

Effects of Green Bio-Catalysts and Ferric Oxide in Cryo-Mesophilic Temperature Biogas Production

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Abstract: - Biogas production at low temperature regimes is annoyingly slow and yields low biogas volumes. Most biogas supplements are expensive and substrate-specific. The effects of two bio-catalysts *Saccharomyces spp.* and *Acanthaceae spp.* and an inorganic catalyst ferric oxide on biogas production using cow dung slurry was investigated. 1.5L batch anaerobic digester operating in unstirred cryo-mesophilic temperature regime of 20.0 -24.5 °C were utilized. The substrate underwent mild synergistic pre-treatment by steaming with 1% sodium hydroxide solution. The alkalinity and volatile acids of the substrates were insignificantly perturbed by inclusion of the additives. Additives *Saccharomyces spp.* and *Acanthaceae spp.* stabilized digestion temperature while ferric oxide stabilized pH drifts. The overall biogas yields produced in the 100-day retention period were in the order of 4615ml (990.34ml/g-Volatile solids) for ferric oxide, 2335ml (494.08ml/g-Volatile solids) for *Saccharomyces spp.*, 1750ml (328.94ml/g-Volatile solids) for *Acanthaceae spp.* and 1030ml (212.37ml/g-Volatile solids) for the control sample. Use of these additives would thus optimize biogas production in cold countries.

Key words: bio-catalysts, cryo-mesophilic temperature, biogas production

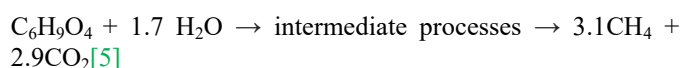
I. BACKGROUND

Greener processes are no longer just an environmental option but a strategic choice in the modern economy. [1] By green chemistry, there is minimization of waste disposal and emissions are channeled to renewable energy. Green chemistry advocates for use of energy with reduced environmental emissions (clean energy). There is unsatisfied desire in use of green catalysts that will not only accelerate Anaerobic Digestion processes while simultaneously reducing any Polycyclic Aromatic Hydrocarbons (PAHs) emissions [2]. Use of biogas fuel is by itself a green chemistry application since the amount of gaseous emissions are reduced. Economic conservancy is a critical pillar of green chemistry. Anaerobic digestion offers excellent opportunities to convert organic waste streams into environmentally safe bio-solid. It also generates renewable energy through the bio-methane produced by the microbiological populations processing the wastes [3].

The anaerobic digestion process is carried out by the involvement of different types of microorganisms which possess a very close syntrophic relationship with production of CH₄ being slow and sensitive. In the presence of water, dry biomass is hydrolyzed anaerobically to volatile acids which are then digested by micro-bacteria archea to form biogas.

Landfill gas is however formed when the biomass has very little water or the C:N ratio is too little. Landfill gas can also be upgraded to biogas [4].

Dry biomass + water → biogas



The final result of anaerobic digestion is similar to feedstock digestion in the rumen of herbivores but with almost full biomass conversion unlike in the rumen where only about 50% conversions are attained [6]. High temperature is required to break down the biomass to bio-syngas. Further catalytic processes are required for biomethane production from the bio-syngas [7]. Any biomass with a Carbon content of 50% and 80% volatile solids and above can produce biogas. Bulky and fibrous biomasses are preferred due to their ease of digestion. They are however associated with high levels of VFAs [8]. The resulting bio-syngas thus has high levels of carbon dioxide (around 35%), water vapor, ammonia and free Nitrogen due to peptides, H₂S, C_xH_y, BTX, siloxanes (C₄H₄S), inorganic and organic contaminants [9].

Compared to other fuels such as charcoal and firewood, biogas is considered a cleaner fuel. Biogas comprise of about 65% methane (CH₄), 30% carbon dioxide (CO₂) and other trace gases such as ammonia and hydrogen sulphide. Whereas methane is fully combusted to form water and carbon dioxide, carbon dioxide is directly emitted into the atmosphere. Thus, higher levels of methane imply lower levels of carbon dioxide in the biogas and therefore less carbon dioxide emission. The molecular formulae of CH₄ implies that methane gas has the largest C:H ratio of 1:4 for all hydrocarbons. A higher C:H ratio implies more direct combustion leading to less CO₂ emission. It is also worth to note that CH₄ in the atmosphere is 4 times more potent than CO₂. More hydrocarbon combustion thus minimizes CH₄ levels in the atmosphere. Biogas emits 80% less CO_x and 60% less NO_x than other hydrocarbons [10].

Presence of carbon dioxide in biogas; lowers power output from engine, takes up space when biogas is compressed for storage in cylinders and cause freezing problems at valves and meter points where the compressed gas undergoes expansion during engine running [11]. Traces of hydrogen sulphide in biogas form sulphuric acid mists which corrode internal pipes and fittings. H₂S in biogas is largely from the sulphate content in the water used and range between 1000 to 2400 ppm. It is

traditionally removed by rapid injection of air into the biogas whereby thiobacilli bacteria oxidize the sulphides to water, hydrogen and sulphur [12].

Moisture in biogas cause corrosion as well as reduce the Heating Value (Calorific value) of the biogas. The amount of saturated water vapor in a gas depends on temperature and pressure; increasing with temperature. When water vapor condenses within a system due to pressure or temperature changes, it can result in clogging of the pipes. Water vapor in the biogas combine with CO₂ or H₂S forming corrosive carbonic or sulphuric acids which also lower the AD pH [13]. The simplest means of removing excess water vapor to dew points that preclude downstream condensate in biogas is through refrigeration [14].

Radicalization of bio-catalysts in biogas production is expected to optimize on biogas substrate potential. This study aimed at exploiting some of these biocatalysts using portable digestors at ambient conditions. A 100-day retention period was chosen to completely monitor the parametric behavior of the biogas systems.

II. METHODS AND MATERIALS

2.1 Materials

All chemicals used were lab grade and sourced from Sigma-Aldrich.

Sulfuric acid, sodium hydroxide pellets, potassium sulfate, anhydrous copper sulfate, anhydrous titanium dioxide, alundum boiling stones, methyl red indicator, methanol, ethanol tributyl citrate (for antifoam), paraffin, lysine monohydrochloride, hydrochloric acid, potassium dichromate, triple-distilled water, ferrous ammonium sulfate, magnesium sulfate, ammonia solution, absolute ethanol, universal indicator solution.

Access to centrifuge and pH meter (Hanna G-114).

2.2 Sample Pretreatment

The representative samples were pretreated before further characterization for smooth analysis. Removal of debris such as pebbles and tiny woody parts was done manually. The dry cow dung was soaked in warm water (30°C) for 1 day prior to feeding in the reactors. Parts of the animal dung containing excessive wastewater and sewerage sludge were dewatered in order not to take too much of the AD volume. Inorganic material was also separated from the organic matter by magnetic separation.

2.3 Sample Characterization

2.3.1 Solid concentration

10.000g of sample was weighed, M₁ using an Analytical balance. The sample were then placed in a furnace conditioned at 105°C for 6 hours before removing, cooling and reweighing. The new mass was recorded as M₂. A different 10.000g sample, M₃ was pre-weighed and placed in a

furnace conditioned at 540°C for 1 hour before removing, cooling and reweighing. The new mass was again recorded as M₄

$$\% \text{ TS} = M_2/M_1 * 100$$

$$\% \text{ VS} = M_4/M_3 * 100$$

The moisture content of the sample was measured by subtracting the percentage TS from 100%.

$$\text{Moisture content} = 1 - \text{TS}$$

2.3.2 Alkalinity and Fatty Acids

A raw sample was hydro-distilled and the distillate titrated against standard 0.05N H₂SO₄ solution up to pH 4.0. The volume of sample solution used was used to determine the concentration of Alkalinity in the sample. Another raw sample was also hydro-distilled and the distillate titrated against standard 0.1N NaOH solution up to pH 8.3. The volume of sample solution used was used to determine the concentration of VFAs in the sample.

2.3.3 Setting pH and temperature

The temperature and sample pH at the onset were determined using a pH meter, Hanna G-114.

2.4 Substrate Preparation

The already pretreated samples were tightly ensilaged in a polythene bag for three days for fibre content reduction. The sample was then slowly steam reformed with 1% CaO followed by 1% NaOH solutions to minimize VFA concentration. They were then dried up for a further 2 days away from direct sunlight to reduce any toxicity of the sample due to acidity. Size reduction was further done by crushing to a size of about 2 inches for ease of agitation before setting in AD.

2.5 Anaerobic Digester Reactor Design and Sample Setting

1.5 litre batch digesters were prepared. Four AD reactors were used in this case. To all, 400.00g of fresh cow dung from cows fed on grass and hay was added. 400.00ml of distilled water was then added and the mixture vigorously agitated by a shaker for 1 hour. 3.25% w/v of catalytic additives *Saccharomyces spp.*, ferric oxide and 5% v/v *Acanthaceae spp.* were then added to the reactors with the fourth reactor being left out as the control sample. 50 ml aliquot samples of the substrates containing additives were again recharacterized. The reactors were then labelled with the above initials. The combustibility of the biogas produced was tested on daily basis.

III. RESULTS AND DISCUSSIONS

3.1 Variation in Solid content over the retention period

The average TS of the samples was around 14.526±1.369% resulting to a water content of about 85.550±1.900% which is well in range with the desired standards of not less than 90% water content. [15] The TS of sample containing ferric oxide

was lower than the rest due to the hygroscopic nature of the additive used. The overall solid content in all samples declined over the retention time as they were being hydrolyzed down to biogas and liquid bio-slurry [16]. Use of the three additives was found to hasten the transformation rate

of solids to liquids. This transformation is an effective measure of the viability of the biogas additives [17]. Variation in sample solid content for the setups is summarized in table 1 below.

Table 1; Solid content of the substrate samples

Parameter	Day	Samples			
		Control	<i>Saccharomyces</i> spp.	Ferric oxide	<i>Acanthaceae</i> spp.
TS (%)	Day 1	14.413±0.008	14.389±0.012	15.890±0.011	13.413±0.014
	Day 50	12.115±0.124	10.090±0.001	9.901±0.233	11.145±1.011
	Day 100	10.245±1.107	8.071±1.223	7.456±0.023	9.111±0.324
VS (%)	Day 1	4.850 ±0.021	4.730 ±0.009	5.320 ±0.011	4.660 ±0.020
	Day 50	4.223±0.965	4.056±0.463	4.123±0.024	4.122±1.023
	Day 100	3.889±0.021	3.347±0.997	3.001±0.489	3.998±0.434
Water Content (%)	Day 1	85.587±0.070	85.611±0.060	84.110±0.070	86.587±0.030
	Day 50	87.885±0.161	89.910±0.161	90.099±0.786	88.855±0.964
	Day 100	89.755±0.132	91.929±0.333	92.544±1.045	90.889±0.789

The reactor with additive *Acanthaceae* spp. had a higher water content since the additive itself was in a syrup form (watery). The VS values were all proportionate to their TS values and ranging at around 4.890±0.570% which is in accordance to standard values (5%) [17]. Volatile solid content also declined over the retention time as the solid biomass was being converted to gas and liquid bio-slurry [16, 18]. A higher VS value in the sample containing additive ferric oxide is an indicator of more volatile matter (most of which happen to be acidic). Gallagos *et al.*, 2017 [19]; showed that the volatile matter in biomass sequentially reduce over time as hydrolysis

occur. This is in relation to the weak acidic nature of the additive.

3.2 Variation in Alkalinity and Volatile Acids over retention period

Alkalinity and volatile acids content is proportional to the composition of the biomass at that instant [20, 21]. Biomass degradation is controlled by several enzymes which are pH dependent and affect the total volatile acids produced [22]. The change in alkalinity and volatile acids across the 100-day retention period is summarized in table 2 below;

Table 2; Alkalinity and volatile acids concentration of the samples

Parameter	Day	Samples			
		Control	<i>Saccharomyces</i> spp.	Ferric oxide	<i>Acanthaceae</i> spp.
ALK (g/L)	Day 1	10.930 ±0.018	10.210 ±0.010	10.300 ±0.012	10.100 ±0.020
	Day 50	10.102±0.111	9.452±0.133	9.235±1.220	9.103±0.111
	Day 100	9.454±1.206	8.665±0.564	8.001±0.678	8.892±0.132
VFAs (g/L)	Day 1	2.670 ±0.020	1.990 ±0.011	1.870 ±0.010	1.950 ±0.011
	Day 50	2.990±1.033	2.221±1.334	2.002±0.000	2.102±0.986
	Day 100	3.421±0.978	2.093±0.888	2.010±0.463	2.457±0.765

The Alkalinity values of the samples were in the range of 10.500 ± 0.500g/L slightly higher than the anticipated and recommended value of 8.0-10.0g/L [23]. This is partly attributable to the pretreatment undertaken by steaming with 1% alkali solution. The change in alkalinity values were highest in the samples containing additives. All the samples with the catalytic additives indicated reduced VFA concentration. It was noted that the volatile acid content of the

ferric oxide sample was less compared to the other samples attaining only 2.010±0.463g/L after 100 days retention period. Shakeri *et al.*, 2017 [24], found out that volatile acids are inversely proportional to the ferric ions content in biomass sample. The ALK:VFA ratio of all the substrates was in the range of 3.6 – 5.0 as recommended. [25]

3.3 pH variation for 100 days Retention Period

All samples were within the desired range of biogas production throughout the entire retention period. There was a slight decrease in the pH of the samples for the first 20 days across all samples attributable to increased volatile acids during the precursor steps of methane formation. Thereafter, the pH gradually rose attaining a range of 7.0 -8.5.

The initial pH of all samples was in the range of 7-8 which is ideal for cow dung inoculum. The pH gradually declined then

increased after about 20 days. pH variation depend on the stage of biomass to biogas conversion i.e from hydrolysis, acidogenesis, acetogenesis and methanogenesis [26]. During hydrolysis, starch is converted to glucose (cellulose and hemicellulose enzymes thriving at neutral pH thus pH still slightly alkaline i.e. 7- 8.5) Lipids and proteins are also broken down to smaller molecules such as peptides, fats and glycerol. Both lipase and peptidase enzymes thrive at weakly alkaline pH. [27]. Figure 1 below illustrates the changes in pH over time.

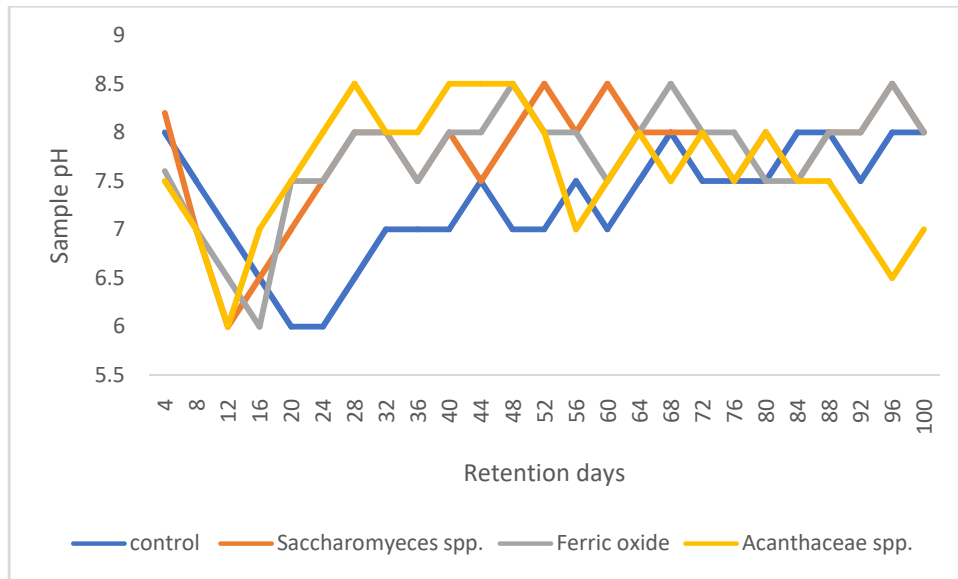


Figure 1; pH variation of substrate samples over the retention period

After the 4th day depending on the catalytic additive used, the pH gradually decrease. The pH decrease is largely attributable to the prevailing two steps; acidogenesis and acetogenesis. [28]. Higher temperature results to more acetic acid [28]. The control sample took the longest time to lower its pH indicating lack of process catalysis. Samples *Saccharomyces spp.* and *Acanthaceae spp.* attained a lower pH quickly indicating enhanced process activation by their additives. During acetogenesis step, the acids formed react to give acetic acid as the major product [28]. Transition of volatile acids to acetic acids further lowers the pH of the samples. Acetogenetic enzymes also dwell in acidic media. While the control sample showed a longer transition period (12 days) between acidogenesis and acetogenesis, sample *Acanthaceae spp.* was more drastic taking only 4 days for the transition. The final stage of biogas production is methanogenesis whereby acetic acid formed during acetogenesis is slowly cleaved to form natural gas and carbon dioxide.

The sample pH was between 7.5 -8.5 for all the samples set after Retention day 17 (ferric oxide), 20 (*Saccharomyces*

spp. & *Acanthaceae spp.*) and 32 for the control sample. From above information it is clear that additive ferric oxide is the quickest in accelerating the HRT for biogas production. Samples with additives *Saccharomyces spp.* and *Acanthaceae spp.* both reduced the HRT by 12 days each. These data is in accordance with the number of days taken to produce combustible gas as discussed under section 3.5 below.

3.4 Temperature variation for 100-day Retention Period

Biogas production is directly proportional to the digester temperature [29]. The ADs largely operated in a psychophilic regime of between 20.0 to 24.5°C. Temperature variation is not directly related to catalysis since the process of anaerobic digestion is very long and controlled by many factors, temperature itself being one of the factors [30]. The kinetics of AD are thus neither extensively endothermic nor exothermic and temperature is only usually taken to determine if the reaction is feasibly progressing. The changes in temperature over the retention period are shown in figure 2 below.

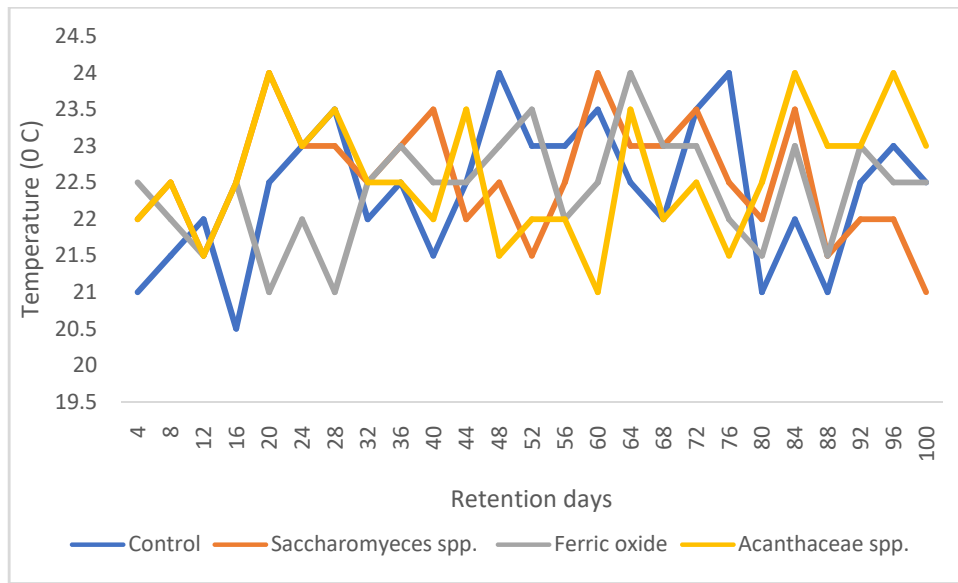


Figure 2; Temperature variation of substrate samples over the retention period

Temperature is directly proportional to both biogas quantity and quality. Since the temperature variation for all the samples remained almost constantly in range, we can only discuss the temperature of the samples before attainment of HRT periods for each of the samples. From the above graph, it is clear that the temperature of samples with additives *Saccharomyces spp.* and *Acanthaceae spp.* are consistent Retention day-26. These temperature conditions are also proportional to their HRT with both attaining HRT after 20 days. The sample with additive ferric oxide indicated a lower temperature as compared to the other samples up to Retention day-33. It is however notable that this sample was the first to attain HRT after only 17 days. The Control sample was the

most inconsistent sample, with its temperature largely influenced by external factors such as the day’s weather conditions. Failure to have a stabilized temperature elongated its HRT to 32 days and lowered its biogas yields.

3.5 Biogas production over 100-day retention period

The overall biogas yields produced in the 100-day Retention period were in the order of 4615ml (990.34ml/g-Volatile solids) for Ferric oxide, 2335ml (494.08ml/g-Volatile solids) for *Saccharomyces spp.*, 1750ml (328.94ml/g-Volatile solids) for *Acanthaceae spp.* and 1030ml (212.37ml/g-Volatile solids) for the Control sample. Biogas yields were monitored and recorded in figure 3 below;

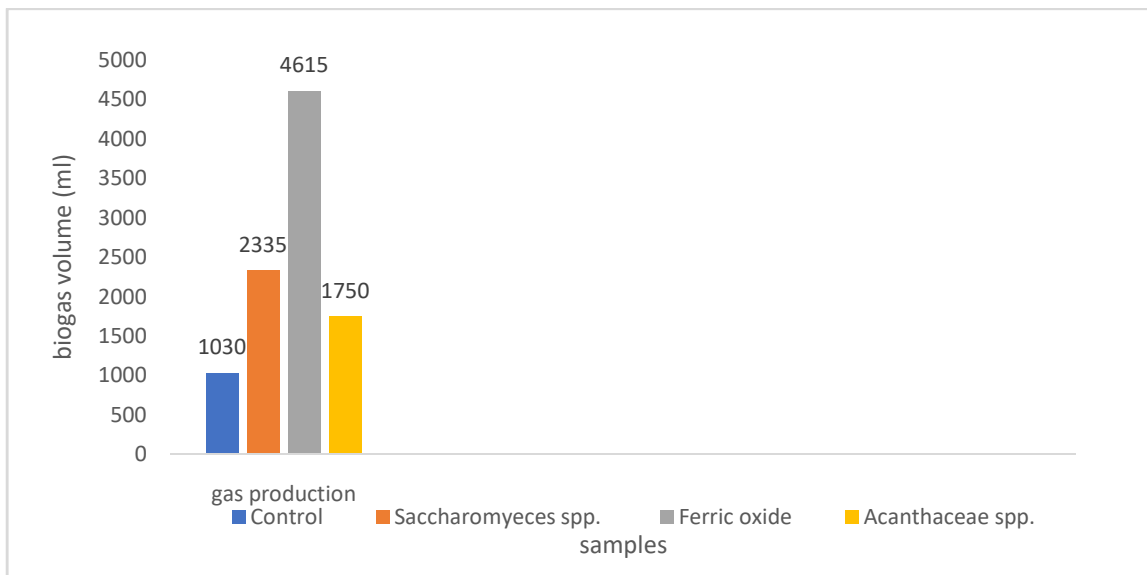


Figure 3; Biogas production yields of samples over the retention period

All the catalytic additives increased the volume of biogas production by 1.7, 2.3 and 4.5 for catalysts *Acanthaceae spp.*, *Saccharomyces spp.* and ferric oxide respectively. It is also worth noting that sample ferric oxide had the earliest Hydraulic Retention Time of 17 days. Sample ferric oxide maintained high biogas yields right from the onset and throughout the entire retention period. Biogas yields for sample ferric oxide were however inconsistent but largely in line with the sample pH expectations for each stage. Maurya

et al., 2015; showed that use of plant extracts in a mixed-consortia biogas setup and biomass pre-treatment increased biofuel yields by appreciable margins [31]. Presence of transition metals is known to increase reactivity of both organic and inorganic reactions due to their formation of low energy intermediate complexes hastening reactions [32]. The ferric oxide sample was thus able to produce more biogas volumes. These variations in biogas production are illustrated in figure 4 below.

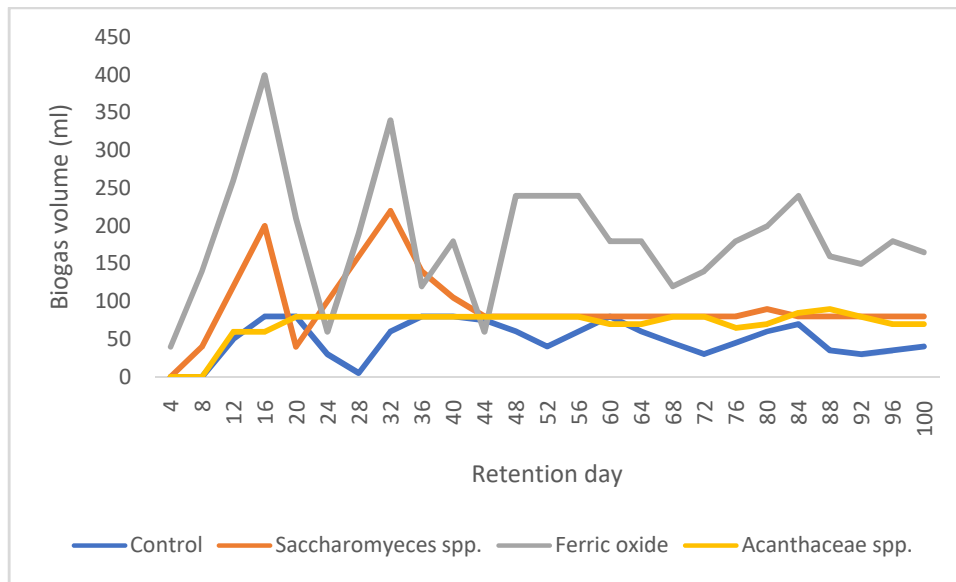


Figure 4; Biogas production patterns over the retention period

An anomaly observed with sample ferric oxide is the relation between its biogas productions with temperature whereby the biogas yields were high when the temperature was below those of the rest. An insight into this anomaly is invited. Sample *Saccharomyces spp.* and *Acanthaceae spp.* which are both purely bio-organic catalysts (BOCs) indicated more biogas yields as compared to the control sample. These additives regulated the temperature and their biogas yields

were consistent immediately after HRT attainment. Routine biogas yields from these samples were highly predictable.

3.6 Combustibility test

The time taken to produce combustible gas was in direct relation to the sample temperature readings. Table 3 below shows the durations taken for combustible gas to be formed.

Table 3; Comparison of duration taken to produce combustible biogas

Sample	Control	<i>Saccharomyces spp.</i>	Ferric oxide	<i>Acanthaceae spp.</i>
Days taken to combust	16	10	11	9

Samples *Acanthaceae spp.* and *Saccharomyces spp.* were thus the first samples to produce combustible gas (equivalent to 60% methane levels) followed by sample ferric oxide whereas the Control sample was last taking 16 days. This test indicates that biogas quality is directly related to AD temperature and catalytic additives *Acanthaceae spp.* and *Saccharomyces spp.* were tentatively raised and stabilized their AD temperature thus higher methane levels quickly attained.

IV. CONCLUSION

Green Bio-Organic Catalysts (BOCs) christened *Saccharomyces spp.* and *Acanthaceae spp.* and an inorganic catalyst ferric oxide were investigated for their effects on biogas production from fresh cow dung. Inclusion of these additives had adverse effects on biogas digester parameters such as pH and temperature leading to increased biogas yields, reduction of biogas digester hydraulic retention time and acceleration of combustibility points (more methane levels). BOCs *Saccharomyces spp.* and *Acanthaceae spp.*

had stabilized the digester temperature values thus attained combustible gas 6 and 7 days prior to the control sample and produced biogas yields 2.3 and 1.7 folds higher than the control sample. Additive ferric oxide was able to normalize pH drifts quickly, reinstating pH 7.5 and above on retention day-17 as compared to retention day-32 for control sample and thereafter fully stabilizing these pH values. Consequently, the sample with additive ferric oxide produced 4.5 folds biogas yields as compared to the control sample. The overall biogas yields produced in the 100-day retention period were in the order of 4615ml (990.34ml/g-Volatile solids) for ferric oxide, 2335ml (494.08ml/g-Volatile solids) for *Saccharomyces spp.*, 1750ml (328.94ml/g-Volatile solids) for *Acanthaceae spp.* and 1030ml (212.37ml/g-Volatile solids) for the control sample.

Integration of these biocatalysts or their sources into biogas digester systems can thus reduce biogas production time and as a result minimize production costs while increasing production yields as well as enable efficient waste biomass conversion to energy.

AUTHORS CONTRIBUTIONS

Bakari Chaka was involved in data collection and writing up of the final findings as well as compiling this research article. Dr Aloys Mosima was involved in designing of the research and reviewing of the findings as well as result analysis and discussion. Both authors approved the work with no conflict of interest.

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DECLARATION OF INTEREST

The authors declare to have no conflict of interest whatsoever.

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