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Isolation, Identification and Characterization of Some Bacteria Associated with Biogas Production from Cow Dung

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Abstract

The industrial and non-industrial nations are both trapped between a rising inhabitants generating substantial amount of waste and the awaiting arrival of hard frontier to non-renewable energy sources. This study was aimed at isolation, identification and characterization of some bacteria associated with biogas generation via anaerobic digestion of cow dung using standard microbiological methods. Fresh cow dung was collected from Lafia abattoir, Nasarawa State, Nigeria. The sample was collected in a 100 g clean plastic container and transported to the laboratory for analysis. The total quantity of biogas produced from the cow dung by anaerobic digestion was 13.180 ml in the fifteenth (15^{th}) day of the digestion period with daily mean production of 878.67 ml. The least volume of about 700 ml was produced in the second day of digestion period. The total aerobic and anaerobic bacteria count ranged from 6.3×10^7 *to* 2.5×10^8 and 1.8×10^7 *to* 2.3×10^7 cfu/ml, respectively. The microorganisms isolated from the digester before, during and after anaerobic digestion include *Escherichia coli*, *Bacillus sp.*, *Pseudomonas sp.*, *Staphylococcus sp*., and *Proteus sp*. The results revealed that the generation of biogas from cow dung is potentially a good, cheap and alternative source of fuel or energy. The biogas technology is a good way of providing solution to the increasing waste management and disposal problems apart from the generation of fuel or energy from renewable energy sources.

Keywords: Cow dung, Biogas, Anaerobic digestion, Renewable energy, waste management

1. Introduction

In more recent time, there has been a huge demand for renewable energy sources worldwide. The industrial and non-industrial nations are both trapped between a rising inhabitants generating substantial amount of waste and the awaiting arrival of hard frontier to non-renewable energy sources. There is therefore the need for a clean and renewable energy sources in the developing world, where its competences could be introduced and maximised [1].

Biogas technology is an alternative option to local energy needs and has the capacity of providing significant benefits to human and the ecosystem [1]. The growing price rate of high-quality petroleum products used for industrial, agricultural and domestic fuels activities has witnessed a drastically increased level due to sudden increase in the activities of the masses. Also, there is paucity report on biogas production in Lafia, Nigeria. These activities make it hard for most individuals and nations to rise beyond subsistence level particularly, Nigeria, as one of the developing countries. These certainties have given rise to an enhancement in the exploration for renewable and sustainable alternative to fossil energies [2].

Animal wastes are known to contain high levels of nutrients which could be used as sources of energy to both macro and micro-organisms, and has the potential of being used as a source of energy to replace the existing non-renewable energy sources (such as crude petroleum, cooking gas); owed to their nature of being abundant all over the world. Nigeria have the capacity of producing over 227,500 tons of fresh waste each day [3], with the capacity of each 1kg of fresh animal waste to produce about $0.03m^3$ of gas daily. This could be inferred that; Nigeria can produce 6.8 million M^3 of biogas daily which corresponded to ca. 3.9 million litres of the petroleum product. Therefore, the production of biogas in Nigeria could provide a particular thrust in both cities and rural areas. A biogas plant does not need sophistication as locally available material could be used for the construction [4].

Cow dung is the undigested remains of already eaten food materials (mostly plants) being ejected by herbivorous animals. It is a mixture of faeces and urine in the ratio of 3:1, it consists primarily of lignin, cellulose and hemicelluloses. It could also be seen to consist of trace amounts of sulphur, iron, magnesium, copper,

cobalt or manganese along with different minerals like potassium, nitrogen etc [5].

Biogas production is promising as an alternative to other energy sources, which is capable of being used to generate electricity or provide energy for cooking, car fuelling among others [6]. Energy consumption rate within a nation represents the index of development and betterment of the standard of living. Globally, the concern is the rate of depletion and exhaustion of fossil energy reserves. This is because the rate of formation or development is not proportionate with that of consumption. Due to this fact, countries struggle for rare available fossil energy, thereby motivating researchers to look for other energy sources such as biogas [7].

Biogas is a combustible, pale gas generated through fermentation of animal, plant, human, industrial and public waste to produce methane (50-70%), Carbon dioxide (20-40%) and traces of other gases such as hydrogen, nitrogen, water vapour, hydrogen sulphide and ammonia in the absence of air (an-aerobic) [8,9,10]. Biogas is said to be produced when bacteria act favourably on organic resources in a process known as anaerobic digestion [10]. The process leads to the production of carbon dioxide gases, methane and a nearly stable residue of other materials [2]. Four stages are involved during anaerobic digestion process, namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The methane production process is attained at mesophilic (30-40°C), psychrophilic (10-25°C) or thermophilic temperatures (50-60°C) and sometimes can transpire under hydraulic flow regimes, a process known as Sequencing Batch Reactor (SBR), Batch Reactor (BR) or Continuous Flow Reactors (CFR). Continuous flow bioreactors are operated as a Completely Stirred Tank Reactor (CSTR) or as Plug Flow Reactor (PFR) [11-14].

The organisms concerned in the processes of biogas production are referred to as hydrolysers, acetogens, acetogens and methanogens [15]. Extracellular hydrolytic enzymes notably cellulase, xylanase, amylase, protease, lipase which the microbes excrete are involved in the hydrolytic stage of biogas production process and helps in hydrolyses of the polymeric resources to monomers while acidogenic bacteria convert sugars and amino acids produced in the first stage into carbon (IV) oxide (CO_2), ammonia (NH_3), hydrogen gas (H_2), and other organic acids or compounds; the more volatile fatty acids are then transformed into acetate ($C_2H_3O_2$), and hydrogen (H_3) by obligate hydrogen-producing acetogenic microorganisms during the process [16]. The accumulation of hydrogen has the ability of inhibiting the proliferation of acetogenic bacteria; thus, the preservation of an exceptionally low pressure is crucial for the acetogenic and hydrogen producing bacteria [16].

The last stage of the process (methanogenic stage) involves the production of methane from acetate ($C_2H_3O_2$) or hydrogen (H_2) and carbon dioxide (CO_2). These microbes (bacteria) are anaerobes in nature and require a lower redox potential for growth than most other anaerobic bacteria [17]. The composition of these microbes on cow dung depends mainly on factors like temperature, pH, substrate composition, mixing or the nature of the anaerobic digester [18].

Biogas emits very negligible amount of smoke or sometimes smokeless, hygienic and more convenient in comparison to other solid fuels [16]. Industrial and kitchen wastes, commonly known as garbage or trash, such as product packaging, grass clippings, furniture, clothing, bottles, food residues, newspapers and other appliances from hospitals, homes, schools and businesses are all reliable substrates for biogas production [19]. The biological treatment processes of biogas plant have advantages over the other energy such as;

(i) Economically available and attractive source of investment,
(ii) Operated easily without sophistication and safe to installation,
(iii) A renewable source of electricity and heat stable, resulting in a reduction of CO_2 emissions
(iv) Methane emissions are less from manure storage [16].
(v) Improved fertilizer quality when used as a source of manure [20],
(vi) Possibility to produce usable biogas that is about 60-80% methane with a fuel value of 17-23.9 MJ/m^3,
(vii) Odourless with reduced solid content when the substrate has been digested due to the degradative action of microorganisms.
(viii) That the digested sludge is been conserved in the digestion process resulting in the enhancement of the fertilizer value.
(ix) That pathogenic microorganisms such as *Salmonella* Sp. and *Brucella* Sp. as well as weed seeds are destroyed during the anaerobic digestion process [21].

The anaerobic digestion of public waste can have positive environmental value since it can combine waste removal and stabilization with net fuel (Biogas) production. The solid or liquid residue can further be used as feed or as biomass briquette for cooking [4,22].

Therefore, this research aimed at isolation, identification and characterization of some bacteria associated with biogas production via anaerobic digestion of cow dung in Lafia, Nasarawa State, Nigeria.

2. Materials and Methods

2.1 Research Area and Period

This study was carried out at Microbiology Laboratory, Department of Microbiology, Federal University of Lafia in Nasarawa State, Nigeria between the month of August 2019 and September 2019. Lafia is in the North central part of Nigeria, lying at latitude 8º29'30''. It has a total of 330, 712 inhabitants according to National Population Census [23,24].

2.2 Sample Collection

Fresh cow dung was collected from Lafia abattoir along Shinge road, Lafia, Nasarawa State, Nigeria. The sample was collected in a large clean plastic container and transported to the laboratory for further analytical work. The sample was air dried and pounded into powder using pistol and mortar. Hyacinth plants were also collected from Amba river along Doma road Lafia, Nasarawa State, dried using hot air oven in a beaker and later chopped into pieces. The two samples were then stored in the refrigerator. The Nutrient agar used in this study were prepared according to manufacturer's instructions and stored in the refrigerator for further use.

2.3 Digester Design

A 2.5 L capacity of amber bottle was used as reactor which contained the prepared slurry. The bottle was inserted into water bath to regulate and maintain the temperature at 35 ℃ - 40 ℃. The reactor was tightly closed air-tight with stopper and channelled to the first conical flask mounted on retort stand using delivery tube that was connected to an air-tight conical flask containing 1 M of sodium hydroxide (NaOH) and a small quantity of phenolphthalein was added as a pH indicator. The indicator turned pinkish violet in dilute solutions having pH above 8.2 and colourless when it was below pH 8.2. This flask served as carbon dioxide scrubber. The carbon dioxide scrubber usually faded when saturated with carbon dioxide. A delivery tubes and rubber tubing were again used to connect the conical flask with the second one containing water and methyl red as indicator. Each valve or connector was further fitted with rubber ring to ensure no air was entering. This served as displacement bottle supporting Mariotte principle of quantifying Methane, and the added drops of methyl orange only simplified the volumetric reading of the water displaced in the graduated cylinder that was channelled from the second conical flask as shown in Plate 1.

Plate 1: Biogas Digester Design

2.4 Preparation of Cow dung Slurry

Cow dung processing was done by methods previously used by [2]. 260 g dried cow dung was weighed using an automated weighing machine and mixed with 520 ml of water in ratio of 1:2 in a plastic calibrated container. The collected ruminant waste from the goat's gut was filtered using fine sieve until 100 ml of liquid inocula was obtained then added to the mixture above and stirred properly. The pH value of the slurry was determined using a digitalized pH reader which was adjusted to be 5.02 pH values. Small quantities of 1 M Sodium hydroxide was continually added with intermittent pH monitoring until pH value of about 7.03 is obtained. Five grams (5 g) of the dried chopped hyacinth plant was added to balances the nitrogen to carbon ratio.

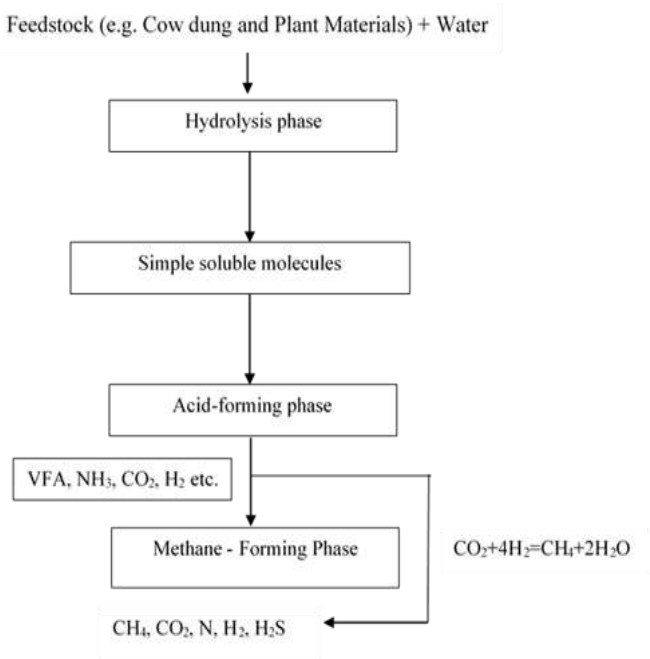

Figure 1: Diagram of biogas production process.

2.5 Feeding of Digester

The prepared organic waste (slurry) was charged into the reactor bottle that was connected to the digester using pipes. The mode of feeding used was a discontinued feeding. This simply means loading the digester at once and maintaining a closed environment throughout the retention period. The mixture of the slurry was charged loaded into the digester and allowed to undergo anaerobic digestion. The daily gas productions were monitored for fourteen (14) days retention time.

2.6 Collection of Biogas

The collection of biogases was by downward displacement of water. The displacement of water technique of biogas collection was a means in which gas could substitute water with equivalent quantity of water displaced and this would be used to establish the volume of gas generated daily. The biogas produced from the reactor bottle was connected to a separate inverted 1000 mL measuring cylinder. The volume of displaced water was recorded as the volume of gas produced.

2.7 Tests for the Presence of Methane

Methane gas (CH_4), which was the major component of the biogas that has combustible characteristic was tested by lighting a match on a Bunsen burner connected to the digester.

2.8 Preparation of Fresh Cow dung and Cow dung Slurry for Bacteriological Analysis

2.8.1 Total Bacterial Count

Preparation for fresh cow dung and cow dung slurry for bacterial analyses was done according to the methods described in reported literature [5]. Cow dung was prepared by serial dilution method. One gram (1 g) of dried cow dung sample was mixed in 10 ml sterilized phosphate buffer and vigorously shaken for about 2 minutes for proper mixing of the sample. Before plating, the sample was incubated at 37 °C for 30-40 min in incubator for activation of microorganism. After the incubation, standard dilution method was used to dilute the sample using a pipette. Nine millilitres (9 ml) of sterilized distilled water was used in the test tubes assembled for the serial dilutions. One millilitre (1 ml) of standardized solution was transferred aseptically into test tube 1 and 1 ml into test tube 2 and the procedure was repeated for each dilution in order to reduce the bacterial load from each test tube. The last 1ml was then discarded, while the 0.1 ml of the second and the fourth dilutions were used for the inoculation of the sample and was dispensed in the prepared medium (Nutrient agar) plate, which was then properly spread and incubated at 37 °C for about 24-48 h under aerobic and anaerobic conditions to obtain total aerobic and anaerobic bacterial counts. After incubation, the numbers of emergent colonies on each plate were counted using colony counter and the results are expressed as colony forming unit per millilitre (cfu/ml). The isolation and identification processes were carried out for both digested and undigested slurries.

2.9 Identification and characterization of bacterial isolates

The previously inoculated plate was checked for bacterial growth; the plates with viable culture were picked and sub-cultured on another media to obtain pure cultures of the colony. The isolates were then classified base on their colonial morphology notably: colour, shape, size, surface, edges, margins, elevation and gram staining as described in previous reports [4,25]. The isolates were further identified by comparing and referring to the identification manual of known taxa using the schemes reported in literature [26].

2.10 Spore Staining Test and Biochemical Characterization of the Isolates

The morphological examinations of the isolates, some spore staining and biochemical tests were carried out on the isolates with the intention to characterize and identify the organisms. The biochemical tests include indole, Coagulase, Urease, Oxidase, Methyl red, Voges-Proskauer, Catalase, Citrate utilization, Hydrogen sulphide according to [27].

3. Results and Discussion

3.1. Results

3.1.1. Anaerobic digestion and Biogas production

The total volume of biogas produced by anaerobic digestion of cow dung was 13,180 ml in the fifteenth (15^{th}) day of digestion period with daily mean production of 878.67 ml. The highest volume of biogas (13,180 ml) was produced in the fifteenth (15^{th}) day while the least volume (700 ml) was produced in the second day of digestion period (Table 3.1).

3.1.2. Bacterial count

The mean bacterial count before and after the digestion process is shown in Table 2. The count of aerobic organisms revealed a decrease trend from 2.5 x 10^8 cfu/ml in the first day of digestion to 6.3 x 10^7 cfu/ml in the fifteenth day. The anaerobic count was found to have an increasing trend from 1.8 x 10^7 cfu/ml in the first day of digestion to 2.3 x 10^7 cfu/ml in the last fifteenth day as can be seen in (Table 3.2).

3.1.3. Morphological, Gram staining, biochemical and identification of bacterial isolates from fresh cow dung slurry before, during and after anaerobic digestion.

The morphological characterization of isolates from fresh cow dung slurry before, during and after anaerobic digestion results are shown in Tables 3.3, 3.5 and 3.7 respectively.

Identification, Gram staining and Biochemical reaction of isolates from fresh cow dung slurry before, during and

after anaerobic digestion results are shown in Table 3.4, 3.6 and 3.8 respectively. The results revealed that before anaerobic digestion, three bacterial to include: *Staphylococcus* sp, *Proteus* sp and *Escherichia coli* were isolated; during anaerobic digestion two bacterial to include: *Pseudomonas* sp and *Staphylococcus* sp were isolated while after the anaerobic digestion, four bacterial to include: *Staphylococcus* sp, *Pseudomonas* sp, *Bacillus* sp and *Escherichia coli* were isolated.

Table 3.1: Biogas Production from Cow dung slurry

Day	Daily Biogas Production (ml)	Cumulative Biogas Production (ml)
1	0	0
2	700	700
3	650	1350
4	885	2235
5	1000	3235
6	950	4185
7	1160	5345
8	1600	6945
9	1650	8595
10	1200	9795
11	900	10695
12	800	11495
13	685	12180
14	600	12780
15	400	13180

Table 3.2: Total Bacterial Counts Before and During the Anaerobic Digestion Process

Days of digestion	TAC (cfu/ml)	TANC (cfu/ml)
0	2.5×10^8	1.8×10^7
7	2.1×10^8	2.2×10^8
15	6.3×10^7	2.3×10^7

Key: TAC = Total aerobic bacterial counts; TANC = Total anaerobic bacterial count; cfu = Colony forming unit; ml = millilitres

Table 3.3: Morphological characterization of isolated organisms found in fresh cow dung slurry before anaerobic digestion.

Characters Observed	1st Isolate	2nd Isolate	3rd Isolate
Forms of Colony	Circular	Circular	Circular
Translucency	Opaque	Opaque	Opaque
Elevation of Colony	Flat	Convex	Convex
Surface of Colony	Smooth	Swarm	Smooth
Pigmentation	Yellow-golden	Greenish-brown	Blue-purple
Cell shape	Coccus	Bacillus	Bacillus

Table 3.4: Identification, Gram staining and Biochemical reaction of isolates from fresh cow dung slurry before anaerobic digestion

Isolate Code	GR	Coag	Ure	Ind	Oxi	Cat	Glu	Mot	H_2S	Gas	Mb	Cit	VP	SP	Bacterial Isolated
1st	+	+	+	-	-	+	+	-	-	-	-	+	+	-	*Staph.* sp
2nd	-	-	+	wk	-	-	+	+	+	-	Wk	+	-	-	*Proteus* sp
3rd	-	-	-	+	-	-	+	+	-	+	Wk	-	-	-	*E*. coli

Key: + = positive reaction, = negative reaction, GR = Gram stain, Coag = Coagulase, Ure = Urease, Ind = Indole, Oxi = Oxidase, Cat = Catalase, Glu = Glucose, Mot = Motility, H2S = Hydrogen Sulphite, Mb = Methyl blue, Cit = Citrate, VP = Voges-Proskeur, SP = Spore, *Staph.* = *Staphylococci*, wk = weak reaction, E = *Escherichia*

Table 3.5: Morphological characterization of isolated organisms found in fresh cow dung slurry during anaerobic digestion

Characters observed	1st isolate	2nd isolate
Form of colony	Circular	Weakly irregular
Translucency/opacity	Opaque	Opaque
Elevation of colony	Convex	Undulate
Surface of colony	Rough	Rough
Pigmentation	Pearlescent	Reddish
Cell shape	Bacillus	

Table 3.6: Identification, Gram staining and Biochemical reaction of isolates from fresh cow dung slurry during anaerobic digestion

Isolate Code	GR	Coag	Ure	Ind	Oxi	Cat	Glu	Mot	H_2S	Gas	Mb	Cit	VP	SP	Bacterial Isolated
2nd	+	-	-	-	+	-	-	+	-	-	+	+	-	-	*Pseudo* sp
3rd	+	-	-	-	-	+	+	+	+	-	-	+	+	+	*Bacillus* sp

Key: + = positive reaction, = negative reaction, GR = Gram stain, Coag = Coagulase, Ure = Urease, Ind = Indole, Oxi = Oxidase, Cat = Catalase, Glu = Glucose, Mot = Motility, H_2S = Hydrogen Sulphite, Mb = Methyl blue, Cit = Citrate, VP = Voges-Proskeur, SP = Spore, *Pseudo.* = *Pseudomonas, Staph.* = *Staphylococci*

Table 3.7: Morphological characterization of isolated organisms found in fresh cow dung slurry after anaerobic digestion

Character observed	1st isolate	2nd isolate	3rd isolate	4th isolate
Form of colony	Circular	Circular	Weakly irregular	Circular
Translucency/opacity	Opaque	Opaque	Opaque	Opaque
Elevation of colony	Flat	Convex	Undulate	Convex
Surface of colony	Smooth	Rough	Rough	Smooth
Pigmentation	Yellowish-golden	Pearlescent	Reddish	Bluish-purple
Cell shape	Coccus	Bacillus	Bacillus	Bacillus

Table 3.8: Identification, Gram staining and Biochemical reaction of isolates from fresh cow dung slurry after anaerobic digestion

Isolate Code	GR	Coag	Ure	Ind	Oxi	Cat	Glu	Mot	H_2S	Gas	Mb	Cit	VP	SP	Bacterial Isolated
1st	+	+	+	-	-	+	+	-	-	-	-	+	+	-	*Staph.* Sp
2nd	+	-	-	-	+	-	-	+	-	-	+	+	-	-	*Pseudo.* Sp
3rd	+	-	-	-	-	+	+	+	+	-	-	+	+	+	*Bacillus* sp
4th	-	-	-	+	-	-	+	+	-	+	wk	-	-	-	*E.* coli

Key: + = positive reaction, = negative reaction, GR = Gram stain, Coag = Coagulase, Ure = Urease, Ind = Indole, Oxi = Oxidase, Cat = Catalase, Glu = Glucose, Mot = Motility, H_2S = Hydrogen Sulphite, Mb = Methyl blue, Cit = Citrate, VP = Voges-Proskeur, SP = Spore, *Pseudo.* = *Pseudomonas, Staph.* = *Staphylococci*, wk = weak reaction, *E* = *Escherichia*

3.1.4. The Presence of Methane in the Biogas Produced

The biogas produced was examined and established that the biogas was combustible (containing methane) with a bluish flame that lasted for 50 s.

3.2 Discussion

Energy is unavoidable when we come to control a nation's economy; it is one of the pointers for sustainable growth and development. The draining nature of petroleum and coal threatens source of fuel throughout the globe. The delinquent of petroleum and coal combustion leads to research work in all corners of human to get access to the alternate sources of energy. However, biogas is different from other renewable energy sources due to its features of using, controlling and collecting organic wastes [28]. The essence of biogas production is to produce gasses that are clean and safe with sparkling properties such as the one obtained in methane (CH_4) and carbon dioxide (CO_2), which generally convenient for lightening and cooking [5].

The finding from this study showed that biogas production of 700 ml was delayed till the seventh day. This could be connected to the fact that most cows nourish on fibrous sources and microbes need a longer time to degrade fibrous materials. This result agrees with previous observations reported [3]. The findings of this study showed that, biogas production was less and gradual in the first week of the research work. This suggests that the biogas producing microbes were in the lag phase of growth where adaptations or acclimatization of the cells take place. This result is similar to the one obtained by [29]. It can also be deduced that biogas production rate is equivalent or dependent on the growth of methanogens like the one obtained in this study, such that the more the growth and number the higher the biogas produced and the less their number, the lower the amount of biogas produced [29].

After the seventh (7^{th}) day of the research work, results showed a progressive increase in biogas production, this continued to the fifteenth (15^{th}) day. This indicated exponential stage in the growth of the methanogens Though, this result was different showing decrease of biogas production after the fifteenth (15^{th}) day as compared to other published works [29,30]. These changes observed may be due to the diverse breeds of

cows found in the different regions and nature of feeds or substrate.

The findings from this research work revealed that about 13,180ml (13.18litres) biogas was generated from anaerobic digestion of 260g dried cow dung in 520g of water in the ratio of 1:2. This result is however similar with the amount of biogas generated (124.3 litres of methane) from 17kg cow dung digested with 34 kg of water in the ratio of 1:2 (cow dung: water) [30]. This process was less efficient possibly due to the quantity of cow dung used in production process or due to the simple laboratory setup of the biodigester system used.

Also, in this research work, the biogas production started with no production in the first day (day 1), low performance from day 2 to day 4 which then rose from day 5 until it reached maximum daily production at seventh (7^{th}) day and increase to its highest volume (13,180 ml) at fifteenth (15^{th}) day. This agrees with a different study, which produced a progressive increase in production from 0 ml to 7.2 ml in day 5 and later increased in the last two remaining days out of seven days retention period [31]. This confirms their report that generally, biogas production increased from the beginning and as the days progressed, it reached an optimal value in a given time and may decrease after maximum gas generation.

In this research work, three bacterial isolates were isolated from fresh cow dung before anaerobic digestion and were identified as *Staphylococcus* sp, *Proteus* sp, and *E. coli* sp. Similar microorganisms including *Proteus, Salmonella, Streptococcus, Staphylococcus* and *E. Coli,* were isolated from cow dung [32]. The research work revealed that *Pseudomonas* sp and *Bacillus* sp were isolated from cow dung slurry during anaerobic digestion. This result concord with Pratik 20, patrik 30, patrik 40, patrik 50, which were later identified as *Pseudomonas* sp, and VBC 1, VBC 2, and VBC 3 identified as bacillus [33].

Furthermore, four bacterial isolates were isolated from cow dung slurry after the anaerobic digestion which included *Staphylococcus* sp., *Pseudomonas* sp, *Bacillus* sp., *and E. coli* spp. This is in accordance with the study where non methanogenic microorganisms from waste matter including *Listeria, Arthrobacter, Pseudomonas* sp., *Citrobacter* sp., *Bacillus* sp., *Escherichia coli, Staphylococcus, Lactobacillus, Flavobacterium,* and *Micrococcus* were isolated from cow dung slurry [34].

Generally, bacterial isolates from the digester before, during and after anaerobic digestion include *Escherichia* coli, *Pseudomonas* sp, *Bacillus* sp, *Staphylococcus* sp, *Bacillus* sp and *Proteus* sp. These microbes maybe accounted for the breaking down of the composite organic substance to intermediates such as unstable fatty acids which were finally changed to biogas. The isolation of *B. licheniformi*s and *E. coli* from biogas digesters were also reported [30,25]. *Bacillus, Yersinia* and *Pseudomonas* species were also responsible for biogas production in cow dung [35].

4. Conclusion

In conclusion, the potential of biogas production from cow dung have been demonstrated as a good, cheap and alternative source of fuel or energy. The production of the gas started on the seventh day and continued to increase till the fifteenth day of the research work. The microorganisms isolated from the digester before, during and after anaerobic digestion included: *Escherichia* coli, *Pseudomonas* sp., *Staphylococcus* sp., *Proteus* sp. and *Bacillus* sp. Biogas production when generated in commercial quantity provides an alternative source of sustainable energy, that can serve as a means of waste management and its disposal problems for the whole world particularly Nigeria.

5. Recommendations

(i) A pilot research work should be done using leaf litre seeded with cow dung as a substrate to produce biogas in large amounts.

(ii) The methanogenic bacteria involved in biogas generation should be isolated, identified and characterized to species and strain levels and then used selectively for biogas generation.

(iii) Digestion of organic waste compound should be encouraged. The reason being that generation of storable energy sources and manufacture of a stabilized deposit that can be transformed as fertilizer.

(iv) Further research work should be done to access the role of anaerobic digestion on microbial pathogens.

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