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Anaerobic digestion of *gliricidia sepium* co-digested with pig manure using automated and portable digester

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Promoting biogas as a renewable energy source is strengthened by decentralized anaerobic digestion systems at the community level, providing an innovative approach to managing organic waste and sustaining energy. The anaerobic digestion of pig dung co-digested with *gliricidia sepium* were experimented with over 30 days all using both fabricated and automated digesters. This study used a mixing ratio of the substrate to an inoculum of 1:1 for a hydraulic retention time (HRT) of 30 days in triplicates. The physicochemical and microbial characteristics of the substrates and digestate were analyzed using standard procedures. A statistical analysis was conducted using ANOVA to assess the data. There was a reduction in cellulose concentration by 2% m.m^{-1} after the thermo-alkaline pretreatment, which indicates a modification in the structure that aids the breakdown of the biomass during digestion Experimental results show that pig dung co-digested with *gliricidia sepium* produced a gas yield of 0.986772m^3 and 0.50845m^3 from the automated and fabricated digester respectively. When comparing treated and untreated *Gliricidia sepium*, the raw sepium had a higher C/N ratio, ranging from 7 to 8. Iron, zinc, aluminum, copper, Biological oxygen demand, Chemical oxygen demand as well as T.alkalinity, T.nitrogen, T.phosphate, T.carbon, potassium, sulfate, calcium, magnesium, and manganese well as total solids, volatile solids all showed an increase after pre-treatment. This research has shown that significant methane content of gas (58.26%) can be generated from *Gliricidia sepium* co-digested pig manure even at small-household levels.

Keywords Anaerobic digestion, *Gliricidia sepium*, Biogas, Digester, Biomass, Pretreatment, Renewable energy, Methane yield

Energy is a significant tool for expansion, yet humanity's dependence on fossil fuels has led to environmental fading, a shift in the climate, and health challenges[1,2]. Insufficient energy supply and environmental degradation pose significant challenges for Nigeria and numerous other emerging nations worldwide[3–5]. The present energy crisis has consequently put a huge strain on the nation's economic growth and development. Increasing energy supply is of great importance in Sub-Saharan Africa (SSA) because currently fewer than 45% of the population has access to power[6]. The national electricity infrastructure in Nigeria serves only 40% of the country's population, and power outages occur 60% of the time[7,8]. Energy is essential for development, and sustainable energy systems are required for long-term development[9]. As fuelwood is still the primary source of energy for over 80% of Nigerians, the country's current energy plan no longer places a high priority on using fuelwood for energy. The adoption of sustainable and renewable energy sources as alternatives to conventional energy sources has increased due to depletion and environmental harm[10]. *Gliricidia sepium*is an example of agricultural residue that can be transformed chemically or biologically[11]. Pig manure, cow dung, and chicken droppings are agriculture classified as animal waste that frequently emits an unpleasant odor and affects the surrounding community's environment[12,13]. In a biogas digester, bio-waste decompose in the absence of oxygen to produce biogas and other hydrocarbons. Biogas can be naturally produced in a variety of environments, including swamps, waterlogged, marine sediment, saturated soils, rice fields, plantations, deep water, sanitary

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landfills, and even ruminant and termite digestive systems[14]. Biogas has been used on a modest scale for heating, cooking, and illuminating in low-tech environments all across the world for a long time. According to the World Biogas Association, biogas served Assyrian baths circa 900 BC. Alessandro Volta, an Italian scientist, is usually associated with discovering methanol in the 1770s. According to legend, Volta's research was inspired by Franklin's investigation into "flammable air"[15]. Anaerobic digestion systems, which may be found on a farm or an industrial site, are the primary source of biogas production. According to[16], the digester is usually made up of a spherical holding tank with a rubber membrane. Methane (CH_4) and dioxide (CO_2) constitute the majority of biogas composition, with the remaining gases being hydrogen (H_2), hydrogen sulfide (H_2S), water vapor (H_2O), nitrogen (N), breathable air (O_2), and ammonia (NH_3).

Waste accumulation poses a number of environmental challenges, related to health and safety hazards, as well as preventing progress in terms of resource recovery and waste material recycling[17]. The use of petroleum and diesel remains the dominant source of energy. In contrast to renewable energy, fossil fuels are non-renewable, highly polluting, and their production is predicted to fall over the next few decades. However, the increasing use of fossil fuels for energy has an environmental impact through increased greenhouse gas emissions, environmental degradation of water, air, and land, and warming temperatures, all of which have a significant influence on people's quality of existence and their health. Hence, it is imperative to establish novel and sustainable energy supply systems capable of satisfying the increasing demand for energy from renewable sources[18]. There is a growing focus on minimizing greenhouse gas emissions by promoting the production of renewable energy.

Lignocellulosic biomass is numerous and can be obtained from a variety of sources, including agricultural, municipal, industrial, and others[19]. Corn chaff, timber sawdust, elephant grass, siam weed, and wild Mexican sunflower are examples of lignocellulose biomass used for biogas production[20–22] *Gliricidia sepium*commonly referred to as 'Agumaniye', is a rapidly growing tree species capable of dispersing seeds up to 40 m from the parent tree. It is versatile and widely utilized for various purposes, including fuel, shade provision, green manure production, and live fencing. It can quickly dominate secondary forests, suppress native species and raising environmental concerns[23,24]. Animal waste is another bioenergy source available in Nigeria[25]. Various studies have been carried out to determine whether animal manure may produce biogas. The biogas digester output from cowpea crops and maize scraping loads was contrasted according to[26] among so many others.

Co-digestion refers to the mixing and treatment of two or more types of organic waste, a practice increasingly adopted to mitigate issues inherent to mono-digestion processes, such as limited feedstock flexibility and process instability[27]. Integrating co-substrates into the digestion process optimizes digester performance, leading to increased biogas production[28]. Co-digestion strategies combining organic substrates with animal waste, lignocellulose, and sewage sludge can effectively balance carbon-to-nitrogen (C/N) ratios[29]. A laboratory-scale bioreactor study investigated biogas production using buffalo dung and poultry manure. The results showed that co-digestion outperformed single-substrate digestion, yielding higher biogas quantities and quality, enhanced biomass biodegradability, and efficient volatile solids (VS) removal[30,31]. There has been little or no work in literature carried out on the production of biogas using pig manure co-digested with *Gliricidia sepium*. This research provides another alternative means of the production of pure energy, as well as utilizing numerous waste resources such as pig manure as well as other alternative means which involves the use of certain materials such as *Gliricidia sepium*. Particularly, the use of pig manure and *Gliricidia sepium* will be used in this research. In addition, the study accesses portable fabricated anaerobic digester viability as a decentralized way of generating biogas for energy use in small household level.

Materials and methods

Materials collection

*Gliricidia sepium*was obtained from within the Omu-Aran community, Kwara State Nigeria, collection of Pig manure at the Landmark University Farms Omu-Aran, Kwara State, Nigeria. Omu-Aran is geographically situated at 8° 8′00″ N latitude and 5°6′00″ E longitude, with an elevation of 564 m above sea level. According to the 2006 Census, the town has a population of 148,610 inhabitants and spans a land area of 73.7 square kilometers[32].

- *Gliricidia sepium*.

*Gliricidia sepium*has grown throughout the arid regions from its original area to support cultivation products such as cocoa. It is now utilized for a variety of various applications such as fence, fodder, fuel, manure, intercropping, and rat poison[33]. Leaves of gliricidia are incorporated into the soil during plowing[24].

- Pig dung.

Fresh pig dung samples were obtained from the pens and transferred using air-tight bags[34]. The collected samples were then transported to the laboratory for further analysis. The pig dung used in this study was collected from the Teaching and Research Farms of Landmark University, Omu-Aran, and refrigerated at 4 °C to prevent degradation.

Pre-treatment processes

Physical treatments like milling, pyrolysis, and mechanical extrusion can effectively increase the surface porosity of lignocellulosic biomass while substantially reducing its cellulose crystallinity and polymerization degree.

A combination of both mechanical and alkaline Pre-treatment was adopted. The biomass was ground using a mill, sun-dried to remove moisture from them and thereafter ground and sieved into fine particles and then a 70 min thermal treatment at 80°C using the EDIBON water bath as shown in Fig. 1, in line with the method of[35].

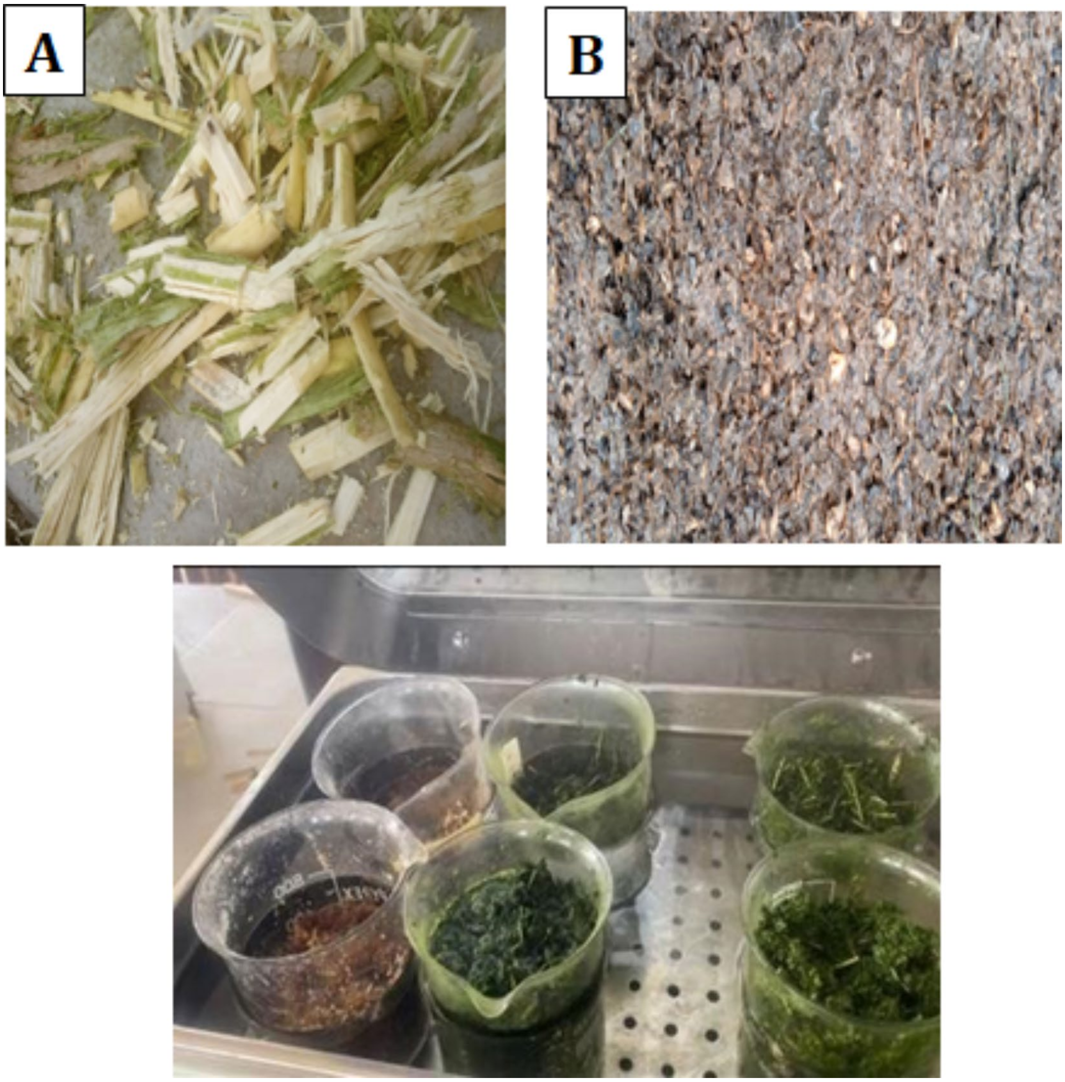

Fig. 1. (a) Gliricidia sepium sample (b) Pig dung slurry used in this study. Hydrothermal pretreatment in CLIFTON, 88579 water bath.

After heating, the alkaline pre-treatment was then carried out using 3 g of sodium hydroxide (NaOH) per 100 g at 55ºC for 24 h. Sodium hydroxide (NaOH) was used to enable breakdowns. In comparison to various pre-treatment methods, alkali treatment demands a lower temperature within a controlled environment, and this pre-treatment involves a duration of days and hours[36]. Various other forms of alkali pre-treatment such as calcium, ammonium, sodium, and potassium hydroxides can be substituted for alkaline Pre-treatment, but among these sodium hydroxides are the most commonly used alkaline Pre-treatment agent[37].

Pre-treatment determination of structural components

Analysis of the structural components (lignin, cellulose, and hemicellulose) and solids (fixed and extractive) was performed for both untreated and treated samples as *Gliricidia sepium* is lignocellulose biomass, with the results displayed in Table 1. The extractable components were evaluated by subjecting the samples to Soxhlet extraction for 6 h, while the fixed solids content was determined by incineration in a muffle furnace[19]. The composition of CPH was analyzed by treating 0.3 g of dried sample with 3mL of 72% (v/v) sulfuric acid in a thermostatic bath at 30 °C for 1 h. This hydrolysis step allowed for the determination of lignin, cellulose, and hemicellulose concentrations. Furfural and HMF levels were evaluated using an adapted version of this method according to the earlier reported method[19].

Determination of physico-chemical of substrate

It has proven quite difficult to determine the best substrate that generate the best gas yield, despite the numerous amounts of potential substrates. The total biogas generated depends on the CH_4content of the material[38]. The mixing of various types of materials will increase and better the chances of digestion until balance is achieved. The physico-chemical parameters of *Gliricidia sepium* and Pig manure were determined before and after

Parameters	Untreated Sepium	Pig Dung	Treated Sepium	Unit
pH	7.84 ± 1.31	7.90 ± 1.02	7.65 ± 0.51	
Total solids	85.6 ± 8.47	29.9 ± 13.33	34.1 ± 21.2	(%)
Fixed solids	26.17 ± 7.23	18.5 ± 4.23	20.6 ± 6.11	(%)
Volatile solids	73.83 ± 7.21	84.2 ± 12.41	79.4 ± 14.1	(%)
C/N	7:01	12:01	8:01	(%)
T Alkalinity	168 ± 5.7	305 ± 12.6	23.5 ± 43.5	(mg/L)
T. Nitrogen	19.6 ± 2.11	20.3 ± 3.66	24.5 ± 4.5	(mg/L)
Cellulose	5 ± 0.6	-	3 ± 1	(%)
Lignin	6 ± 1.3	-	4 ± 0.52	(%)
Hemicellulose	9 ± 0.5	-	3 ± 0.2	(%)
T. Phosphate	1.41 ± 0.29	2.08 ± 0.11	2.35 ± 0.33	(mg/L)
T. Carbon	137.3 ± 2.71	248.6 ± 6.54	198.7 ± 33.2	(mg/L)
Potassium	2.9 ± 0.18	4.2 ± 0.20	4.3 ± 0.7	(mg/L)
Phosphate	0.35 ± 0.21	1.36 ± 0.31	1.2 ± 0.122	(mg/L)
Sulphate	42 ± 5.23	56 ± 3.15	60 ± 9	(mg/L)
Calcium	36 ± 6.54	44 ± 5.32	50 ± 12.2	(mg/L)
Magnesium	2.8 ± 0.13	32 ± 2.17	40 ± 2.12	(mg/L)
Manganese	0.019 ± 0.01	0.028 ± 0.003	0.032 ± 0.002	(mg/L)
Iron	1.8 ± 0.25	3.8 ± 0.6	3.68 ± 0.24	(mg/L)
Zinc	6.2 ± 2.71	12 ± 1.32	11.6 ± 0.11	(mg/L)
Aluminum	0.23 ± 0.12	0.36 ± 0.04	0.33 ± 0.04	(mg/L)
Copper	1.84 ± 0.20	2.3 ± 0.323	2.15 ± 0.42	(mg/L)
BOD	32 ± 2.50	234 ± 10.20	76 ± 6	(mg/L)
COD	186 ± 8.31	974 ± 30.54	390 ± 32	(mg/L)

Table 1. Physical and chemical characteristics of *Gliricidia sepium* and pig-dung.

pretreatment to assess the effect of the treatment process on the substrate. Total solids and Volatile solids were computed using Eqs. 1 and 2, respectively as adopted by[39,40].

$$\text{Total Solid\%} = \frac{z_3 - z_1}{z_2 - z_1} \times 100 \tag{1}$$

$$\text{Volatile Solid\%} = \frac{(z_3 - z_1) - (z_4 - z_1)}{z_2 - z_1} \times 100 \tag{2}$$

Where,

$$z_2 - z_1 = \text{weight of wet sample}$$

$$z_3 - z_1 = \text{weight of the sample after drying @ } 105^\circ \text{ C}$$

$$z_4 - z_1 = \text{weight of sample after drying @ } 550^\circ \text{ C}$$

The other chemical characteristics of each substrate sample such as estimation phosphates, sulfates potassium, magnesium, calcium, iron, copper, zinc, aluminum and manganese were determined using the Pallintest Advanced Digital Readout Photometer (Model 7500PHOT.1.1.AUTO.75, Camlad, Cambridge, United Kingdom) as adopted by[41,42]. Prior to anaerobic digestion, chemical assessments were carried out to determine the concentration of key elements and nutrients. The analyses were conducted in the specialized laboratories of Landmark University, Omu-Aran, Nigeria, specifically in Environmental Engineering and Soil Mechanics/ Geotechnics.

Experimental setup of digester

25 L-biogas digester tank were fabricated from 25-litre gallon container, with each tank measuring 0.5 m in height and 0.25 m in diameter, capable of withstanding the pressures generated by the mixed substrates, as shown in Fig. 2. The substrates were thoroughly mixed to achieve homogeneity, before being poured into the digester[19]. Each tank is airtight and positioned above ground level, linked to a gas collection system through a plastic hose, enabling the biogas to be channeled into a storage tube[43]. A storage tube of 19-inch diameter with a blend of natural and synthetic materials and a thickness of 1.2 mm was infused with hose to the digester which enables the collection of biogas as it is produced as shown in Fig. 3.

Anaerobic co-fermentation was carried out for the pretreated samples and this was done using the Batch digester (EDIBON, United Kingdom)[17] as shown in Fig. 4. The feedstock (*gliricidia sepium*) was inoculated with pig dung and decomposed in the digester at mesophilic temperatures. The fabricated digester made use of eco-

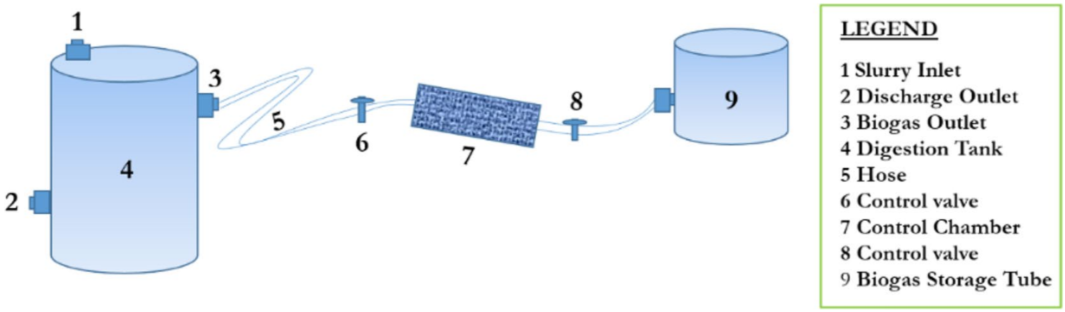

Fig. 2. Schematic design of Fabricated Anaerobic Digestion setup.

Fig. 3. The set-up for Fabricated Anaerobic Digestion.

friendly materials that are easily accessible in the community for the setup, however, the automated digester is a controlled experimental setup that is not accessible to the community. After the experiments, the data from all digestion setups was processed and analyzed to identify the primary constituents of the biogas (CH_4, CO_2, and H_2S) Gas chromatography analysis using (Clarus 580GC, PerkinElmer, USA).

In this study, using mixing ratio of the inoculum to the substrate of 1:1 for hydraulic retention time (HRT) of 30 days.

Daily monitoring of gas

To evaluate the efficacy of anaerobic treatment, gas monitoring and parameter evaluation were performed. This comprised daily weight-based measurements of gas generation, as well as observation and chemical analysis of digestates. This was done daily with a measuring scale, and the pH was measured in the morning and evening to get an average value with pH meter model PHS – 3 C. Gas chromatography (Clarus 580GC, PerkinElmer, USA) was used to characterize the gases[32].

Data and statistical analysis

Collected data were analyzed by descriptive statistics. ANOVA was carried out to determine the level of significance using IBM SPSS V.22 software. Statistical significance tests were performed with a significance level of 0.05. Results are presented as mean values with standard error (SE) margins.

Fig. 4. Automated Anaerobic Digestion set-up.

Results and discussion

Physio-chemical analyses of untreated and thermo-alkaline pre-treated *gliricidia sepium* and pig dung

The outcomes of the chemical examination of the structural parameters performed on the untreated and thermo-alkaline-pretreated substrates utilized in the digestions are reported in Table 1. The results of the thermo-alkaline treated substrate compared to the untreated showed a decrease in the concentrations of the structural elements, particularly those of hemicelluloses, cellulose, and klason lignin. The observed lignin content for untreated sepium is 6 ± 1.3 which reduced significantly after treatment to 4 ± 0.52. The concentration of cellulose for untreated sepium is 9 ± 0.5 compared to 3 ± 0.2 for treated sepium which indicate a after the thermo-alkaline pretreatment similar to the result observed in a study by[19]. Iron, zinc, aluminum, copper, BOD, COD, as well as T.alkalinity, T.nitrogen, T.phosphate, T.carbon, potassium, sulfate, calcium, magnesium, and manganese well as total solids, and volatile solids all showed a slight increase after pre-treatment as shown in Table 1.

The co-digestion process showed considerable improvement as the ratio of carbon to nitrogen (C/N) for the digestions. The observed C/N ratio is 12 which is similar to 15 reported by[13]. Pig dung had the highest COD and BOD value of 974 ± 30.54 mg/L and 234 ± 10.20 mg/L respectively.

T. Carbon was highest in Pig dung (248.6 ± 6.54 mg/L) while Untreated Sepium had the lowest value (198.7 ± 33.2 mg/L). Mineral elements such as Iron, Zinc, Aluminum and Copper, the highest values were documented for Pig dung (3.8 ± 0.6, 12 ± 1.32, 0.36 ± 0.04 and 2.3 ± 0.323 mg/L) respectively. The presence of these metals may be attributed to their inclusion in the piggery feed, as various materials are typically added during the feed production process. The physicochemical characteristics of the substrate used in this study are similar to those of piggery manure earlier reported (13).

The elevated levels of certain elements in the digestates can be attributed to microbial activity, which facilitated the extensive breakdown of complex substrate molecules into monomers, releasing previously bound nutrients. This trend is consistent with results from similar studies on various biomass substrates, including Arachis hypogaea, Chromolaena odorata, Telfairia occidentalis, Tithonia diversifolia, and Carica papaya peels (21).

Biogas generated and process parameter from *gliricidia sepium* co-digested with pig-dung

The production of biogas began in the digester gradually on the second day for the automated bio-digester and the fourth day of loading the fabricated bio-digester. Gas levels increased progressively, achieving its highest values on the 10th and 19th days, respectively. Biogas production in the automated bio-digester dropped off progressively after day 16, aside from a short-lived increase on the 14th and 16th days. The automated bio-digester produced the highest daily biogas yield of 0.06512m^3 compared to 0.041m^3 obtained from the fabricated bio-digester as shown in Fig. 4. The controlled system of the automated bio-digester might be responsible for the variation in biogas produced. In the fabricated bio-digester, Biogas production experienced a steady decline after the 19th day, apart from a brief surge on the 22nd day. There were fluctuations in biogas production volume during the anaerobic digestion period as shown in Fig. 5.

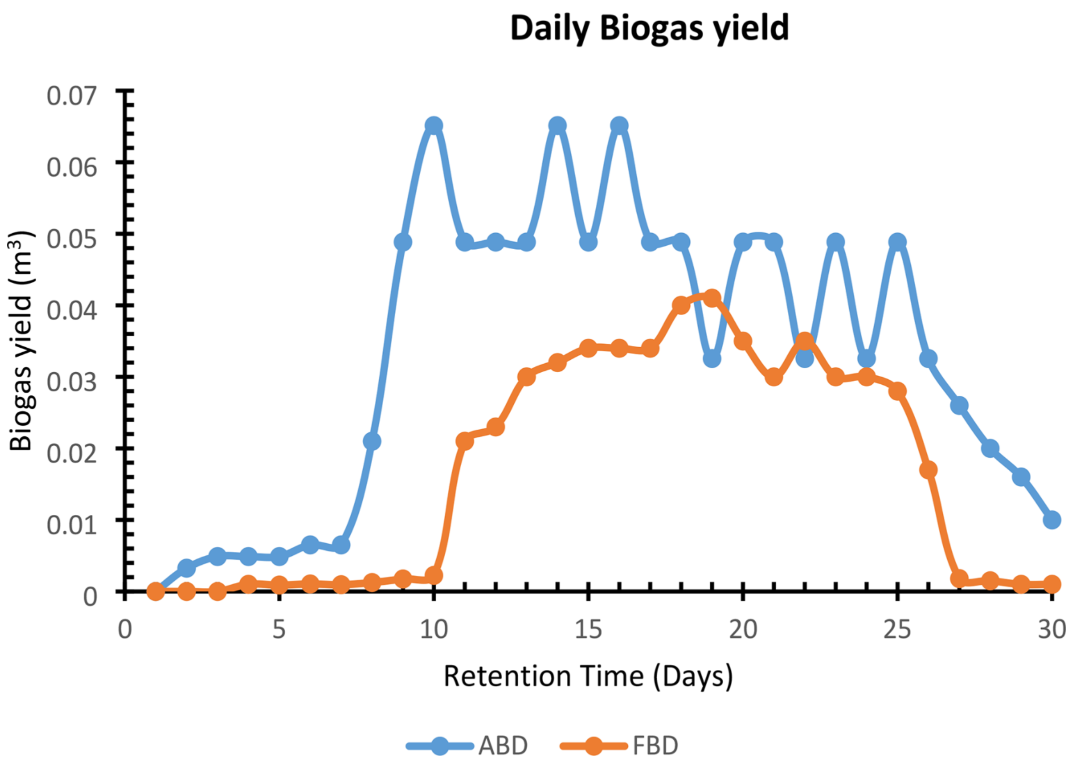

Fig. 5. Daily gas generated in the anaerobic degradation of the two bio-digesters of pig dung co-digested with *Gliricidia Sepium*.

The early stages of digestion (days 1–4) were characterized by low biogas production, likely resulting from oxygen trapped in the reactors during startup. This observation is in consistent with (13) study, where maximum biogas production occurred on day 9 in a food waste, cow dung and piggery dung co-digestion system.

Change in pH with anaerobic retention time

All the substrates that were digested had a somewhat acidic medium at the beginning of the digestion process. In other words, there was little variation in the pH of the biomass. The medium's pH changed steadily from acidic to slightly alkaline, with fluctuations within an optimal pH range of 6 to 8.5. These average pH levels fall within the range needed for effective anaerobic digestion. A comparable analysis by[30] revealed that these values displayed a similar trend. The digester produced the highest pH reading of 8.86 as shown in Fig. 6.

The pH range in the automated bio-digester is slightly basic with an average value of 7.5 while fabricated bio-digester is slightly acidic with average value of 6. There is no significant difference in the pH of the two bio digester. The pH falls with the observed pH range in a study by[13].

Gas production

The composition of CO_2 and CH_4 in the biogas was quantified through sampling and analysis after a 30-day retention period. The automated bio-digester yielded a peak methane content of about 58.85% at a 30-day retention time, as illustrated in Table 2. The fabricated bio-digester had methane content of 58.26% which is slightly lower compared to the automated bio-digester yield. Similar study using same digester set up using different feedstock produced higher methane yield, according to[22]. The substrate might contribute to the observed methane yield for both bio-digester. Carbon dioxide which affects the calorific value of biogas yield is slightly higher in the fabricated digester (24.90%) than automated digester (23.83%), however it is not significant. The observed CO_2value is also similar to recent study by[44]. Results showed a composition of 58.26% methane; 24.90% carbon dioxide and 0.52% for hydrogen sulfide for anaerobic digestion of *Gliricidia Sepium* and pig dung in the fabricated bio-digester as shown in Table 3. The methane yield in the fabricated digester is quite higher than reported methane yield in a study that used another fabricated digester, according to[45]. High methane yield: 58.85% (automated) and 58.26% (fabricated) after 30-day retention period shows that both the automated and fabricated bio-digesters achieved efficient biogas production with high methane content.

Conclusion

This study has revealed that Anaerobic Digestion of *Gliricidia sepium* with Pig dung is promising and suitable for biogas generation. The hydrothermal pretreatment aids the considerable breakdown of the biomass structure by reducing lignin and cellulose composition. The cumulative biogas of 0.986772 m^3 and 0.50845 m^3 was produced by the co-digestion of *Gliricidia sepium* and Pig dung using automated and fabricated bio-digester respectively. The methane content of 58.85% and 58.26% was obtained from the co-digestion of *Gliricidia sepium* and Pig

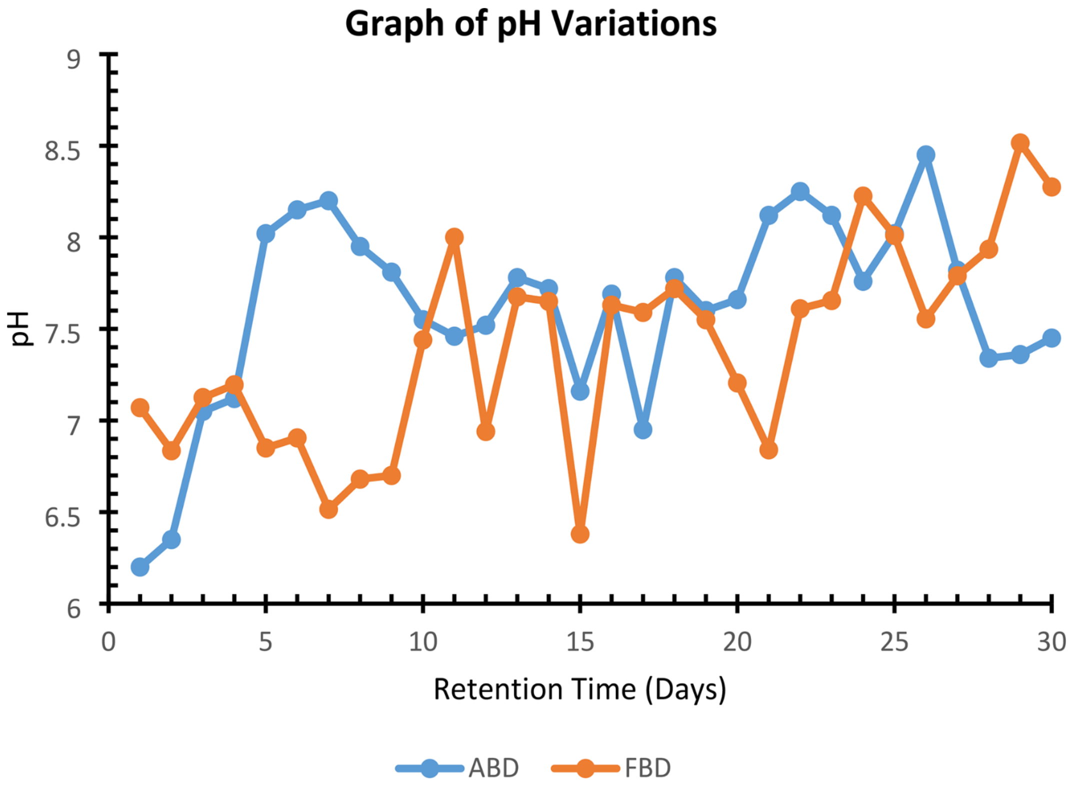

Fig. 6. Variation in pH values of pig dung co-digested with *Gliricidia Sepium* using automated bio-digester and fabricated bio-digester.

PEAK	RT	NAME OF GAS	MOLECULAR FORMULA	MOLECULAR MASS	PEAK AREA	% COMPOSITION
1	19.23	Ethane	C_2H_6	30	4.59	5.43
2	13.09	Oxygen	O_2	32	1.18	1.40
3	14.85	Carbon dioxide	CO_2	44	21.03	24.90
4	41.93	Nitrogen	N_2	28	2.47	2.92
5	25.36	Methane	CH_4	16	49.21	58.26
6	34.12	Carbon monoxide	CO	28	3.42	4.05
7	15.12	Ammonia	NH_3	20	1.28	1.52
8	9.05	Hydrogen	H_2	2	0.85	1.01
9	20.96	Hydrogen Sulphide	H_2S	34	0.44	0.52

Table 2. Characterization of biogas from pig dung co-digested with *Gliricidia Sepium* using fabricated bio-digester.

Peak	RT	Name of Gas	Molecular Formula	Molecular Mass	Peak Area	% Composition
1	19.3	Ethane	C_2H_6	30	4.69	5.55
2	23.4	Oxygen	O_2	32	1.08	1.28
3	26.7	Carbon dioxide	CO_2	44	20.13	23.83
4	28.8	Nitrogen	N_2	28	2.49	2.95
5	36.2	Methane	CH_4	16	49.71	58.85
6	25.9	Carbon monoxide	CO	28	3.52	4.17
7	5.8	Ammonia	NH_3	20	1.49	1.76
8	17.8	Hydrogen	H_2	2	0.85	1.01
9	39.0	Hydrogen Sulphide	H_2S	34	0.51	0.60

Table 3. Characterization of biogas from pig dung co-digested with *Gliricidia Sepium* using automated bio-digester.

dung using automated and fabricated bio-digester respectively. In other to enhance biogas yield from *Gliricidia sepium*, future research should focus on optimizing factors such as substrate mixing, organic loading, and feedstock pre-treatment, and consider co-digestion with other substrates.

Data availability

All data generated or analysed during this study are included in this published article.

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