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Comparative Biogas Generation from Cow Rumen Waste and Human Fecal Slurry at Different Retention Times

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A B S T R A C T

The generation of biogas from cow rumen waste and human fecal slurry at different retention times was investigated. Microbial growth, pH, and the volume of biogas produced from the two substrates were monitored over a 60-day retention period. Temperatures were maintained within the mesophilic range. Both microbiological and physicochemical analyses were conducted. The results revealed the presence of *Citrobacter* sp., *Moellerella* sp., *Providencia* sp., *Shimwellia* sp., *Micrococcus* sp., *Bacillus* sp., *Proteus* sp., *Paenibacillus* sp., *Budvicia* sp., *Solibacillus* sp., *Photobacterium sp.*, and *Breuundimonas* sp. The pH values steadily decreased alongside the total viable count, leading to a decline in biogas production from both substrates. Peak biogas volumes of 251.226 cm^3 on the 45th day and 226.103 cm^3 on the 48th day were recorded for cow rumen waste and human fecal slurry, respectively. In cow rumen waste, the pH decreased from 8.1 to 4.61, indicating a shift from slightly alkaline to acidic. In human fecal slurry, the pH dropped from 8.1 to 6.80, indicating a shift from slightly alkaline to nearly neutral. GC-MS analysis after the 60-day retention period showed that biogas from cow rumen waste comprised 50.39% methane, 19.21% carbon dioxide, 6.52% hydrogen sulphide and 5.33% ammonia. In contrast, biogas from human fecal slurry contained 57.99% methane, 17.12% carbon dioxide, 1.80% hydrogen sulphide and no detectable ammonia. The absence of ammonia in the human fecal slurry was attributed to its high moisture content of 99.39%, as indicated by the results of the proximate analysis. It can therefore be concluded that these waste products, which are typically environmental nuisances, can be effectively managed through conversion into biogas.

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1. INTRODUCTION

The environmental consequences of relying on fossil fuel are numerous and severe. For instance, fossil fuels release CO_2, CH_4 and other greenhouse gases, contributing to global warming and climate change. Combustion of fossil fuels emits NO_X, SO_2 and particulate matter, which can cause acute respiratory health problems. Their extraction and storage can lead to oil spills and leakages, contaminating water bodies and harming aquatic life. Loss of biodiversity and habitat fragmentation are also some among the consequences of fossil fuel dependency. These issues can impose significant economic costs, infrastructure damage, and loss of ecosystem services. To mitigate these challenges, transitioning toward renewable energy sources for a sustainable and safe future is imperative (Angeles et al., 2021; Holechek et al., 2022).

Biogas is typically generated through anaerobic digestion using biodegradable organic compounds during the treatment of organic waste materials (Girotto et al., 2017). Anaerobic digestion (AD) is a multi-step biological process that converts organic waste into biogas through four major biochemical reactions: hydrolysis, acidogenesis acetogenesis, and methanogenesis (Mata-Alvarez et al., 2014; Eboibi, 2020). A wide range of microbial consortia interact to degrade organic materials in the absence of oxygen, resulting in the production of biogas (Yang et al., 2019). Biogas typically consists of methane (40–75%), carbon dioxide (25–60%), nitrogen (< 2%), hydrogen sulfide (< 2%), ammonia (< 0.1%), and trace pollutants such as siloxanes and volatile organic compounds (Franco-Morgado et al., 2018; del Rosario Rodero et al., 2019).

Several factors influence the efficiency of anaerobic digestion, with temperature and pH control being particularly critical for maintaining stable processes. Slight temperature fluctuations may not significantly affect the conversion to acetic acid but can impact the conversion of acetate to methane (Matheri et al., 2016). According to Grimberg et al. (2015), anaerobic microbial systems are greatly affected by ambient temperature, which influences substrate solubility, ionization equilibria, and metabolic activity. Higher temperatures promote the growth of hydrogen-producing and spore-forming bacteria and affect the activity of hydrogenotrophic methanogens during anaerobic digestion. The optimal temperature range for mesophilic digestion is generally between 35 and 37°C, while 55°C is considered ideal for thermophilic digestion (Meegoda et al., 2018).

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Acids such as butyric, propionic, and acetic acids are more likely to be produced at low pH levels (around pH 4.0), while ammonia production predominantly occurs at pH values above 8.0. According to Matheri et al. (2016), the presence of volatile fatty acids tends to lower the pH and can reduce methanogenic activity, thereby limiting biogas production. Fermentative bacteria are less sensitive to pH fluctuations due to their broad optimal pH range of 4.0 to 8.5, whereas methanogens thrive in neutral to slightly alkaline environments and are inhibited under acidic conditions. Once, the anaerobic digestion process stabilizes, the ideal pH range for optimal performance is between 7.0 and 8.5, with values close to 7 being most favorable (Sun et al., 2022). Deviations from these optimal ranges can result in reduced biogas output, increased volatile fatty acid concentrations, inhibited microbial activity, and decreased process stability.

Sustainable energy sources offer numerous benefits. Biogas can be generated locally from readily available organic waste, making it cost-effective and simultaneously addressing waste management challenges. The resulting digestate is nutrient-rich and can be used as fertilizer or soil conditioner (Kovačić et al., 2022). Biogas provides a steady and safe supply of energy, which can be stored in low-pressure tanks or underground facilities and used when needed. It can be used for domestic heating, as vehicle and cooking fuel, and in industrial applications (Garlapati et al., 2021).

Despite its potential, biogas production faces several challenges. Biogas contains corrosive compounds such as hydrogen sulfide, which can damage equipment. Therefore, upgrading is often necessary to remove impurities and increase methane content – an energy intensive and costly process. Constructing a biogas facility can also be capital-intensive, which may limit adoption in small-scale settings or in underdeveloped countries. The biological degradation of nitrogenous materials, primarily urea and proteins, results in ammonia production. Among anaerobic microbes, methanogens are the least tolerant of ammonia and are the first to be inhibited. According to Jiang et al. 2019, methanogenic activity in granular sludge decreased by 56.5% as ammonia concentrations rose from 4051 to 5734 mg NH_3 N L^{-1}, while acidogenic populations remained largely unaffected.

Ammonia inhibition can be mitigated using adsorbents or ionic exchangers. Natural zeolite and glauconite, for instance, show high selectivity for ammonium ions (Yenigun & Demirel, 2013).

Ammonia production can disrupt microbial balance, alter acid-base production of acids and bases and lower the pH. The breakdown of ammonia also leads to the formation of volatile fatty acids, further reducing pH levels. In addition, biogas production can generate unpleasant odors and contribute to air pollution if not properly managed. The process involves pathogens and may pose health risks, if handled improperly. Therefore, public education and awareness campaigns are essential to address concerns and promote acceptance. Renewable energy sources are vital because they emit fewer greenhouse gases and are naturally replenishable, making them infinite resources. As the world transitions to a low-carbon economy, the role of renewable energy will become increasingly significant. Cow rumen waste and human fecal slurry were selected as feedstocks for this study due to their sustainability benefits in waste management and their potential to reduce environmental and health risks associated with inadequate sanitation. These substrates are abundantly available, especially in urban areas with existing sanitation infrastructure, and they possess high organic content.

2. EXPERIMENTAL

Two sets of 54-liter capacity steel tanks were used as digesters for the samples, labeled A1, A2, B1, and B2. Equal concentrations of the samples were charged into each digester. The digesters were sealed tightly to prevent leakage and a transparent plastic delivery tube was attached to convey the produced gas. Biogas quantification was carried out using the water displacement method as described by Sarker and Møller (2013).

A transparent plastic pipe was connected to a nozzle on the biodigester, filled with water leaving approximately 10% of the volume empty and then inverted. The initial water level was recorded. As biogas was generated, it displaced the water in the pipe; the displaced volume was measured daily at 12noon. The rate of biogas production was calculated by multiplying the volume of displaced water by the frequency of measurement.

A steel stirrer was installed at the top of each digester to ensure proper mixing of the contents, promoting uniform decomposition and preventing scum formation. A digital thermometer was also installed to monitor temperature of the biogas system. Anaerobic digestion proceeded over a retention period of 60 days, with daily gas measurements recorded at 12 noon. The pH and total viable count (TVC) were accessed every three days. Values of pH, TVC and gas volume were consistently recorded. Upon biogas production, aliquots of the digestate were collected and subjected to microbiological analysis, including Gram staining and subsequent biochemical tests, in accordance with the procedures outlined by Cheesbrough, (2006).

2.1 Proximate Analysis

The physiochemical properties of cow rumen waste and human fecal slurry were determined to access their suitability for biogas production. The parameters analyzed included total volatile solids, total solids, ash content, nitrogen content, protein content, moisture content, fat content, organic matter, and organic carbon content. The proximate composition of the substrates was determined according to the method described by AOAC, (2010).

3. METHOD

3.1 Methodology

3.1.1 pH, total viable count and Gas chromatography

The pH during degradation was assessed using a pH100 analyzer (Search Tech, Model PHS 3C, USA). The electrode was calibrated using buffer solutions with pH values of 4.00 and 6.87. After each insertion into the buffer solutions, the electrode was thoroughly rinsed. Following calibration, the electrode was inserted into the samples, and the readings were recorded. Test samples were collected in sterile sample bottles through a pipe outlet located beneath the digester after appropriate stirring. The composition of the evolved biogas was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent 6890 Gas Chromatograph (Agilent Technologies, Model 1100, USA) following the method described by Westerholm et al., (2019).

3.1.2 Microbiological analysis

A 10-fold serial dilution was performed on the samples using peptone water broth. A 0.1mL aliquot of suitable dilutions was inoculated onto Nutrient Agar, MacConkey Agar, and Brain Heart Infusion Agar (BHIA) plates. The inoculum was spread evenly on the agar surface using a sterile glass rod. The Petri plates were inverted, labeled and incubated at 35–37ºC for 24–48 hours for both Nutrient Agar and MacConkey Agar media. All plates were prepared in triplicate. BHIA plates were incubated in an anaerobic jar for 5–7 days using an anaerobic gas pack sachet containing carbon (Becton, Dickinson and Company, Sparks, USA) to evacuate residual oxygen, as described by Cross et al., (2019). Visible colonies were sub-cultured into stoppered tubes containing Nutrient Agar slants. Colonies were further characterized and identified using biochemical tests (Cheesbrough, 2006; Barnhart et al., 2016). The number of colonies appearing on the plates was quantified and recorded as colonies-forming units per milliliter (CFU/mL) of sample. To obtain pure isolates, colonies were sub-cultured onto fresh agar plates. Total viable counts were enumerated using the standard plate count technique described by Cheesbrough (2006). Morphological characteristics of the bacterial isolates were noted. Gram-staining and biochemical tests were conducted on pure isolates to enable accurate identification. The biochemical tests included catalase, Voges Proskauer, citrate utilization, coagulase, indole, methyl-red, hydrogen sulfide (H_2S) production, and sugar fermentation tests, following the methods outlined by Cheesbrough (2006). Results obtained were entered into the search dialogue of the Advanced Bacterial Identification Software (ABIS), available at https://www.tgw1916.net/bacteria_logare.html, to determine the probable identities of the isolates. Data were analyzed using one- way analysis of variance (ANOVA) with Origin Pro 2022 statistical software. Mean values and standard deviations were separated using Bonferroni, Tukey, Sidak and Fisher test methods at a significance level of P = 0.05.

3.1.3 Sample collection and Preparation

Samples of fresh cow rumen waste and human fecal slurry were collected from a central abattoir and a residential septic tank, respectively using sterile containers. The samples were immediately placed in ice packs at -10ºC and transported to the digester facility for further analysis. A total of 37.5 liters of each sample was measured and mixed with 2.5 liters of water to homogenize and loosen the materials prior to loading into the digesters.

4. RESULTS AND DISCUSSION

Table 1 presents the results of the identification and characterization of bacterial isolates obtained from the substrates**.** The analysis revealed the presence of a diverse microbial community, comprising *Citrobacter* sp., *Moellerella* sp., *Providencia* sp., *Shimwellia* sp., *Micrococcus* sp., *Bacillus* sp., *Proteus* sp., *Paenibacillus* sp., *Budvicia* sp., *Solibacillus* sp., *Photobacterium* sp., and *Breuundimonas* sp.

The bacterial isolates identified in this study were predominantly members of the *Enterobacteriaceae* family, which are known to play key roles as microbial indicators in the anaerobic digestion of organic waste. Previous research by Pietruszka et al. (2023), reported that genera such as *Citrobacter*, *Bacillus* and *Moellerella* are commonly present during the initial hydrolytic stages of anaerobic digestion. In alignment with these findings, the current study confirms a high abundance of *Enterobacteriaceae* in both cow rumen waste and human fecal slurry. These microorganisms are instrumental in the digestion process, contributing to syntrophic interactions, breaking down complex organic compounds into simpler molecules, and participating in metabolic activities that lead to the production of carbon dioxide and methane. Notably, *Bacillus* species demonstrate resilience in fermentation processes due to their spore-forming capability and the production of diverse hydrolytic enzymes (Prenafeta-Boldù et al., 2017). Supporting this, Abuhena et al. (2022) highlighted *Bacillus subtilis* as a promising candidate for bioaugmentation in anaerobic digestion systems, owing to its rapid growth on inexpensive substrates, efficient protein secretion, strong degradative potential and extended survivability.

Table 1. Biochemical test results of bacterial isolates

Isolate	Morphology	Gram Reaction	Catalase	Coagulase	Citrate Utilization	Oxidase	Methyl Red	Voges Proskauer	Sulphur	Indole Production	Motility	Glucose	Surcrose	Lactose	Mannitol	Maltose	Xylose	Probable Organism
1	Tiny rods in clusters	-	+	+	-	-	+	-	+	+	+	A	A	A	A	A	A	*Citrobacter* sp.
2	Rod in clusters	-	-	-	-	+	+	-	+	+	-	A	A	A	-	A	A	*Moellerella* sp.
3	Rod in chains	-	-	-	-	-	+	-	-	+	+	A	-	-	-	-	-	*Providencia* sp.
4	Rod in chain	+	+	-	+	-	+	+	+	+	+	-	A	-	-	-	-	*Shimwellia* sp.
5	Cocci in clusters	+	+	-	-	-	-	-	-	-	-	-	-	-	-	A	-	*Micrococcus* sp.
6	Rod in singles	+	+	-	-	-	-	-	-	-	-	A	A	-	-	A	-	*Bacillus* sp.
7	Rod in chains	-	+	-	-	-	+	-	-	-	-	A	A	-	-	A	-	*Proteus* sp.
8	Rod in clusters	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	*Bacillus* sp.
9	Rod in chains	+	+	-	-	-	+	+	-	-	+	A	A	A	-	-	-	*Paenibacillus* sp.
10	Cocci in clusters	-	+	-	+	-	-	-	-	-	-	A	A	A	A	A	-	*Budvicia* sp.
11	Rod in chains	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	*Solibacillus* sp.
12	Tiny rods in chains	-	+	-	-	+	+	-	-	-	-	A	A	-	-	-	-	*Photobacterium* sp.
13	Rod in chains	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	*Breuundimonas* sp.

Furthermore, positive results from the citrate utilization and methyl red tests in this study suggest the presence of metabolic pathways that convert citrate into oxaloacetate and acetate, subsequently leading to the formation of pyruvate and carbon dioxide – key intermediates in acidogenic and acetogenic stages of biogas production. *Moellerella* species, identified as acid-tolerant anaerobes, are capable of surviving in environments such as human fecal slurry and can outcompete other microbial species thereby dominating the microbial community in the digester (Zhang et al., 2018).

Members of the *Enterobacteriaceae* such as *Citrobacter*, *Bacillus*, and *Moellerella*, contribute to the degradation of proteins into peptides and amino acids through syntrophic relationships with other microorganisms. Additionally, both *Bacillus* and *Moellerella* are known to produce lipases that hydrolyze lipids into fatty acids and glycerol, while also participating in carbohydrate degradation and the generation of hydrogen gas, a key substrate for methanogenic archaea. In our study, biogas production showed a dynamic pattern correlated with microbial activity. Initially, a high microbial count coincided with increased biogas output. However, as anaerobic digestion progressed, a slight increase in gas production was followed by a subsequent decline, paralleled by a decrease in microbial population. These changes are likely attributable to pH fluctuations inherent to anaerobic digestion, which can inhibit sensitive microbial populations. Nevertheless, acidophilic organisms may continue to thrive under these conditions and sustain biogas generation. The observed relationship between microbial count and biogas yield underscores the complex interplay between microbial succession, substrate degradation, and metabolite accumulation over time.

Interestingly, biogas was produced more rapidly from human fecal slurry than from cow rumen waste. This can be attributed to several factors: human fecal slurry generally has a higher content of nutrients such as nitrogen and phosphorus, a more favorable carbon-to-nitrogen (C/N) ratio, and a near-neutral pH (~7), all of which support optimal conditions for methanogenic activity (Kumari & Das, 2017).

In contrast, cow rumen waste may require additional conditioning or co-digestion to enhance its biodegradability and biogas potential. Table 2 presents the result of the proximate analysis of the substrates.

As the anaerobic digestion process progressed towards the methanogenesis stage, a decline in total viable counts observed, as shown in Table 3. This reduction in microbial population can be attributed to the continued depletion of available nutrients in the substrates, which likely led to increased competition among bacterial populations, ultimately resulting in a decrease in their number (Ren et al., 2016; Wang et al., 2017). Additionally, a steady decrease in the pH of the substrates was observed across all digesters, as depicted in Figures 1 and 2. This drop in pH created conditions of microbial stress, which further contributed to the decline in microbial viability.

Extreme acidic conditions are known to be detrimental to bacterial populations, and their reduction over time in the digester can be attributed to this pH shift (Mao et al., 2015; Lackner et al., 2020). Notably, lower pH values can suppress the growth of competing microorganisms, thereby favoring acidophilic methanogens, which are capable of thriving in such environments. These methanogens are able to dominate the microbial community and continue the process of biogas production (Franke-Whittle et al., 2014; Bahira et al., 2018; Ali et al., 2019). A study by Ai et al. (2018), examined the role of acid-producing species, specifically *Bacillus* sp. and *E. coli*, in decreasing pH levels during anaerobic digestion of waste–activated sludge (WAS). The research indicated that the presence of *Enterobacteriaceae* increased the acidity of the material, contributing to a decline in pH. These findings align with the results of the present study, as pH of the digesters steadily decreased over time, corresponding with increased retention time. Furthermore, the degradation of proteins and urea in the rumen material is likely to have contributed to the observed pH reduction, as the dissociation of ammonia into ammonium and hydroxide ions leads to the formation of an acidic environment (Jiang et al., 2019; Yellezuome et al., 2022).

Biogas production increased as the retention time progressed, reaching an optimal value at a certain point before declining after the maximum gas yield was achieved. During the first three weeks of anaerobic digestion of cow rumen waste, biogas production increased from 12.61 cm^3 to 117.239 cm^3, while the total viable count decreased from 164 x 10^7 cfu/ml to 66 x 10^7 cfu/ml. At the peak biogas yield of 251.226 cm^3, the microbial count was 62 x 10^7 cfu/ml. Similarly, in the first three weeks of anaerobic digestion of human fecal slurry, biogas generation rose from 10.968 cm^3 to 92.116 cm^3 while the total viable count dropped from 90 x 10^7 cfu/ml to 56 x 10^7 cfu/ml. At the highest yield of 226.103 cm^3, the microbial count was 30 x 10^7 cfu/ml. These findings indicate that biogas production was slow and gradual during the first three weeks of the research. This phase corresponds to the microbial lag phase, where cells adapt or acclimatize to a new environment (Ya'aba & Ramalan, 2020). It can also be inferred that the biogas production rate is closely linked to microbial growth, with higher microbial numbers correlating with greater biogas volumes. Figure 3 below presents the GC-MS analysis of the biogas components generated from the samples on the 60th day. Human fecal slurry exhibited a higher methane content of 57.99 % compared to 50.39 % in cow rumen waste, although both values falls are within the expected range (Xie et al., 2020).

Table 2. Proximate analysis of the substrates

Parameters	Cow rumen waste (%)	Human fecal slurry (%)
Moisture content	98.67	99.39
Ash content	1.09	0.034
Fat content	0.113	0.041
Protein content	0.475	0.363
Nitrogen content	0.076	0.058
Organic Carbon content	0.391	0.094
Organic Matter content	0.673	0.162
Total Solids	1.33	0.61
Total Volatile Solids	0.24	0.576

Table 3. Cumulative biogas yields at different retention times for cow rumen waste and human fecal slurry

Cow rumen waste				Human fecal slurry			
Retention Time (Days)	**pH Value**	**Biogas yield (ml)**	**Total viable count (cfu/ml) x 10^7**	**Retention Time (Days)**	**Biogas yield (ml)**	**pH Value**	**Total viable count (cfu/ml) x 10^7**
3	8.1	12.561	164	3	10.468	8.1	90
6	7.9	31.403	142	6	27.216	8	86
9	7.4	41.871	143	9	41.871	7.8	71
12	7.1	83.742	103	12	56.526	7.9	69
15	6.5	100.490	99	15	69.087	7.4	65
18	5.8	108.865	81	18	81.648	7.21	62
21	5	117.239	66	21	92.116	7.05	56
24	5.08	142.362	66	24	113.052	6.72	56
27	5.19	157.016	65	27	127.707	6.27	52
30	4.92	177.951	63	30	146.549	6.7	49
33	5	192.607	69	33	159.110	7.05	47
36	5.08	200.981	68	36	171.671	6.72	31
39	5.19	217.729	70	39	188.420	6.27	29
42	4.92	247.039	65	42	209.355	6.7	28
45	4.82	251.226	62	45	221.916	6.57	26
48	4.85	215.636	62	48	226.103	6.73	30
51	4.87	205.168	60	51	200.981	6.94	32
54	4.76	198.887	56	54	177.952	6.86	30
57	4.65	188.420	56	57	133.987	6.85	30
60	4.61	167.016	42	60	125.613	6.80	28

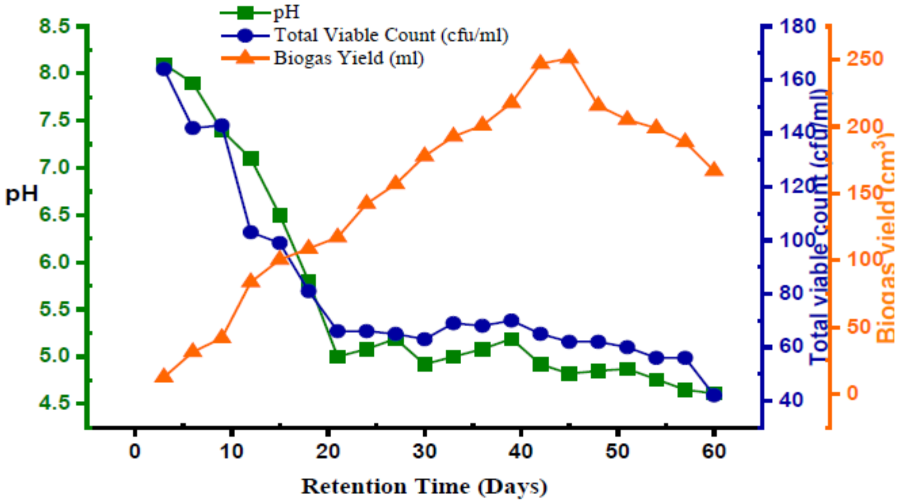

Figure 1. Relationship of pH, retention time, total viable count, and biogas yield using cow rumen waste substrate

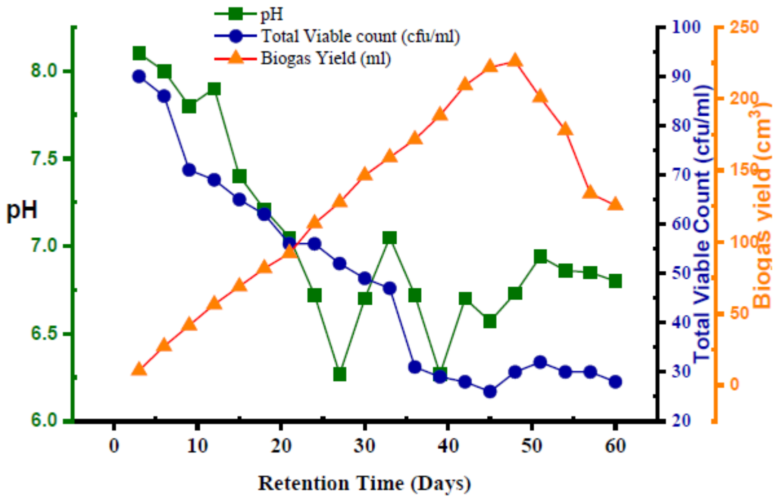

Figure 2. Relationship of pH, retention time, total viable count, and biogas yield using human fecal slurry substrate

This aligns with findings from Dhungana et al. (2019) and Soyingbe et al. (2019), who reported methane contents of 53.11 % and 40 % respectively, using human fecal slurry. Other components were found in trace amounts and would require specific treatments for removal to enhance the calorific value of biomethane (Ahmed et al., 2020). Ammonia is often cited as a major component of human fecal slurry, which could hinder sustained biogas production (Zhang et al., 2014; Wang et al., 2015; Adjama et al., 2022). However, in this study, no ammonia content was detected in the human fecal slurry after 60 days of retention time. According to Zhang et al. (2017), Fuchs et al. (2018), and Kupper et al. (2020), ammonia can be lost through dilution, which supports the findings of the proximate analysis that showed 93.39 % moisture content in the human fecal slurry.

5. STATISTICAL ANALYSIS

Statistical analysis was conducted using the Origin Pro 2022 statistical package. A one-way analysis of variance (ANOVA) was performed to compare the biogas yields between the two substrates.

The null hypothesis assumes that the means of all groups are equal, and that at a significance level of 0.05, there is no significant difference. As shown in Tables 4 and 5, the P–value obtained was 0.0731, which is greater than 0.05. Therefore, the null hypothesis is not rejected, and it can be concluded that there is no significant difference between the biogas yields from the two substrates. As shown in Table 6, the grouping letter is same signifying that the groups are not significantly different from each other.

5. CONCLUSIONS

The performance of cow rumen waste and human fecal slurry demonstrates that both can serve as viable and cost-effective alternative sources of energy. The biogas generated from these substrates can be further upgraded by removing carbon dioxide, carbon monoxide, and ammonia using suitable, environmentally-friendly zeolites. However, human fecal slurry presents a more sustainable resource due to its higher biomethane content and the absence of ammonia.

Bacterial isolates from these substrates can be further characterized at the species and strain levels using molecular techniques. These isolates can then be purified and selectively used as microbial inoculants in biogas production and bioaugmentation processes involving organic waste. This research aligns several United Nations Sustainable Development Goals (SDGs): Goal 2, (Zero Hunger) by supporting sustainable agricultural practices, Goal 6, (Clean Water and Sanitation) by improving waste management and reducing water pollution, Goal 7, (Affordable and Clean Energy) by increasing access to renewable energy, and Goal 13, (Climate Action) by reducing greenhouse gas emissions and mitigating the impacts of climate change.

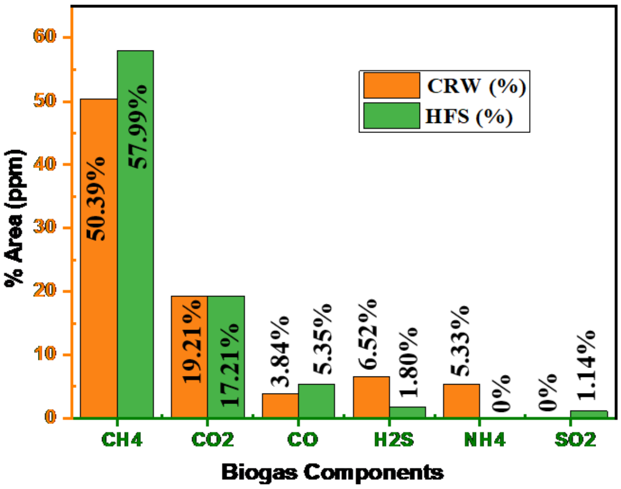

Figure 3. Comparison of GC-MS results in % area on the composition of biogas generated in both substrates

Table 4. Overall ANOVA

Overall ANOVA					
	df	**Sum of squares**	**Mean square**	**F value**	**Prob >F**
Model	1	15108.51218	15108.51218	3.27317	0.07301
Error	116	535439.7452	4615.85987		
Total	117	550548.25738			

Table 5. Mean Comparisons

Mean comparisons	Mean difference	SEM	t/q value	Prob.	Alpha	Sig.	LCL	UCL
Bonferroni test	-22. 6308	12.50879	-1.80919	0.07301	0.05	0	-47.40603	2.14444
Tukey test	-22. 6308	12.50879	2.55858	0.07301	0.05	0	-47.40604	2.14445
Sidak test	-22. 6308	12.50879	-1.80919	0.07301	0.05	0	-47.40603	2.14444
Fisher test	-22. 6308	12.50879	-1.80919	0.07301	0.05	0	-47.40603	2.14444

Table 6. Grouping letter table

Grouping letter table	Mean	Groups
Cow rumen waste biogas yield	152.14708	A
Human fecal slurry biogas yield	129.51629	A

6. ACKNOWLEDGEMENT

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REFERENCES

1. Abuhena, M., Azim, F., Barman, N.C., Khan, N.M., Kabir, G., Rasul, N.M., Huang, Z., Akter, S. & Hug, A. (2022). Optimization of *Bacillus subtilis*-based fermentation of anaerobic digestate and biohazard-free application in endophyte-assisted hardening of micro propagated plantlets for increasing survivability. *Biocatalysis and Agricultural Biotechnology*, 45, 102512. https://doi.org/10.1016/j.bcab.2022.102512
2. Adjama, I., Derkyi, N., Uba, F., Akologo, G. & Opuko, R. (2022). Anaerobic co-digestion of Human feces with rice straw for biogas production: A case study in Sunyani. *Hindawi Modelling and Simulation in Engineering, 2022,* 7. https://doi.org/10.1155/2022/2608045
3. Ahmed, S.A., Zhou, S., Tsegay, A.S., Zhu, Y., Malik, A., Ahmad, N. & Legese, Z. (2020). Effects of CO_2 ratio in biogas on performance, combustion and emissions of dual- fuel diesel engine. *International Energy Journal*, 20, 15-38. http://www.rericjournal.ait.ac.th/index.php/reric/article/view/2112
4. Ai, S., Liu, H., Wu, M., Zeng, G. & Yang, C. (2018). Roles of acid-producing bacteria in anaerobic digestion of waste activated sludge. *Frontiers of Environmental Science and Engineering*, 12, 1-11. https://doi.org/10.1007/s11783-018-1050-y
5. Ali, S., Hua, B., Huang, J.J., Droste, R.L. Zhou, Q, Zhao, W. & Chen L. (2019). Effect of different initial low pH conditions on biogas production, composition and shift in the aceticlastic methanogenic population. *Bioresource Technology*, 289, 121579. https://doi.org/10.1016/j.biortech.2019.121579
6. Angeles, K., Patsialis, D., Taflanidis, A. A., Kijewski-Correa, T. L., Buccellato, A. & Vardeman, C. (2021). Advancing the design of resilient and sustainable buildings: An integrated life-cycle analysis. *Journal of Structural Engineering, 147* (3), 04020341. https://doi.org/10.1061/(ASCE)ST.1943-541X.0002910
7. AOAC (2010). Association of Official Analytical Chemists official Methods of Analysis of the Association of Official Analytical Chemists. 18th Edition, AOAC International, Washington, DC. https://www.aoac.org/official-methods-of-analysis/
8. Bahira, B.Y., Baki, A.S. & Bello, A. (2018). Effect of varying pH on Bigas generation using Cow dung. *Direct Research Journal of Biology and Biotechnology*, *4* (3), 28-33. https://directresearchpublisher.org/drjbb/abstract/effect-of-varying-ph-on-biogas-generation-using-cow-dung/
9. Barnhart, E. P., Weeks, E. P., Jones, E. J., Ritter, D. J., McIntosh, J. C., Clark, A. C., Ruppert, L.F., Cunningham, A.B., Vinson, D.S., Orem, W. & Fields, M. W. (2016). Hydro-geochemistry and coal-associated bacterial populations from a methanogenic coal bed. *International Journal of Coal Geology*, *162*, 14-26. https://doi.org/10.1016/j.coal.2016.05.001
10. Cheesbrough, M. (2006). District laboratory practice in Tropical countries. Part 2. 2nd Edition, Cambridge University Press, New York. https://www.medbox.org/preview/5255d6e1
11. Cross, K. L., Campbell, J. H., Balachandran, M., Campbell, A. G., Cooper, C. J., Griffen, A., Heaton, M., Joshi, S., Klingeman, D., Leys, E. & Yang Z. (2019). Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nature Biotechnology, 37* (11), 1314-1321. https://doi.org/10.1038/s41587-019-0260-6
12. del Rosario Rodero, M., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., García-Encina, P. A. & Muñoz, R. (2019). Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor. *Bioresource Technology, 279,* 43-49. https://doi.org/10.1016/j.biortech.2019.01.110
13. Dhungana, S., Adhikari, B., Shrestha, S.D. & Shrestha, B.P. (2019). Enhanced biogas production from fecal sludge by iron metal supplementation: iron enriched fertilizer as a byproduct. IOP Earth and Environmental Science, 301, 012028. https://doi.org/10.1088/1755-1315/301/1/012028
14. Eboibi, B.E (2020). Anaerobic digestion of cow manure: influence of hydraulic retention time on biogas yield and properties of digestate. *LAUTECH Journal of Engineering and Technology*, *14*(1), 87-97. Retrieved from https://laujet.com/index.php/laujet/article/view/366
15. Franco-Morgado, M., Toledo-Cervantes, A., González-Sánchez, A., Lebrero, R., & Muñoz, R. (2018). Integral (VOCs, CO_2, mercaptans and H_2S) photosynthetic biogas upgrading using innovative biogas and digestate supply strategies. *Chemical Engineering Journal*, *354*, 363-369. https://doi.org/10.1016/j.cej.2018.08.026
16. Franke-Whittle, I.H., Walter, A., Ebner C. & Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management*, *34,* 2080- 2089. https://doi.org/10.1016/j.wasman.2014.07.020
17. Fuchs, W., Wang, X., Gabauer, Ortner, M. & Li, Z. (2018). Tackling ammonia inhibition for efficient biogas production from chicken manure: status and technical trends in Europe and China. *Renewable and Sustainable Energy Reviews*, *97*, 186-199. https://doi.org/10.1016/j.rser.2018.08.038
18. Garlapati, V. K., Sharma, S. & Sevda, S. (2021). Photosynthetic biogas upgrading: an attractive biological technology for biogas upgrading, In Emerging Technologies and Biological Systems for Biogas Upgrading Academic Press, pg. 383-409. https://doi.org/10.1016/b978-0-12-822808-1.00014-3
19. Girotto, F., Lavagnolo, M. C. & Pivato, A. (2018). Spent coffee grounds alkaline pre-treatment as biorefinery option to enhance their anaerobic digestion yield. *Waste and Biomass Valorization*, *9*, 2565-2570. https://doi.org/10.1007/s12649-017-0033-8
20. Grimberg, S. J., Hilderbrandt, D., Kinnunen, M. & Rogers, S. (2015). Anaerobic digestion of food waste through the operation of a mesophilic two-phase pilot scale digester–assessment of variable loadings on system performance. *Bioresource Technology*, *178*, 226-229. https://doi.org/10.1016/j.biortech.2014.09.001
21. Holechek, J. L., Geli, H. M. E., Sawalhah, M. N. & Valdez, R. (2022). A global assessment: Can renewable energy replace fossil fuels by 2050? *Sustainability*, *14*, 4792. https://doi.org/10.3390/su14084792
22. Jiang, Y., McAdam, E., Zhang, Y., Heaven, S., Banks, C. & Longhurst, P. (2019). Ammonia inhibition and toxicity in anaerobic digestion: a critical review. *Journal of Water Process Engineering*, *32*, 100899. https://doi.org/10.1016/j.jwpe.2019.100899
23. Kovačić, Đ., Lončarić, Z., Jović, J., Samac, D., Popović, B. & Tišma, M. (2022). Digestate management and processing practices: a review. *Applied Sciences*, *12*(18), 9216. https://doi.org/10.3390/app12189216
24. Kumari S. & Das, D. (2017). Improvement of biohydrogen production using acidogenic culture. *International Journal of Hydrogen Energy*, 42(7), 4083- 4094. https://doi.org/10.1016/j.ijhydene.2016.09.021
25. Kupper, T., Eugster, R., Sintermann, J. & Hani, C. (2021). Ammonia emissions from an uncovered dairy slurry storage tank over two years: Interactions with tank operations and meteorological conditions. *Biosystems Engineering*, *204*, 36-49. https://doi.org/10.1016/j.biosystemseng.2021.01.001
26. Lackner, N., Wagner, A.O., Markt, R. & Illmer, P. (2020). pH and phosphate induced shifts in carbon flow and microbial community during thermophilic anaerobic digestion. *Microorganisms*, *8*(2), 286. https://doi.org/10.3390/microorganisms8020286
27. Mao, C., Feng Y., Wang X. & Ren G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable Sustainable Energy Review*, *45*, 540-555. https://doi.org/10.1016/j.rser.2015.02.032
28. Mata-Alvarez, J., Dosta, J., Romero Güiza, M.S., Fonoll, X., Peces, M. & Astals, S. A. (2014). Critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, *36*, 412-427. https://doi.org/10.1016/j.rser.2014.04.039
29. Matheri, A. N., Belaid, M., Seodigeng, T., & Ngila, C. J. (2016). Modelling the kinetic of biogas production from co-digestion of pig waste and grass clippings. In *24th World Congress on Engineering (WCE 2016) London-UK*. https://www.iaeng.org/publication/WCE2016/WCE2016_pp813-820.pdf

30. Meegoda, J. N., Li, B., Patel, K., & Wang, L. B. (2018). A review of the processes, parameters and optimization of anaerobic digestion. *International Journal of Environmental Research and Public Health*, *15*(10), 2224. https://doi.org/10.3390/ijerph15102224

31. Pietruszka, A., Maslanko, M. & Ciecholewska-Jusko, D. (2023). Sanitization of biomass in agricultural biogas plants depends on the type of substrates. *Animals*, *13*(5), 855. https://doi.org/10.3390/ani13050855

32. Prenafeta-Boldù, F.X., Fernandez, B., Vinas, M., Lizardo, R., Brufau, J., Owusu-Asiedu, A., Walsh, M.C. & Awati, A. (2017). Effect of *Bacillus* spp. Direct-fed microbial on slurry characteristics and gaseous emissions in growing pigs fed with high fiber-based diets. *Animal (Basel)*, *11*(2), 209-218. https://doi.org/10.1017/S1751731116001415

33. Ren, G., Xu, X., Qu, J., Zhu, L., Wang, T. (2016). Evaluation of microbial population dynamics in the co-composting of cow manure and rice straw using high throughput sequencing analysis. *World Journal of Microbiology and Biotechnology*, *32*, 1-11. https://doi.org/10.1007/s11274-016-2059-7

34. Sarker, S. & Møller, H. B. (2013). Boosting biogas yield of anaerobic digesters by utilizing concentrated molasses from 2nd generation bioethanol plant. *International Journal of Energy and Environment (Print)*, *4*(2), 199-210. *ISSN 206-2895. https://www.ijee.ieefoundation.org/vol4/issue2/IJEE_04_v4n2.pdf*

35. Soyingbe, A.A., Olayinka, O., Bamgbose O. & Adetunji M.T. (2019). Effective management of faecal sludge through co-digestion for biogas generation. *Journal of Chemical Society of Nigeria*, *44*(5), 858-870. https://dx.doi.org/10.4314/jasem.v23i6.25

36. Sun, C., Guo, L., Zheng, Y., Yu, D., Jin, C., Zhao, Y., Tao, Z., Gao, M. & She, Z. (2022). Effect of mixed primary and secondary sludge for two-stage anaerobic digestion (AD). *Bioresource Technology*, *343*, 126160. https://doi.org/10.1016/j.biortech.2021.126160

37. Wang, X., Oehmen, A., Freitas, E. B., Carvalho, G. & Reis, M. A. (2017). The link of feast-phase dissolved oxygen (DO) with substrate competition and microbial selection in PHA production. *Water Research*, *112*, 269-278. https://doi.org/10.1016/j.watres.2017.01.064

38. Wang, X., Zhang L., Xi, B., Sun W., Xia X., Zhu C., He, X., Li, M., Yang, T., Wang P., Zhang Z., (2015). Biogas improvement and C/N control by natural clinoptilolite addition into anaerobic co-digestion of *phragmites australis*, feces and kitchen waste. *Bioresource Technology*, *180*, 192-199. https://doi.org/10.1016/j.biortech.2014.12.023

39. Westerholm, M. & Schnürer, A. (2019). Microbial responses to different operating practices for biogas production systems, pp. 1-37. In Banu R.J. (ed.), Anaerobic digestion, Intech Open, London United Kingdom. DOI: http://dx.doi.org/10.5772/intechopen.82815

40. Xie, L., Xu, J., Zhang, Y. & He, Y. (2020). Biogas upgrading, *Advances in Bioenergy*, 5, 309-344. https://doi.org/10.1016/bs.aibe.2020.04.006

41. Ya'aba, Y. & Ramalan, A.S. (2020). Isolation, identification and Characterization of some bacteria associated with biogas production from cow dung. *Equity Journal of Science and Technology*, *7*(2), 91-99. ISSN 2354-1814. https://www.ajol.info/index.php/equijost/article/view/214414/202224

42. Yang, Q., Wu, B., Yao, F., He, L., Chen, F., Ma, Y., Shu, X., Hou, K., Wang, D. & Li, X. (2019). Biogas production from anaerobic co-digestion of waste activated sludge: co-substrates and influencing parameters. *Review on Environmental Science and Biotechnology*, *18*, 771–793. https://doi.org/10.1007/s11157-019-09515-y

43. Yellezuome, D., Zhu, X., Wang, Z. & Liu, R. (2022). Mitigation of ammonia inhibition in anaerobic digestion of nitrogen-rich substrates for biogas production by ammonia stripping: a review. *Renewable and Sustainable Energy Reviews*, *157*, 112043. https://doi.org/10.1016/j.rser.2021.112043

44. Yenigun O. & Demirel, B. (2013). Ammonia inhibition in anaerobic digestion: a review. *Process Biochemistry*, *48*, 901-911. https://doi.org/10.1016/j.procbio.2013.04.012

45. Zhang, Q., Hu, J., Lee, D.J., Cheng, Y. & Lee, Y.Y. (2017). Sludge treatment: current research trends. *Bioresource Technology*, *243*, 1159-1172. https://doi.org/10.1016/j.biortech.2017.07.070

46. Zhang, W., Xing, W. & Li, R. (2018). Real-time recovery strategies for volatile fatty acid-inhibited anaerobic digestion of food waste for methane production. *Bioresource Technology*, *265*, 83-92. https://doi.org/10.1016/j.biortech.2018.05.098

47. Zhang, W.Q., Wei, Q.Y., Wu, S.B., Qi, D.D., Li W., Zuo, Z. & Dong, R.J. (2014). Batch anaerobic co-digestion of pig manure with dewatered sewage under mesophilic conditions. *Journal of Applied Energy*, *128*, 175-183. https://doi.org/10.1016/j.apenergy.2014.04.071