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HARNESSING BIOGAS PRODUCTION POTENTIAL OF *Jatropha curcas* SEED CAKE ALONE AND IN COMBINATION WITH COW DUNG AND GUTTER SLUDGE

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ABSTRACT
Agricultural wastes continually generated on daily basis unarguably results in environmental pollution. Converting the wastes to clean energy sources in large quantities is of paramount importance. This research aimed at generating and up-scaling biogas production using Jatropha seed cake as a substrate and cow dung and gutter's sludge as inocula. Samples of Jatropha cake, cow dung and gutter sludge were collected and physicochemical parameters which include pH, temperature, total solids, volatile solids, carbon-nitrogen ratio and organic matter of substrate and inocula were determined. Initially, 1.5L digesters were fabricated and fed for the pilot digestion then later 25L digesters for the upscale using the optimum conditions from the pilot studies. The gas produced was monitored weekly using a gas analyzer and the retention time lasted 25 days. Result revealed Jatropha seedcake had the least values of physicochemical parameters and cow dung having the best. The combination of Jatropha seedcake, cow dung, and gutter's sludge produced the highest percentage of methane 41.8% and Jatropha plus cow dung produced the least at 4.2% methane percentage. Result from the 25L anaerobic digester rapidly produced biogas after 24h but started depleting after 48h and was gone within a week.. Anaerobic microorganisms were isolated from the substrates and inocula but methanogenic colonies were isolated from lower dilution of gutter's sludge only which appeared in clusters, smears and confined colonies on the plate. This study confirms suitability of gutters sludge as inoculum in biogas production using Jatropha seed cake as substrate. Upscaling methane production requires further studies to determine the reasons behind methane loss.
Keywords: Jatropha, cow dung, gutters sludge, methane, biogas, methanogens.

INTRODUCTION

In order to minimize related global warming and climate change impacts, it is critical to explore and exploit new renewable and eco-friendly energy sources. Anaerobic digestion of crops, residues, and wastes is garnering traction as a method of reducing greenhouse gas emissions and to facilitate a sustainable development of energy supply (Weiland, 2010). Biogas production (Anaerobic digestion) provides a versatile carrier of renewable energy, as its end-products can be used for the replacement of fossil fuels in both heat and power generation and as a vehicle fuel (Weiland *et al*., 2009; Budiyono *et al*., 2018). The process produces gases principally methane (CH_4) and carbon dioxide (CO_2) which have a positive environmental impact as its reduction reduces global warming (Chibueze *et al*., 2017).

Recently, *Jatropha curcas* was discovered as one of the most promising sources, among many other species, that has relative high yield for biodiesel production. However, due to the presence of toxic materials such as crucin, sapronins, etcetera in the cake, it can neither be used as farm fertilizer nor as animal feed (Periyasamy and Nagarajau, 2012). This classifies it as a toxic waste. Jatropha seed cake is rich in organic matter and can be used as feedstock for energy generation. Thus, to solve the disposal issue in benefit of energy, proper exploitation of the cake for biogas production is necessary. The required volume or yield of Jatropha seedcake as a substrates has not been fully utilized. Hence, this study aimed at harnessing its potentials as substrate in biogas production and cow dung, and gutter's sludge as inocula.

MATERIALS AND METHODS

Sample Collection and Processing

For this study, the substrate; Jatropha seed cake and inocula; Cow dung and gutter's sludge were sourced locally. Cow dung sample was collected from a local cattle market located at Hauren Shanu in Gwale Local Government Area, Kano using hand with gloves into a clean polythene bag. Gutter's sludge sample was fetched from a gutter in the residential area of Dorayi Chiranchi located in Kumbotso Local Government Area, Kano using a shovel and introduced into a 20L container via funnel. While Jatropha seedcake sample was obtained from the Association of Jatropha Farmers, Processors and Marketers located at Zone B Kate, near Airport, Ibadan, Nigeria in a clean polythene bag. After which itwas taken to Plant Biology Department, B.U.K., Kano for Scientific Identification at the Herbarium Unit.All samples collected were measured and labelled accordingly before biogas set up and feeding.

Physicochemical parameters of the substrate and inocula

Physicochemical parameters which includes pH, Temperature, Total and volatile solids, moisture content, carbon/nitrogen ratio and Organic matter were analyzed individually for the substrate and inocula. pH was determined using method adopted by Wyasu, (2020). The pH meter was checked and calibrated before each measurement and the electrode of the pH meter inserted in the samples. pH value was measured by reading appearing on the pH meter. Temperature was measured using method described by TaiwoandOsoa, (2003). A mercury in glass thermometer was inserted deep into the samples and the reading was recorded after five (5) minutes. Moisture content and total solids were determined according to the method from WHO-IRCWD, (1978) while volatile solids from method previously described by Victorin, (2016). The Walkley-black's procedure method was used for the determination of Organic matter and Kjeldahl method for total carbon and nitrogen previously described by Hach, (2016).

Designing, Fabricating, Feeding and Running of the Pilot Anaerobic Digesters

Anaerobic digesters were constructed using 1.5L transparent plastic containers. Each container was channeled tightly to a gas collection unit (urine bags) using a connecting tube and candle wax for air sealing (Yusuf *et al.*, 2020). To modify, the digesters were sealed in sterile black polythene bags, to prevent light reflection on the transparent bottles which might lead to algae growth (Plate 1).

A total of four digesters (in duplicate) were set up labelled digester A to D, each containing different mix ratio as follows:

Digester A: 200 g of Jatropha seed cake + 1L water
Digester B: 100 g of Jatropha seed cake + 100 g of cow dung + 1L water
Digester C: 200 g of Jatropha seed cake + 1L gutter's sludge
Digester D: 100 g of Jatropha seed cake + 100 g of cow dung + 1L gutter's sludge

Cow dung and gutter's sludge served as inocula for the digestion process and Jatropha seedcake as substrate. The anaerobic digesters were allowed to ferment for an average of 25 days under ambient temperature. The percentage composition of the gas produced; methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) were determined using a Geo Tech Gas Analyzer (Biogas 5000).

Designing, Fabricating, Feeding and Running of the 25L Anaerobic Digester

A 25L black drum was connected to a plastic gas collection unit (balloon tyre) and a standing stove. This was achieved with the help of pipes, stoppers and gas controllers at the appropriate joints. The set-up was kept on a flat surface in an area with enough sun exposure. Guidelines were carried to ensure no leakage from the digesters as that would disrupt the anaerobic process.

The digester was fed with content of the pilot digester which produced the highest value of methane. The quantity of feeding was as follows;

3.3 kg Jatropha seedcake + 3.3 kg Cow dung + 10L Gutter's sludge + 8L water.

The digestion lasted 7 days and observations were recorded.

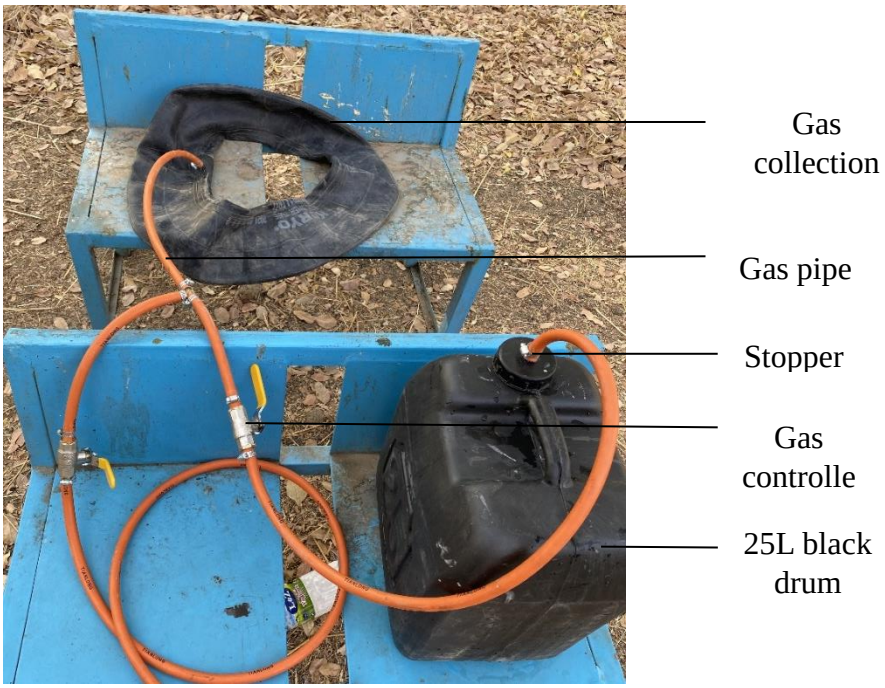

Plate 1: 25L biogas set up

Microbial Analysis of the Substrate and Inocula

Isolation of Methanogenic Bacteria

The isolation of methanogenic bacteria was carried out following the previously described method employed by Pratiksha and Gireesh, (2012) but with some modifications. About 1g/ ml of the substrate/inocula were weighed and poured into test tubes containing 9 ml of autoclaved brewer thyglycollate media. The medium consisted of 1.0 g beef extract, 2.0 g yeast extract, 5.0 g peptone, 5.0 g glucose, 5.0 g sodium chloride, 0.002 g methylene blue, and 1.0 g agar agar at pH 7.2±0.2 per litre (Murunga *et al.,* 2016). The tubes were incubated under anaerobic condition for 72 hours at 35^{o}C to enhance the growth of the anaerobic methanogenic bacteria. Anaerobic jar was evacuated by placing a kindled candle in it which immediately quenched the left over oxygen.

Following incubation, the test tubes were manually agitated to form a uniform solution and allowed to stand for a few seconds to allow the larger particles to settle. The 72-hour-old bacteria dilution was then serially diluted into ratios of 10^{-1} to 10^{-5}. A volume of 1 ml of each dilution was introduced into labelled sterile petri-dishes and brewer thyglycollate media with modification was individually pour plated into petri dishes containing the sample. The plates were arranged in an anaerobic jar and incubated at 35^{o}C for 24 hours.

Identification of Methanogenic Bacteria

Colonies of methanogenic bacteria were identified on the petri-dishes using blue-green fluorescent test which is unique to the metabolic group of bacteria (methanogens) (Dhadse*et al.,* 2012). Strains of Methanogenic bacteria synthesizes a low-molecular compound; F240 in which in an oxidized form fluoresces when excited by long-wave ultraviolet light (Cheeseman *et al.,* 1972). With the compound confined to the colony, glowing spots appear when exposed to long-wave UV light indicating the presence of methanogens. Non-methanogenic colonies differ from the white-yellow fluorescence normally observed with methanogenic bacteria as they appear with no glow under ultraviolet light and plainly visible under visible light.

RESULTS

The result of the physicochemical parameters of the bio-digesters is presented in Table 1. The result showed that cow dung had the highest pH (6.81) followed by that of gutter's sludge (6.31) and Jatropha seedcake (5.47) respectively. Cow dung was also observed to have the highest value of C/N ratio while that of Jatropha seedcake and cow dung appeared the same. The organic matter, moisture content and total carbon ratio of gutter's sludge was higher than that of cow dung and Jatropha seedcake. The highest temperature value was observed in cow dung and lowest in gutter's sludge. Total nitrogen of Jatropha seedcake and gutter's sludge was higher than that of cow dung by a highly significant amount.

Table 1: Physico-chemical Parameters of Jatropha seedcake, Cow dung, and Gutter's sludge.

Parameters (Unit)	Jatropha seedcake	Cow dung	Gutter's sludge
pH	5.47	6.81	6.31
Temperature (^{o}C)	29.5	30.7	22.0
Moisture content (%)	4.57	43.59	64.17
Total solids (%)	95.43	56.41	35.83
Volatile solids (%)	19.2	17.2	17.8
TC (mg/L)	3.2	1.96	3.23
TN (mg/L)	320	2.6	228
C/N ratio	0.01	0.75	0.01
OM (%)	3.10	3.37	5.56

KEY: TC= Total carbon; TN= Total nitrogen; C/N ratio= Carbon/nitrogen ratio; OM= Organic matter

Methane Yield at Respective Peak Days of Production.

At respective peak retention time (days of production), the percentage amount of methane produced in the biogas was measured and result presented in Table 2. Anaerobic digester D produced the highest percentage of methane; 41.8% on day 14, followed by digester C with a methane percentage of 18.0% on day 19. Digester A produced a low methane percentage of 7.1 also on day 19 while Digester B produced the lowest percentage of methane of 4.2% on day 14.

Table 2: Methane Yield in the Pilot Anaerobic Digesters (A-D) at their Peak Day of Production.

Type of gas (Unit)	A	B	C	D
CH_4(%)	7.1±2.5	4.2±0.7	18.0±5.3	41.8±1.0
CO_2 (%)	0.5±0.2	24.8±1.3	14.8±3.8	13.6±0.2
O_2 (%)	19.9±0.0	15.4±0.1	18.8±0.2	9.1±0.0
H_2S (ppm)	41	387	297	141

KEY: A= Jatropha seedcake only; B= Jatropha seedcake + Cow dung; C= Jatropha seedcake + Gutter's sludge; D= Jatropha seedcake + Cow dung + Gutter's sludge. Result was presented by taking the readings appearing on the biogas analyzer.

Flow of Methane production along Retention time (days)

The effect of methane yield in percentage against anaerobic digestion in days is presented in Figure 1. Digester D was observed to have the highest and fastest flow of methane production starting with a methane percentage of 14.3% as early as day 6 but dropping rapidly from day 19 (14.4%), to 25 (1.1%). Digester B was observed to have the lowest methane production rate on a consistent level as digestion process started at day 6 with a methane value of 0.2%, peaking at day 14 (4.2%) and ending on day 25 with 0.1% methane. Digester C produced methane percentages of 5.9% on day 6 and peaking at day 19 with 18.0% methane. While Digester A was observed to have the slowest production of methane starting on day 11 (1.6%) and rapidly peaking at day 19 (7.1%). The production ended miserably on day 25 with a methane value of 0.4%.

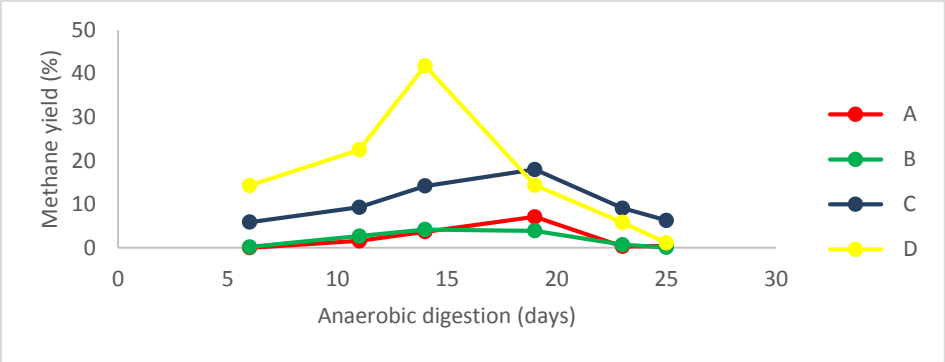

Figure 1: Effect of Methane yield (%) against Anaerobic digestion (days).
KEY: A= Jatropha seedcake only; B= Jatropha seedcake + Cow dung; C= Jatropha seedcake + Gutter's sludge; D= Jatropha seedcake + Cow dung + Gutter's sludge.

Result of Biogas Upscale (25L Anaerobic Digester)

The result from the upscale of biogas production using Jatropha seedcake, cow dung amd gutter's sludge is presented in the plates 2, 3 and 4. After 24 hours the gas chamber connected to the anaerobic digester was highly inflated. This inflation (Plate 2) confirms the rapid production of biogas in the chamber. However, a decrease in the inflation was observed at day 2; after 48 hours (Plate 3). The chamber was completely deflated at day 7 (plate 4) indicating no biogas present.

Plate 2: Gas collector after 24 hours.

Plate 3: Gas collector after 48 hours.

Plate 4: Gas collector after a week (7 days)

Isolation of Methanogens from the Substrates

The result from the isolation of methanogens from Jatropha seedcake, cow dung and gutter's sludge is presented in Table 3. Colonies of methanogenic bacteria were only identified on lower dilutions of gutter's sludge. 44 methanogenic glows (colonies) were observed and counted from 10^{-4}dilution (plate 6) while 41 methanogenic colonies appeared on 10^{-5} dilution (plate 8). However, anaerobic microorganisms were isolated from all plates (substrate and inoculum dilutions) which all appeared too numerous to count (TNTC).

Table 3: Recovery of Methanogenic Microorganisms.

Source	Dilution	Visible light	Long-wave ultraviolet light	Total colonies	Methanogenic colonies
Jatropha seedcake	10^{-1}	+	-	TNTC	0
	10^{-2}	+	-	TNTC	0
	10^{-3}	+	-	TNTC	0
	10^{-4}	+	-	296	0
	10^{-5}	+	-	228	0
Cow dung	10^{-1}	+	-	TNTC	0
	10^{-2}	+	-	TNTC	0
	10^{-3}	+	-	TNTC	0
	10^{-4}	+	-	TNTC	0
	10^{-5}	+	-	TNTC	0
Gutter's sludge	10^{-1}	+	-	TNTC	0
	10^{-2}	+	-	TNTC	0
	10^{-3}	+	-	TNTC	0
	10^{-4}	+	+	TNTC	41
	10^{-5}	+	+	282	44

KEY: TNTC=Too Numerous to Count.

Plate 5: 10^{-4} Gutter's sludge under visible light

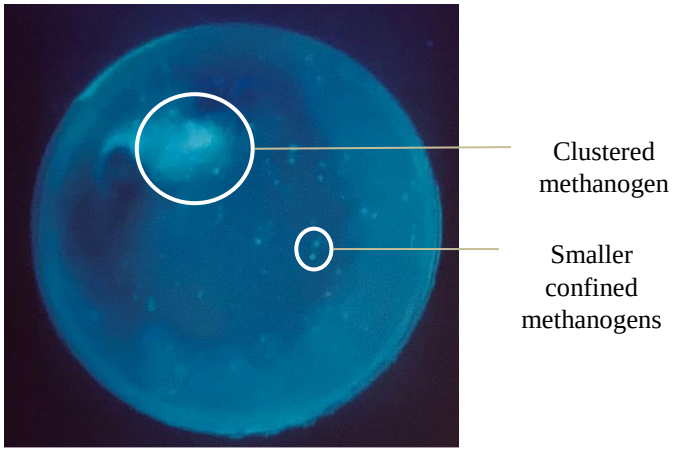

Plate 6: 10^{-4} Gutter's sludge under long-wave ultraviolet light showing a big clustered methanogenic colony and smaller confined ones

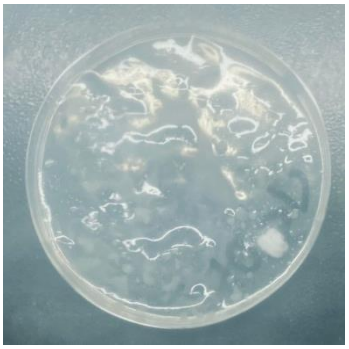

Plate 7: 10^{-5} Gutter's sludge under visible light

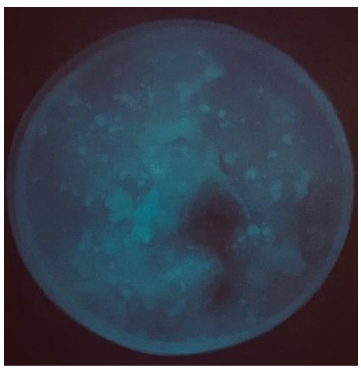

Plate 8: 10^{-5} Gutter's sludge under long-wave ultraviolet light showing methanogens present in smears

DISCUSSION

The physicochemical parameters of the substrate and inocula; Jatropha seedcake, cow dung and gutter's sludge were analyzed. In this study, the pH value of Jatropha seedcake (5.47) was considerably low compared to the other substrates. Jatropha seedcake on normal basis due to the presence of organic acids and phenolic compounds, has a typical low pH, ranging from 4.5-5.5. This goes in line with study from Praptiningsih *et al.* (2013) where the average pH of Jatropha seedcake was calculated at 5.33.

The presented pH is not a full ideal condition for biogas microbial growth as the optimal pH for methanogenic bacteria is 6.7-7.5 (Deublein and Steinhauser, 2008). To fully optimize the use of Jatropha seedcake as a substrate for biogas production, a strategy like co-digestion with substrates of higher/alkaline pH was employed. Research proved the mixture of Jatropha seedcake and cow dung produced more significant methane than each of the substrate alone (Raheman and Mondal, 2012). In this study, the pH of cow dung and gutter's sludge was obtained at 6.81 and 6.31 respectively. The co-digestion of Jatropha seedcake with gutter's sludge and Jatropha seedcake with both inocula produced more methane by a large gap of 18.0% and 41.8% respectively compared to Jatropha alone having 7.1% methane. However, co-digestion of Jatropha with cow dung alone in this study produced the least amount of methane percentage compared to the other substrate combination (4.2%).

Temperature is crucial to the actions of methanogens as the potential of them generating biogas depends on the digester's temperature (Kulkani and Ghanegaonkar, 2019). The temperature of cow dung (30.7ºC) falls within the stipulated temperature range for optimum methane production. However, again the anaerobic digester containing cow dung as an inoculum performed poorly as it produced the lowest percentage of methane at 4.2%. Jatropha seedcake produced an average temperature of 29.5ºC and gutter's sludge 22ºC. Logically, the average between the two gives a mesophilic temperature of 25.8ºC. This falls within the temperature requirement of methanogenic microorganisms for methane production and might explain why the digester containing Jatropha and gutter's sludge performed way better than Jatropha only. According to the mix ratio of Digester D (J+CD+GS), the volume of gutter's sludge against Jatropha and cow dung was by five folds. This coupled with the high content of organic matter (5.56) and volatile solids (17.8) compared to Jatropha and cow dung from the physicochemical parameters result, should justify the action of the mix ratio producing the highest value of methane 41.8%.

The highest value of methane was observed from digester D containing Jatropha seedcake, cow dung and gutter's sludge with methane production observed as early as a day after feeding. This rapid production was also observed in the digester containing Jatropha and gutter's sludge (digester C). Therefore, it is safe to assume this rapid energy generation is influenced by the gutter's sludge. However, this is in contrast with other studies involving waste water sludge where significant biogas production started in the first 15-18 days (Demirbas *et al.,* 2019). The rapid production in respect to gutter's sludge can be speculated to be as a result of some of the stages involved in biogas production already taking place in the sludge prior to feeding. This includes the breakdown of carbohydrates, amino acids and propionate. Hence the methanogenesis kicks start at its natural environment immediately after feeding in the bio-digester. Furthermore, biogas was observed on day 6 in the digester containing Jatropha alone. However, methane production only started at day 11. The same scenario was observed with the digester containing cow dung and Jatropha seed cake. This observation can be attributed to the high amount of degradable biomass contained in the substrate and inoculum and also goes in accordance with result from (Ogunkunle *et al.,* 2019) where methane generation by mix ratios of Jatropha seedcake and cow dung were observed from day 6.

The 25L anaerobic digester up-scale rapidly produced biogas after 24 hours but started depleting after 48 hours. Leakage test was carried out to ensure there was no error in the set-up which proved negative and the gas chamber got completely deflated after a week. This strange action can be attributed to the actions of methanotrophs or by extreme temperature by the sun on the gas chambers. Methanotrophs are subsets of a physiological group of bacteria that are unique in their ability to utilize methane as their sole source of carbon and energy source (Hanson and Thomas, 1996) by oxidizing it into methanol or formaldehyde (Scheller *et al.,* 2010). In the absence of oxygen, these organisms have the ability to alternate electron acceptors such as nitrate or sulfate to carry out anaerobic methane oxidation. Methanotrophs naturally survive in rice, soils, landfills, sewage treatment facilities, and most especially in ruminant animals including cow (Hutchens *et al.,* 2004; Jiang *et al.,* 2011). This is interesting as if we recall regardless of having high and optimum parameters needed for methane production, the digester containing cow dung as an inoculum performed very poorly. It is safe to state that this is as a result of methanotrophs present in the digester which hindered the inoculum from doing its work efficiently. To support this, study from Nguyen *et al.,* (2019) showed methanotrophs ultimately decrease methane content which in turn leads to a decrease in biogas production. The retraction in the gas chamber can also be attributed to extreme temperature in regards to Charles law about the volume of a fixed amount of gas being inversely proportional to its absolute temperature. Hence, as the temperature becomes more extreme, the gas retracts and eventually vanishes from the tube.

Methanogenic bacteria were successfully isolated from the lower dilutions of gutter's sludge. Some modifications were made in the method of Pratikshaand Gireesh, (2012) where the method was adjusted from spread plating method to pour plating method. This proved more efficient in isolating the organisms as they appeared more prominent and visible prior to the previous method in which the isolates combined with the media making them difficult to identify under fluorescence. Additionally, in the absence of 1.1g of sodium thioglycollate (responsible for making the media anaerobic with no oxygen) for the preparation of the media referenced from (Murunga *et al.,* 2016). Anaerobic jar without oxygen (evacuated with a kindled candle) was substituted which sufficed. In regards to modification of the media after the first stage of incubation, media was solidified by additional addition of agar agar, followed by autoclaving and eventually pour plating into the petri dishes.

CONCLUSION

All substrates combination yielded methane gas. However mixture of Jatropha seedcake, Cow dung and gutter's sludge yielded the highest percentage of methane at 41.8%.Cow dung as an inoculum for Jatropha is a poor inoculum in terms of methane yield compared to gutter's sludge or in combination of both. This could be due to the natural presence of methanotrophs in the inoculum. Anaerobic bacteria were isolated from all substrates but methanogenic colonies were isolated from lower dilutions of gutter's sludge alone. This study confirms suitability of gutters sludge as inoculum in biogas production using Jatropha seed cake as substrate.

RECOMMENDATIONS

1. Due to the presence of Hydrogen sulfide in the biogas produced, a rotten egg smell gets released in the process of exploiting the gas which is highly unpleasant. This can be eradicated by the introduction of additives such as Iron, copper, or magnesium sulfate in the gas chambers. The biogas produced can also be put through air membrane filters by gas treatment, which could separate the components before usage.
2. The digestate (left over feedstock) from the anaerobic digestion can be used for agricultural purpose such as application as fertilizer.
3. Actions of methanotrophs should be extensively studied to disable any hindrance caused by them for biogas upscale before set up.
4. Upscaling methane production requires further studies to determine the reasons behind methane loss.

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