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Evaluation of Anaerobic Microbial Community and Physicochemical Parameters in Small Scale Biodigesters within Uasin Gishu County

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Abstract

Anaerobic digestion is a sequential biological activity that accepts the efficient capture of methane for energy production. The dependence on fossil and wood fuels as a primary energy source has led to multitudes of problems such as global warming, environmental degradation and human respiratory health complications. The objective of this research was to characterize, identify and study physicochemical requirement of the digesters in relation to methanogenic bacteria identified from cow dung and improve their efficiency in biogas production. Six study sites were selected within Uasin Gishu County, opande, energy, beta farm, radar, nettos and langas, which varied in both volume and biogas production capacity. The cow dung which had been fed to biodigesters were collected aseptically in sterile jars and bacteria were isolated in Biotechnology laboratory, University of Eldoret under anaerobic conditions in a Gas pak jar at a mesophilic temperature of 35°C for seven days. Pure isolates were obtained using streak plate method and evaluation of physicochemical parameters were done in situ. Identification of isolates was done using cultural, morphological and biochemical characteristics. Laboratory scale set up of anaerobic digester for biogas production was done to evaluate their efficiency in biogas production. Three, 500ml erlernymer flask were half filled with cow dung and inoculated with Methanococcus sp. and Methanosaeta sp. separately and a combination of both isolates. This was done in triplicate with different inoculum ratios at 10:500, 20:500 and 30:500 mls, respectively and a control experiment with cow dung alone and allowed to run for 31 days. Gas generated was collected by volume displacement of water and measured at an interval of 0:10, 11:21 and 21:31 days. The temperature and the pH were regulated and monitored regularly. The study identified 7 different anaerobic bacteria species as Methanococcus, Sulfolobus, Methanosaeta, Methanospirillium, Methanosarcina, Methanomicrobium and Methanothrix species. The most predominant methanogenic bacterial strains, which occurred in at least 5 digesters were Methanococcus sp. and Methanosaeta sp which also produced the highest volume of biogas with maximum production being observed in 21-31 days at a ratio of 30:500. Methanococcus sp. and Methanosaeta sp. synergistic activity yielded the highest gas production of 74.23 mls versus 22.50 mls in control and 64.23 mls versus 50 mls from Methanosaeta and Methanococcus sp. respectively. In conclusion, study on the 6 bio digesters showed that physicochemical parameters plays a paramount role in biogas production and should be maintained at an optimum range. The study shows that predominant methanogens Methanococcus sp. and Methanosarcina sp. when inoculated in the digesters increase the quantity of biogas produced. Research recommends that digesters encompasses diverse group of methanogens which works in syntrophic association in the fermentation process thus should be maintained for increase in biogas production.

Keywords: Bacterial Consortia, Biogas, Biodigesters

INTRODUCTION

The quest for renewable energy facilities to replace fossil fuels has gotten a lot of attention in recent years (Arutyunov & Lisichkin, 2017). Anaerobic digestion is a dual-function technology widely used in the treatment of various organic wastes and wastewaters (Awasthi et al., 2020). Anaerobic digestion is the most environmentally friendly technique of organic disposal due to its significant volume reduction and stability levels (Kadam & Panwar, 2017).

Depending on the qualities of these wastes, they can be converted into energy without the use of any extra fuels, and finally, anaerobic digestion. So far, natural fuels such as coal, oil, and natural gas have given power to developing nations while simultaneously supporting the technologically advanced modern world (Barisa et al., 2020). However, fossil fuels are limited, and their ongoing usage has an influence on our ecosystem and the global climate owing to greenhouse gas emissions. Furthermore, oil and gas supplies are running low. To prepare for the transition to a more dependable energy source, appropriate conserving, augmenting, and exchanging technologies must be investigated (Shindell & Smith, 2019). In this sense, waste products have been shown to boost energy supply, aid in lowering growing dependency on fossil fuels, and ameliorate environmental and health concerns that have arisen as a result of the use of fossil fuels in many third-world and industrialized countries.

Biogas digesters in Kenya have used inputs from various sources, including slaughterhouses waste, trash from municipal landfills, and bagasse from sugar manufacturers, plants such as water hyacinth, and animal as well as human excreta. Others include human waste, such as utilizing a public toilet block in Kibera, Kenya, where biogas reactors have been erected (Sharma & Biswas, 2016). However, issues have developed as a result of the poor quality of the installed units. Users' insufficient operational and maintenance capabilities has decreasing performance, resulting in the abandonment of biogas digesters (Wassie & Adaramola, 2020) In other circumstances, the demonstration effect has discouraged rather than encouraged the use of biogas.

The issue of global warming due to greenhouse gases and the high cost of fossil fuel is an area of concern that needs to be addressed. There is a need to explore a renewable energy source that is self-reliant and environmentally friendly. Kenya's population is quickly growing, creating large disparities in energy demand and supply. In the absence of proper disposal techniques, livestock manure, particularly cow dung, can pose environmental risks, including pathogen contamination, airborne ammonia, odour, and greenhouse gas emissions (Sharma et al., 2022; Sharma & Biswas, 2016). On the other hand, the expanding demand for fossil fuels, a key energy source, depletes them on a daily basis, necessitating a significant capital expenditure.

Biogas offers the most potential as a low-cost domestic energy source since it is renewable, simple to create, simple to use, and economical. Several publications on biogas (methane) generation from cow dung are available today, but very little work has been done on assessing the potential of methanogenic bacteria composition for effective biogas production from cow dung. This research therefore was aimed at characterizing and evaluating the effective bacterial consortia for efficient biogas production on small scale bio digester in Uasin Gishu County.

METHODOLOGY

Study Area

The research study was carried out in the Biotechnology laboratory at the University of Eldoret situated in Uasin Gishu County, at longitude 35° 18' 13" E and latitude 0° 34' 35" N and an altitude of 2180 m. The region receives between 900 mm and 1600 mm of rain from March to September, with two

distinct peaks in May and August, and the average temperature is around 24°C.

Sampling Techniques

Purposive sampling was used to select six sampling sites within Uasin-Gishu County. The sampling sites were radar, opande, nettos, beta farm, energy and langas. The digesters under study varied in both their sizes and biogas production capacity. Thus, they were categorized into small, medium and big size. The cow dung were collected aseptically from the six identified digesters in a sterile 250 ml containers. The bioreactor contents were mixed before each sampling. Physical parameters of biogas digester including the condition of biogas digester, temperature, and pH were recorded. In each sampling days, at least 250 ml was collected in a sterile sampling bottle through a clean funnel and immediately closed with a sterile stopper and transported to the laboratory in a keep cool box containing ice packs, stored at 4°C-8°C and processed within 24 hrs. Sampling was done three times a week during morning hours for three consecutive weeks. The bio digesters from which the samples were taken, from, different sites had the characteristics shown in Table 1.

Table 1: Characteristics of the six digesters under study

Digester	Location	Size	Type	No. of cows	Gas prodn	Uses
BIG		150 m^3	Fixed dome	10-15	155 m^2D	Cooking
(N)	Nettos					
(R)	Radar	150 m^3	Fixed dome	10-15	150 m^2D	Cooking
Medium		135 m^3	floating gas holder	5-6	130 m^2D	Cooking
(O)	Opande					
(E)	Energy	135 m^3	Floating gas holder	4-6	131 m^2D	Cooking
SMALL		120 m^3	Plastic bag holder	2-3	121 m^2D	Cooking
(P)	Langas					
(B)	Beta farm	120 m^3	Plastic bag gas holder	2-3	121 m^2D	Cooking

Sample Processing

Isolation and Identification Studies

The samples of the cow dung that were initially collected and stored in a fridge at a temperature of 4ºC. They were later removed to thaw and attain room temperature. These were then serially diluted using sterile distilled water to 10^5 and then cultured on sterile methanogenic media. The isolates were coded as E1m and E2m, for isolates from energy. R1m, R2m, R3m, R4m and R5m for isolates from radar. B1m, B2m, B3m and B4m for the isolates from beta farm. O1m, O2m and O3m for the isolates from opande, P1m and P2m for the isolates from langas. N1m, N2m, N3m and N4m for the isolates from nettos.

Spread Plate Method

A 14 gram-formulation of methanogenic agar was dissolved into 500 ml of distilled water, it was boiled on a hot plate to homogenate the media then transferred to the autoclave for sterilization. This was done at a set temperature of 121ºC for 15 minutes. The media was dispensed aseptically into sterilized petri plates. They were labelled and left to solidify after which 1 ml of the serially diluted sample of 10^5 was pipetted and inoculated into the solidified media. A sterile bend glass rod was used to spread the inoculum onto the media and let to stand for 5 minutes which allowed the media to absorb the inoculum as described in Bergey's manual 9th Edition (Whitman et al., 2015). The plates were labelled and packed into an anaerobic jar, this allowed anaerobic condition (Plate 1). The jar was then placed into the incubator set at a mesophilic temperature of 35ºC to 55ºC for 3-4 days. The colonies were observed and photographed.

Plate 1: Anaerobic jar packed with plate ready for incubation.

Isolation in Axenic Culture by Streak Plate Method

After 3-4 days, the plates showed mixed bacterial colonies. They were differentiated based on their morphological and cultural characteristics. The colonies were subcultured into sterilized plates containing methanogenic media by streak plate technique. A sterile inoculating loop was used to pick a pure colony differentiated by its colour from the mixed population and streaked on the solidified media in the plate and labeled. It was then incubated anaerobically for 3-4 days.

Morphological Studies

From the pure colonies morphological characteristics were noted using the key described in Bergey's Manual of determinative bacteriology 9th Edition (Whitman et al., 2015). The morphological characteristics included, colour, elevation, form, surface, margin, shape and Gram reaction.

The pure isolated colonies were subjected to the Gram staining technique. This was done to differentiate between gram positive and negative bacteria cells. A sterile inoculating loop was used to pick a colony from a pure streak plate, a thin smear was made on a grease-free glass slide and allowed to dry by passing it on a heat source. A drop of crystal violet stain was added to the smear and allowed to stand for one minute. It was then washed off using slow running tap water. Lugols iodine was added for one minute and washed off. The smear was then decolourized by absolute alcohol briefly for 30 seconds after which safranin stain was added for one minute and washed off, the slide was dried and observed at ×100 oil-immersion-objective-lense. The positive bacteria cell were identified by the bluish black colour while negative cells were identified by the pink coloration.

Methyl red-Vogues Proskauer Test

The Methyl red-Vogues proskauer broth was prepared as per manufacturers descriptions. 5 mls of the broth was poured into tubes and autoclaved the tubes were divided into two pairs. The test organism was inoculated into each tube pair and labelled appropriately. The tubes were incubated at 37ºC for 48 hrs. Five drops of methyl red indicator were put to one pair of tubes after 48 hours and assessed. To the other pair, ten drops of VP 1 reagent and 2-3 drops of VP 11 reagent were

added. To finish the reaction, the tubes were gently shaken and plugged with cotton wool for 15-30 minutes. The methyl red test detects microorganisms that produce stable acid end products via glucose mixed acid fermentation. The vogues proskauer test is used to detect an organism's ability to produce acetoin, a neutral end product of glucose fermentation (Mallick, 2019). Positive test in methyl red is indicated by the tube maintaining the red colour while negative test is indicated by turning of the tube media to yellow.

Determination of Physicochemical Parameters

In each of the digester the substrate was mixed with distilled water in ratio of 2:1 and pH taken using a digitilized pH meter. This was done in replicates to determine the mean pH value and recorded. It was determined for three consecutive days each morning in every digester. The temperature was taken from the cow dung waste for all the digesters under study. Wet bulb thermometer was used. This was taken daily for three consecutive days to determine the mean temperature and recorded. Moisture content was determined using the Association of analytical chemist method (1999). The crucible dish was initially dried at a 98ºC-100ºC temperature in a hot air oven for two hours. It was then cooled in a desiccator to attain room temperature. Fifteen-grams of slurry was weighed into the dried crucible dish and then heated at 98ºC-100ºC for two hours. After the time elapsed, the dish was removed and transferred to a desiccator weighed at room temperature and data was recorded. The moisture content was calculated using the formula;

Moisture content = (mass of the crucible +sample)-(mass of dry crucible)

The volatile solids were determined using America Public Health Association (2005) (Hobbs et al., 2018). Fifteen grams of the slurry were put in a crucible dish and burned for 30 minutes in a muffle furnace at 500°C-550°C. The crucible was removed from the muffle furnace, slightly cooled in air, and placed in a desiccator for a few minutes before being weighed and recorded.

Volatile solid was computed using formulae, shown below;

Volatile solids = (mass of the crucible + sample)-(mass of crucible + sample after ignition) / mass of the empty clean and oven dried crucible

Total solid was determined by weighing 10 gm of freshly collected sample and putting it into the dish. For 1 hour, the crucible was put in a hot air oven set at 105°C. It was cooled in a desiccator to a room temperature and weighed. It was analysed according to the methodology described by American public health association (2005) (Hobbs et al., 2018).

Total solid was computed using the formulae;

Total solids-(mass of crucible + sample after drying) – (mass of empty oven dried crucible) / (mass of silica crucible + sample) – (mass of the empty clean and oven-dried crucible)

Data Analysis

Total Shannon diversity test was used to determine the diversity and abundance of the isolated methanogens in each bio digester under study. Chi-square test was employed on characterization of the isolated methanogens on morphological parameters e.g. elevation, margin, surface, shape and gram stain and to calculate their p value in each parameter. The data from the physicochemical parameters was statistically analysed using Genstat discovery (10th edition 2008). Duncan Multiple range test (DMRT) and the means separated by Turkey's 95% level of coefficient. Analysis of variance (ANOVA), Statistical Package for Social Sciences. SPSS, (2007) version 16.0.

RESULTS

Characteristics of Anaerobic Microbial Community Involved in Biogas Production in Six Biodigester within Uasin Gishu County

Morphological Characterization of Bacterial Isolates

There were seven morphological characteristics that were observed for the anaerobic microbial community involved in biogas production in six biodigester within Uasin Gishu County. These were colour, form, margin, elevation, surface, shape and Gram stain. There were five notable colours that were identified in the morphological characterization of bacterial isolates. These were cream, orange, pink, white and yellow. White color was found in majority 6 (30%) of the isolates (E1m, R1m, N4m, O1m, B3m, R5m) followed by cream 5 (25%), and yellow 5 (25%) while orange was found in only 1 (5%) isolate (R4m) with a significant difference ($\chi^2 = 20.00$, d.f = 4, p = 0.0005) as portrayed in Table 2. There were five forms that were noted in the morphological characterization of bacterial isolates. The punctiform dominated the form accounting to 9 (45%) of isolates characterizing *Methanosaeta* sp*., Methanococcus* sp*, Methanosarcina barkeri* and *Sulfolobus* bacteria species while concentric rings had 1 (5%) isolate with *Methanosarcina barkeri.* Differing in significant difference ($\chi^2 = 32.0$, d.f = 3 d.f p = 0.0000). Three types of margins were noted in the study which included entire, lobate and undulate. Majority of isolates (75%) had entire margin characterizing, *Methanosaeta* sp*., Methanospirillium* sp., *Methanococcus* sp*., Sulfolobus* sp*., Methanothrix* sp*.,* and *Methanosarcina barkeri* bacteria species, while undulate types of margins characterized *Methanomicrobium* with a significant difference ($\chi^2 = 78.5$, df=2, p= 0.0000). Raised elevation dominated many of the isolates 12 (60%) characterizing *Methanospirillium sp., Methanosaeta sp., Methanosarcina barkeri, Sulfolobus sp., Methanosaeta sp.* and *Methanomicrobium sp.* ($\chi^2 = 70.0$, d.f=3, p= 0.0000). Majority of the isolates had smooth and glittering surface which characterizes *Methanospirillium sp., Sulfolobus sp., Methanosaeta sp., Methanomicrobium sp., Methanococcus sp* and *Methanothrix sp.* in terms of shape, cocci dominated majority of the isolates whose bacteria species were *Methanococcus sp., Methanosarcina barkeri and Sulfolobus sp.* majority of the isolates were gram positive as summarized in Table .2.

Table 2: Morphological characterization of bacterial isolates

Morphological characterization	Attribute	f	%f	Chi square (χ^2)
Colour	Cream	5	25.00	$\chi^2 = 20.0$, df=4, p= 0.0005
	Orange	1	5.00	
	Pink	3	15.00	
	White	6	30.00	
	Yellow	5	25.00	
Form	Circular	5	25.00	$\chi^2 = 32.0$ df=3 d.f, p= 0.0000
	Concentric rings	1	5.00	
	Irregular	5	25.00	
	Punctiform	9	45.00	
Margin	Entire	15	75.00	$\chi^2 = 78.5$ df=2 p= 0.0000
	Lobate	2	10.00	
	Undulate	3	15.00	
Elevation	Convex	4	20.00	$\chi^2 = 70.0$

	Flat	3	15.00	df=3 p= 0.0000
	Pulvinate	1	5.00	
	Raised	12	60.00	
Surface	Dry / powdery	3	15.00	$\chi^2 = 110.0$ df=3 p= 0.0000
	Rough	1	5.00	
	Smooth	2	10.00	
	Smooth / glitter	14	70.00	
Shape	Cocci	13	65.00	$\chi^2 = 54.5$ df=2 p= 0.0000
	Lobed cocci	1	5.00	
	Rods	6	30.00	
Gram stain	Negative	3	15.00	$\chi^2 = 49.0$ df=1 p= 0.0000
	Positive	17	85.00	

Distribution of Methanogenic Bacteria from the Sample Biodigesters

Methanogens distribution in the study sites namely opande, nettos, radar, energy, beta farm, and langas were as shown in Figure 1. The seven genera of anaerobes isolated from the different bio digester were *Methanococcus* sp. *Methanomicrobium* sp, *Methanosarcina* sp, *Methanosaeta* sp, *Sulfolobus* sp, *Methanothrix* sp and *Methanospirrilium* sp. The bio digester located in Radar showed the highest bacterial population having 7 of the bacterial genera identified in this study. It was further noted that the highest proportion was with *Methanosaeta sp* which was more prevalent while the least was *Methanospirillium sp* and *Methanomicrobium sp* isolated from Radar and Energy bio digester respectively. Langas had the highest mean abundance of bacteria 2.5 followed by energy at 2.0, beta farm at 1.75, opande and nettos had the same abundance of 1.66 and radar had the lowest of 1.142.

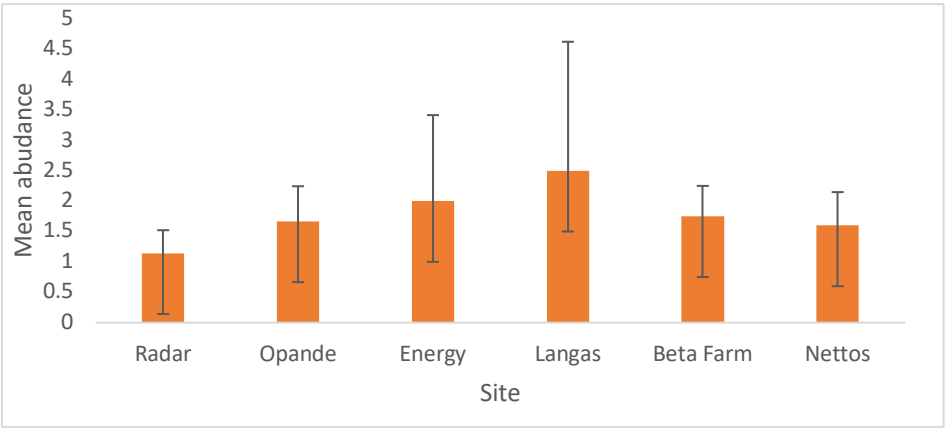

Figure 1: Mean abundance of bacteria in different sites.

Diversity of Methanogenic Bacteria from the Sample Biodigesters

Total Shannon Weiner diversity of the bacteria was 1.76 H'. In terms of sites, radar had the highest diversity (1.90 H') with seven genera recorded. The sites with the lowest diversity were energy (0.56 H') and langas 0.50 H') as illustrated in Figure 2, there was a significant difference in Shannon Weiner diversity between radar and opande

(t=1.9062, p=0.0259), radar and energy (t=1.6021, p=0.0342), radar and langas (t=4.4233 p=0.0001), radar and beta farm (t=1.4245), p=0.0359), energy and beta farm (t=5.9782, p=0.0001), and langas and beta farm (t=1.9352, p=0.0001).

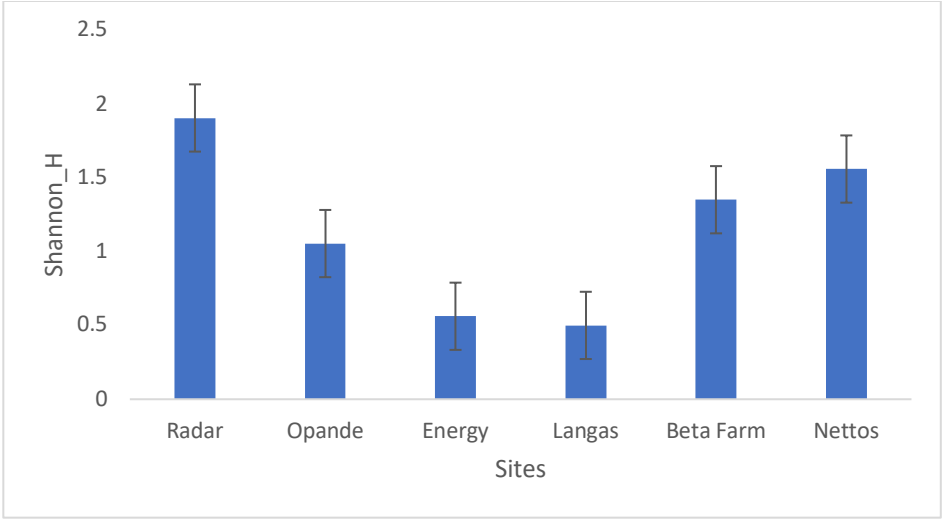

Figure 2: Shannon Weiner diversity.

Physicochemical Parameters of the Anaerobic Microbial Community Involved in Biogas Production in the Different Biodigesters within Eldoret

The highest pH was recorded at beta farm (7.06±0.78) followed by opande (7.20±2.00) and radar (7.20±0.92) while the lowest was recorded in Energy (6.86±1.22) with no signficant difference (p=0.0035). The recorded temperature were highest in radar (37.87±4.67) and nettos (37.80±8.89) but lower in energy (34.90±3.12) with a notable significant difference (p=0.0225). Mean significant difference was between beta and langas,nettos and opande. The mean volatile solids ranged from a lowest of 0.20±0.00 gm recorded at beta farm to a maximum of 0.81±.00 gm recorded at energy which was found to differ significantly amongst the sites except in the case of radar, beta farm and nettos (p=0.0213). The measured moisture content ranged from a lowest of 11.82±1.56 gm recorded at energy to a highest of 13.84±2.45 gm recorded at radar with a significant variation noted among all the sites studied (p=0.1315). Total solids reported was in the range of 6.90±1.34 gm in opande and 9.46±1.32 gm in nettos which was significantly different among the sites (p=0.0784) as illustrated in Table 3.

Table 3: Mean value of physicochemical parameters

Site	pH	Temp (°C)	VS (g)	MC (g)	TS (g)
Beta	7.06±0.78a	35.10±5.02ab	0.20±0.00a	12.72±1.89c	9.25±2.89c
Energy	6.86±1.22a	34.90±3.12a	0.81±.00d	11.82±1.56a	7.77±1.56ab
Langas	7.00±1.45a	36.23±6.67b	0.32±0.01b	13.19±1.25d	8.40±1.90c
Nettos	6.96±0.99a	37.80±8.89c	0.25±0.00a	13.46±2.75e	9.46±1.32c
Opande	7.20±2.00a	35.57±6.89ab	0.42±0.00c	12.47±2.56b	6.90±1.34a
Radar	7.20±0.92a	37.87±4.67c	0.22±0.00a	13.84±2.45f	8.64±1.62bc
p value	0.0035	0.0225	0.0213	0.0131	0.0078

Key: VS-Volatile solids, MC-Moisture content, TS-Total solids. Figures of means followed by the same letters are not significantly different at the 5% level of significance according to Duncan's Multiple Range Test (DMRT).

DISCUSSION

Characteristics of Anaerobic Microbial Community Involved in Biogas Production in Six Biodigesters within Uasin Gishu County

Morphological Characterization of Bacterial Isolates

Results established different Morphological characterization of bacterial isolates which included colour, form, margin, elevation, surface, shape and Gram stain. Results established that some bacteria were white others were cream, orange, pink, and yellow. Kern et al. (2016) indicated that there are some bacteria capable of producing pigment with different varieties of colours. *Methanosaeta* sp. has distinct characteristics such as cream colour, the form is circular and the margin with entire their elevation while surface is pulvinate. Its' small cocci shaped and gram reaction is positive. The findings indicated that different bacteria produce different colours with example of *Methanosaeta* sp. producing cream colour. Masaki et al. (2016) also added that *Sulfolobus* sp. are highly concentrated with yellow pigment due to sulphur element in them.

Bacteria can also be classified in terms of forms. The punctiform was the dominate form characterizing *Methanosaeta* sp., *Methanococcus* sp, *Methanosarcina barkeri* and *Sulfolobus* sp bacteria species while concentric rings isolate characterized *Methanosarcina barkeri.* Three types of margins were noted in the study which included entire, land undulate. The finding established that entire margin characterize *Methanosaeta* sp., *Methanospirillium* sp., *Methanococcus* sp, *Sulfolobus* sp., *Methanothrix* sp. and *Methanosarcina barkeri.* bacteria species while undulate types of margins characterized *Methanomicrobium* sp. these findings are in line with those of (Norell *et al.,* 2018). Raised elevation dominated many of the isolates characterizing *Methanospirillium* sp., *Methanosaeta* sp., *Methanosarcina barkeri, Sulfolobus* sp, *Methanosaeta* sp, and *Methanomicrobium* which is similar with the findings of Wang et al. (2018). Majority of the isolates had smooth and glittering surface which characterizes *Methanospirillium* sp., *Sulfolobus* sp., *Methanosaeta* sp., *Methanomicrobium* sp., *Methanococcus* sp., and *Methanothrix* sp. in terms of shape, cocci dominated majority of the isolates whose bacteria species were *Methanococcus* sp, *Methanosarcina barkeri and Sulfolobus* sp. majority of the isolates were of gram positive which concurs with the findings of (Kern et al., 2016).

Biochemical Characterization of Bacterial Isolates

The biochemical properties of microorganisms were investigated in order to determine the genus and species of an unknown bacteria. Microorganisms are extremely adaptable, with a wide range of metabolic capabilities (Brzeszcz & Kaszycki, 2018). These traits can be used to illustrate the tremendous metabolic diversity. *Methanosaeta sp.* and *Methanococcus sp.* were positive for indole, whereas the other isolates tested negative. These results were comparable to the study done by Khanthong et al. (2021) on test on methanogens ability to deaminate amino acids

Methanogenic Bacteria from the Sample Biodigesters

There were seven genera of bacteria isolated from the different bio digester these were Methanococcus sp. *Methanomicrobium* sp, *Methanosarcina* sp, *Methanosaeta* sp, *Sulfolobus* sp, *Methanothrix* sp and *Methanospirrilium* sp. These results are consistent with those of Sharma et al. (2022) who stated that the anaerobic transformation of organic wastes is a process that involves numerous different kinds of bacteria, including hydrolyzing, acidifying, acetogenic, and methanogenic bacteria. *Methanococcus* and *Methanosaeta* sp. The findings of having similar levels of abundance could have been contributed by the minimal distances between the sampling sites. The findings are supported by those of Bongaerts et al. (2021), that proximity of sampling sites to each other reduces

heteroscedastic of the samples corrected due to continuity of variations.

Physicochemical Parameters of the Anaerobic Microbial Community Involved in Biogas Production in the Different Biodigesters within Eldoret

The present study looked at the moisture influence in biogas production. According to Seruga et al. (2020), water concentration is a crucial factor influencing solid waste anaerobic digestion. There are two fundamental reasons behind this, water enables bacteria to travel and grow, and hence facilitating nutrient dissolution and transport while the second reason is that water reduces the mass transfer limitation of non-homogeneous or particulate substrate. Overall, the moisture content of the digester increased as the amount of volatile solids and total solids decreased. Micro-organisms are categorized as per their optimal pH range (Ananthu, 2019) and to maximize the CH_4 yield, pH typically varies from 6.85 to 7.2 with optimal values of 7.0 - 7.2.

The present study observed the different pH within the digesters the mean range was within 6.8-7.20. According to Yerima et al. (2018), the methanogenus grow best when the pH is between 6.8 and 7.8, and Garba and Sambo indicated that the ideal pH range for biogas production is between 6 and 7. According to the research findings by Hossain et al. (2022) the measured pH was largely within the permitted range for anaerobic digestion during the whole operation at mesophilic conditions. The pH levels are low during the start of digestion; initially, the acid-forming bacteria will break down the organic matter and release volatile fatty acids. The methanogenic species are the most pH sensitive. Acid buildup suggests a pH drop, and since methanogens require a pH of over 6.5, this could result in less methane being created. Since acetate is not utilized, this reduction is thus accompanied by a larger buildup of acids. (Wainaina et al., 2019). An acidic pH might cause the sequence of biological events to halt during digestion, resulting in overall acidity of the digesting material; as a result, the pH will fall below neutral. This value enhances process stability and, as a result, the digester's proper operation.

The volatile solid to total solid ratio determines physical impediment caused by inorganic matter buildup within the bioreactor. According to (Chae et al., 2011), the digester's volatile solid to total solid ratio is a good indicator of the buildup of undesired materials and the appropriateness of the mixing mechanism (Patel, 2017). According to this research the ratio of volatile solid to total solid was 0.37: 8 as a result, these data indicate that the bioreactor is adequately mixed this study concur with the one done with Hobbs et al., (2018), when methane emission increases, total solids and volatile solids decrease. Although there is still a propensity for further total solid and volatile solid decline with low or non-biogas generation, this is most likely due to the inherent scarcely biodegradable ingredients, and increased ammonia concentrations result in process inhibition. According to McVoitte (2018), the animal slurry employed in this study, such as cattle dung, contains lignocellulosic rich components, rendering anaerobic digestion unsuitable.

The current study examined total solids for optimal gas generation. These findings were consistent with those Sun et al. (2017). The ideal solid concentration for biogas production was discovered to be between 7 and 9 percent. Another research Hagos et al. (2017), shows that there is no further increase in the volume of biogas generated at some point when the % total solids increases. Furthermore, Dalkılıc & Ugurlu (2022) discovered that biogas production was unstable below a total solids level of 25% (of manure) and more acidic than lower total solid concentrations in his study. Slurries with higher total solid concentrations were more acidic than those with lower total solid concentrations. The amount of methane produced is governed by the number of volatile compounds in the waste, the amount

of solids in the waste, and the degradability of the solids.

The current research found temperature variations in gas generation the noted temperature was 34.9ºC-37.87ºC. This concurs with the study by Abbas et al. (2020) which showed that the biogas unit's temperature varies from 32°C to 37°C. Temperature has a significant impact on the anaerobic degradation process. Anaerobic digestion reactors are typically operated in the mesophilic (20°C-42°C) and thermophilic (42°C-75°C) temperature ranges. Temperature has little effect on the hydrolysis and acidogenesis processes. However, fewer specialized bacteria undertake the processes or stages of acetogenesis and methanogenesis; they are more temperature sensitive. Harirchi et al., (2022) noted that the variety of methanogens is reflected in the many growing environments. *Methanosarcina*, *Methanobacterium*, and the majority of *Methanococcus* are just a few methanogens that exhibit a mesophilic temperature spectrum. The temperature within the digester has a big influence on the biogas generating process.

CONCLUSION AND RECOMMENDATIONS

From the study 7 different methanogens were identified which differed in both cultural, morphological and biochemical characteristics. These were *methanococcus* sp*, sulfolobus* sp*, Methanosaeta* sp*, Methanospirillium* sp*, Methanosarcina barkeri* sp*, Methanomicrobium* sp*, and Methanothrix* sp. Study on the 6 bio digesters showed that physiochemical parameters play a paramount role in biogas production and should be maintained at an optimum range. The mean values for Temperature is 34.9^0C to 37.87^0C, pH is 6.86 to 7.2, volatile solids are 0.2 to 0.81, total solids are 8.4 to 9.25, and moisture content 11.82 to 13.84. Digesters encompasses diverse group of methanogens which works in syntrophic association in the fermentation process thus for increase in biogas production they should be maintained. Anaerobic methanogens in bio digesters operates best at a mesophilic temperature of 34.9°C to 37.87°C and at an alkaline pH of around 6.86-7.2. This must be kept constant and monitored regularly.

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