bacteria: *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacterium cloacae*, *Streptococcus bovi*, and methanogenic bacterial: *Methanobrevibacter smithii*,. *Methanospirillum hungatii and Methanobacterium* formicicum, were a new recorded in Yemen.

Table 1: biochemical test for bacterial isolates.

NO.	IMViC Test								Catalase Test		Oxidase Test		TRI Test	Gas production	
	Indole test		MR Test		VP Test		Citrate Test								
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve		+ve	-ve
1		✓		✓		✓	✓		✓		✓		Y\Y	✓	
2		✓	✓			✓	✓			✓	✓		R\Y	✓	
3	✓		✓			✓		✓	✓			✓	Y\Y	✓	
4		✓	✓			✓		✓		✓	✓		Y\Y	✓	
5	✓		✓			✓	✓			✓	✓		Y\Y	✓	
6		✓	✓		✓		✓		✓			✓	Y\Y		✓
7	✓		✓			✓	✓		✓			✓	R\Y	✓	
8		✓	✓			✓	✓		✓			✓	R\Y		✓
9		✓		✓	✓		✓		✓			✓	Y\Y	✓	
10		✓	✓			✓		✓	✓			✓	Non		✓
11		✓	✓		✓		✓			✓		✓	Non		✓
Total (100%)	**27%**	**73%**	**82%**	**18%**	**27%**	**73%**	**73%**	**27%**	**64%**	**36%**	**36%**	**64%**	**Y\Y=55% R\Y=27% Non=18%**	**64%**	**36%**

4. Discussion

4.1 *Production of biogas:*

In general, biogas production rate tend to obey sigmoid function (S curve) as generally occurred in batch growth curve and as also has be resulted by Budiyono et al., (2010), Al Imam et al., **[1]** and Bassey et al., **[4]** This is pointed out that the production of biogas stages in batch pass the same stages of growth of the bacteria, for example; biogas production is very slow at the beginning and the end period of observation that mainly due to the lag phase of microbial growth.

Also this is predicted because biogas production rate in batch condition is directly equal to specific growth of methanogenic bacteria. In the around of the second day, biogas production is significantly increases due to exponential growth of microorganisms; in the end of production of biogas tend to decrease and this is predicted tend due to stationary phase of microbial growth **[17]**.

In this study, biogas production were carried out during winter and summer season, the amount of production has been observed in the summer more than winter. This is due to high temperature in the summer, hence the digester walls absorb or loose heat depending on the temperature gradient between the digester and its immediate environment, this implies that seasons affect the rate of heat loss or gain from the digester which in turn affects the microbial activities in the slurry at each stage **[17]**.

The increase of pH after biogas production is predicted due to degradation of protein to give ammonia **[12]**. Also high concentrations of pH due to the decomposition of substrate for bacteria to act on during anaerobic digestion **[18]**.

In addition, the process of bio-methanation is sensitive to changes in temperature **[7].** The intensity of microbial activity is a function of the of the environmental temperature, especially in methanogenesis, where in the degradation rate increases with temperature **[19].**

4.2 *Isolation of bacteria:*

Different results were obtained by Kavitha et al., **[3].**, as they isolated *Methanosarcina thermophile* to stimulate biogas production, and they recommends that, the microbes play crucial role in anaerobic digestion of each and every stage.

27% from all isolates were positive for indole test and 72% were negative test, Iodole is a nitrogen metabolism test. Positive to indole test representing, bacteria that it can act upon amino acids and undergo
deamination and hydrolysis leads to the formation of pyruvic acid and ammonia which leads to the production of methane and CO2 which is main function of methanogens **[11]**. Carbohydrate metabolism of isolates was assessed by conducting tests involving Methyl red (MR), Vogues Proskauer (VP). Methyl red test is used to identify bacteria that produce stable acid end products by means of mixed acid fermentation of glucose, whereas 82% from isolates were positive to MR test, 27% were positive VP test, positive to MR test shows that they use mixed acid pathway to metabolize pyruvic acid to other acids such as lactic, acetic acids which indicates their presence and growth in acid phase during biogas production, Negative to MR test indicates that it use butylene glycol pathway to metabolize pyruvic acid to neutral endproducts. VP test is used to determine the ability of organism to produce a neutral end product, acetoin from glucose fermentation **[11]**.

5. Conclusion

The production of biogas from cow dung has shown that flammable biogas can be produced from these wastes through anaerobic digestion for biogas generation. Thus, biogas production from cow dung is a good and cheap alternative source of energy.

The climate is key factor in biogas production, particularly the temperature, in which the biogas digester is operating, i.e. in warm temperatures the digestion rate is higher than in lower temperatures.

Various bacterial isolates from BGS which prepared from cow dung, have an important role in biogas production. Comparing the values with those obtained in aqueous, it was found that gemini micelles catalyze the reaction more. The use of a quite small quantity of the geminis provides less environmental impact when carrying out the reaction. An important point to be noted is that, at present reaction conditions, a small amount of organic solvents was sufficient to accelerate the reaction rate than that of pure water.

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مقالة بحثية

الهضم اللاهوائي لروث البقر لإنتاج الغاز الحيوي وعزل بكتريا الميثان وغير الميثان

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الملخص

روث البقر هو مصدر رئيسي لإنتاج الغاز الحيوي، وتلعب الكائنات الحية الدقيقة دورًا حيويًا في الإنتاج. عملية الإنتاج تتم بواسطة الهضم بدفعة واحده (نموذج صغير الحجم)؛ تم قياس الغاز الحيوي المتكون بطريقة الإزاحة السائلة.

أظهرت نتائج هذه الدراسة أن ناتج إنتاج الغاز الحيوي خلال المواسم الحارة والباردة كان 2880 مل و377 مل على التوالي. تم عزل أنواع بكتيرية مختلفة من طين الغاز الحيوي، والذي أُعد من روث البقر. أجريت دراسة مظهريه ميكروسكوبية للتعرف على البكتيريا المعزولة. في الظروف اللاهوائية تم عزل كلاً من بكتيريا الميثان وغير الميثان مثل

(Pseudomonas putida, Pseudomonas aeruginosa, E. coli, Bacillus subtilis, Proteus mirabilis, Proteus vulgaris, Enterobacterium cloacae, Streptococcus bovi, Methanobrevibacter smithii, Methanospirillum hungatii and Methanobacterium formicicum)

الكلمات الرئيسية: الغاز الحيوي، روث البقر، طين الغاز الحيوي، بكتيريا الميثان.