Biogas Production from Co-digestion of Cattle Manure, *Oryza sativa* and *Phaseolus vulgaris* in Plug Flow Digester

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The possibility for producing biogas from the simultaneous co-digestion of *phaseolus vulgaris*, *oryza sativa*, and cattle dung in a plug flow digester was examined. *Oryza sativa* and *phaseolus vulgaris* are very common food waste found in Nigeria. The market-purchased food waste was ground in a kitchen processor to a thickness of less than 2 mm. Three digesters with effective capacities of 0.0083 m^3 were created. The digesters were fed with food waste and cattle dung which served as the inoculum at different feeding rates of 0.05 kg every three days, 0.1 kg every three days, and 0.3 kg every three days. During the trial period, chemical oxygen demand (COD), total solids (TS), and volatile solids (VS) were measured to ascertain the system's effectiveness. The parameter utilized to gauge the process stability was pH. When feeding at a rate of 0.1 kg every three days, digester 2 produced the most gas. The least amount of biogas was generated by digester 3 at a feed rate of 0.3 kg every three days. After 19 days, the effluent for digesters 1, 2, and 3 had COD values of 810.49 mg/l, 940.17 mg/l, and 987.68 mg/l, respectively. After 20 days,

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the effluent from digesters 1, 2, and 3 had amounts of TS and VS that were, respectively, 1.82%, 6.24%, 10.76%, and 10.70%, 6.24%, 42.81%. The measured pH range was 7.98 to 8.86, and there was no discernible difference across the three digesters (p>0.05). The ideal feed rate for the digestion of waste and biogas production was determined to be 0.1 kg every three days.

1. INTRODUCTION

The method of turning biodegradable organic waste, such as household garbage, into electricity is becoming more and more financially feasible [1]. One of the most readily available and ecologically favorable forms of renewable energy is biogas [2]. It is a renewable biofuel that aids in reducing worries about the sharp rises in energy consumption, as well as the subsequent greenhouse gas emissions and their devastating downstream effects, including climate change and deteriorating public health [3,4]. Methane (CH_4) and carbon dioxide (CO_2) make up the majority of this biogas, with smaller quantities of water vapor (H_2O), hydrogen sulfide (H_2S), hydrogen (H_2), and siloxanes also present [5]. Anaerobic digestion of organic materials results in the production of biogas. This process is carried out by a complex microbial population and involves several intricate biochemical interactions [6].

Biogas is produced using sophisticated technology, and its output is primarily utilized to generate power, cook food, and also to value-add to organic waste [7]. The biogas is produced by the anaerobic digestion of biodegradable materials such as biomass, sawdust, green waste from cow dung, animal waste from food processing, and other agricultural plant residues like cassava and sugarcane, among others [8].

Anaerobic digestion produces biogas while leaving behind nutrient-rich organic wastes known as digestates. The sustainability of the biogas generation process will increase if these digestate are used in plant production to recycle nutrients already in the nutrient cycle [9]. Anaerobic digestion, which produces biogas as a beneficial byproduct, is a good process for the treatment of wastewater and organic wastes, according to Jantsch and Mattiasson [10].

Igoni et al. [11] investigated the impact of municipal solid waste (MSW) total solids content on the biogas generated in an anaerobic continuous digester. The pH, temperature and efficiency of the microorganisms in the decomposition process are all impacted by the total solids (TS) content of the waste. In order to identify the ideal conditions for gas generation, they experimented with different concentrations of the TS of MSW in an anaerobic continuously stirred tank reactor (CSTR) and the corresponding quantities of biogas generated. The findings demonstrate that there is a geometric rise in the amount of biogas generated in an anaerobic continuous digestion process as the percentage total solids (PTS) of municipal solid waste increases. Due to the high calorific content and nutritive value of kitchen waste for microorganisms, the efficiency of biogas production may be increased in several orders of magnitude. Kitchen trash is an underutilized energy source that is often burned, dumped in the open, or allowed to decay in landfills. Among the sources of kitchen trash are homes, lodging facilities, dining establishments, supermarkets, and many other places [12].

According to Dhanariya et al. [13], a significant amount of biodegradable wastes, including food and animal manure, are utilized to create biogas, a potent greenhouse gas. In the absence of oxygen, these wastes are composted via a process called anaerobic digestion (AD), which creates biogas that may be utilized to produce heat and electricity. Using biodegradable garbage to generate renewable energy helps in the fight against the global energy problem. Lama et al. [14] conducted research at Kathmandu University with an emphasis on producing biogas from the biodegradable kitchen waste generated on campus as an alternative energy source. The average maximum carbon dioxide was measured at 58%, while the highest methane gas was recorded at 65%. The pH value of the slurry was found to range from 5.48 to 6.7, and the daily temperature within the digester was determined to range from 25 to 34°C. 173 L/day on average of gas production was discovered. The gas could burn for up to 62 minutes per day at its maximum, with an average burning time of 26 minutes per day.

In this research, the generation of biogas in a plug flow digester employing food scraps (*Oryza*

sativa and *Phaseolus vulgaris*) and cattle manure as the inoculum was examined. The plug flow bioreactor was created, built, and fed at a specified pace. The amount of biogas produced was measured and compared to biogas produced by digesters with various feeding rates. Investigations were done on the effluent's water quality.

2. MATERIALS AND METHODS

2.1 Preparation of Substrate and Innoculum

The bovine dung used in the seed sludge came from an Aluu settlement in Nigerian slaughterhouse. In order to entirely release the biogas before usage and to allow the dung's organic content to deteriorate, it was anaerobically kept for 30 days. The garbage that would serve as the substrate was purchased from the Aluu community's local market and crushed in a food processor to decrease the particle size to less than 2 mm before being evenly blended. To apply the mixture to each of the three digesters, the mixture was weighed using an electronic weighing scale. Before the food waste was ground up, non-biodegradable components and bones were physically removed. The mixture's total solids (TS) and volatile solids (VS) contents are 24.8 % and 97.2 % respectively.

2.2 Experimental Design

In their study, Anand et al. [15] first employed 50 kg/day of leaf biomass for a 5 m^3 digester and subsequently raised it to 100 kg/day. So, in this research, the daily feed stock allowance is 10 kg per day per 1 m^3 of total digester capacity.

The daily feed supply is specified as 0.083 kilogram per day approximately 0.1 kg per day since the digester capacity is 0.0083 m^3. Lower and higher feed rates of 0.05 kg/day and 0.3 kg/day, respectively, were used. This project was in operation for 25 days. Table 1 shows the design of the experiment for each digester.

2.3 Experimental Setup

The digesters were constructed using pipe that was easily accessible in the local market. Since the pipe was 6 inches in diameter, installation and storage space requirements were made easier. The digester measured 18 inches in length. The ratio of length to breadth is 3:1. This complies with the length to breadth ratio established for plug flow digesters based on manure [16]. The digester capacity used was thus 508.994 inches^3 or 0.0083 m^3.

A 3/4 inch PVC pipe was used to create the digester's input and output. Plastic barrels were utilized to construct the gasholder, and plastic tubes with a diameter of 3/8 inches were used to create the input and exit. The three digesters received the produced inoculum, pulverized food substrates, and water in varied ratios. The digester and gasholder were then sealed with adhesive to make sure it was airtight before being covered. Each digester had a plastic delivery tube that was used to transfer the generated gas from the digester into the gasholder, a 3 liter plastic container, which was attached to each digester. Distilled water was poured into the gasholder. For the purpose of measuring the volume of gas, a second plastic delivery tube that was attached to the top of the gasholder was used to transfer water from the gasholder to the water collection chamber. By using the water displacement technique, the volume of the created gas was calculated by equating it to the volume of water released into the water collecting chamber. Fig. 1 displays the digester's schematic diagram.

2.4 Experimental Analytical Procedure

These tests were performed over a period of continuous operation. We looked at the volatile and total solids content of feed supplies. The substance that was released from the exit was liquid. The COD, pH, total solids, and volatile solids content of the effluent were all measured. The first step in determining COD in water is the reaction of the water sample with a strong oxidizer, which oxidizes the organic matter present. The effluent samples were digested in a digester at 150°C for 120 minutes using potassium dichromate in an acidic medium. The materials were evaluated in a Spectrophotometer model HACH DR/2010 after they had cooled to room temperature.

The feedstock's organic and inorganic matter is represented by total solids. It is the quantity of solid that remains in the sample after the water molecules have been removed. In order to calculate the percentage of solids, the sample's known weight was heated in a pre-weighted crucible at 105°C for around 12 hours, or until a steady weight was attained. Samples were chilled in desiccators, and their ultimate weights

were recorded [17]. The proportion of the whole solid is written as Equation (1):

$$\%\text{Total Solid} = \frac{W_1 - W_2}{W_3 - W_2} \times 100\% \qquad (1)$$

w_1 represents weight of dried crucible + dried residue, w_2 is the weight of crucible, and w_3 represents weight of wet sample (substrate) + crucible.

The volatile solid is the solid remaining after evaporation or filtration are dried, weighed, and ignited at 600 °C. The percentage volatile solid was calculated using the Equation (2).

$$\%\text{Volatile Solid} = \frac{W_1 - W_4}{W_1 - W_2} \times 100\% \qquad (2)$$

where w_1 represents weight of dried crucible + dried residue, w_2 is the weight of crucible, w_3 represents weight of wet sample (substrate) + crucible, and w_4 is the weight of crucible + weight of residue after ignition.

The pH of the effluent was analyzed using MI 160 pH meter. pH is an indication of the process stability of biogas production.

2.5 Measurement of Biogas Produced

The water displacement technique was used every day to measure the biogas. This technique included connecting the gasholder's gas connection to the digester's gas line (plastic). The gasholder had an airtight lid on its mouth and was filled with water. The cap was given a hole through which the pipe could be inserted. To move the water from the gasholder to the water collection chamber for the measurement of gas volume, a plastic delivery tube with a 3/8-inch diameter was mounted on top of the gasholder. The volume of water pushed into the water collecting chamber was measured using a graduated measuring cylinder (1000 ml).

3. RESULTS AND DISCUSSION

3.1 Cumulative Biogas Volume

After a retention period of 25 days, the cumulative volumes of biogas produced by digesters 1 through 3 were found to be 19720 ml, 27575 ml, and 9957 ml, respectively. Figs. 2 through 4 represent this.

Table 1. Composition of feedstock for each digester

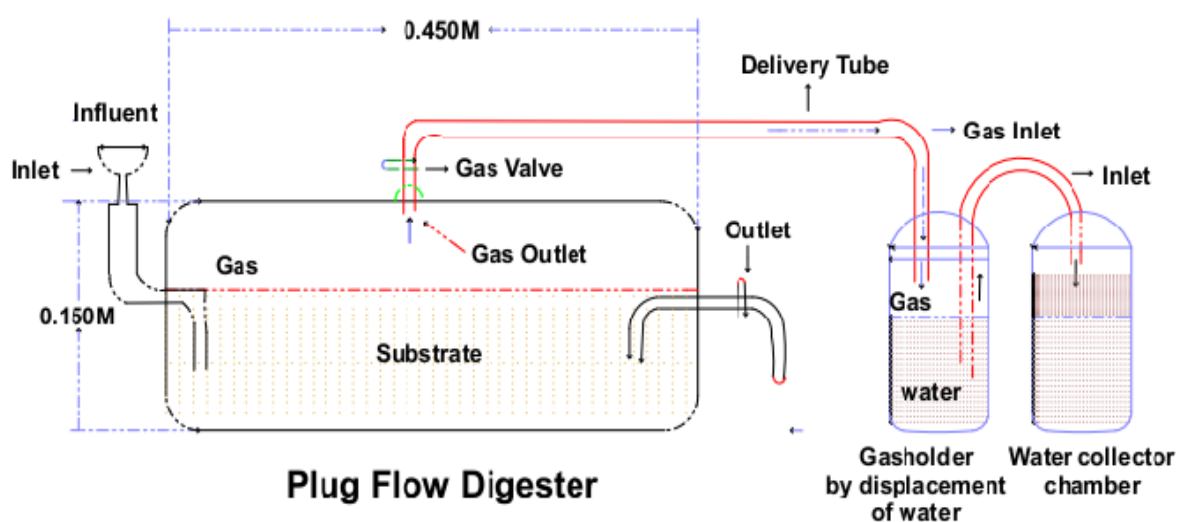| Digester	Loading rate (kg per 3 days)	*Oryza sativa* (g)	*Phaseolus vulgaris* (g)	Water (g)	Operating time (days)
01	0.05	50	50	100	25
02	0.1	50	50	100	25
03	0.3	50	50	100	25

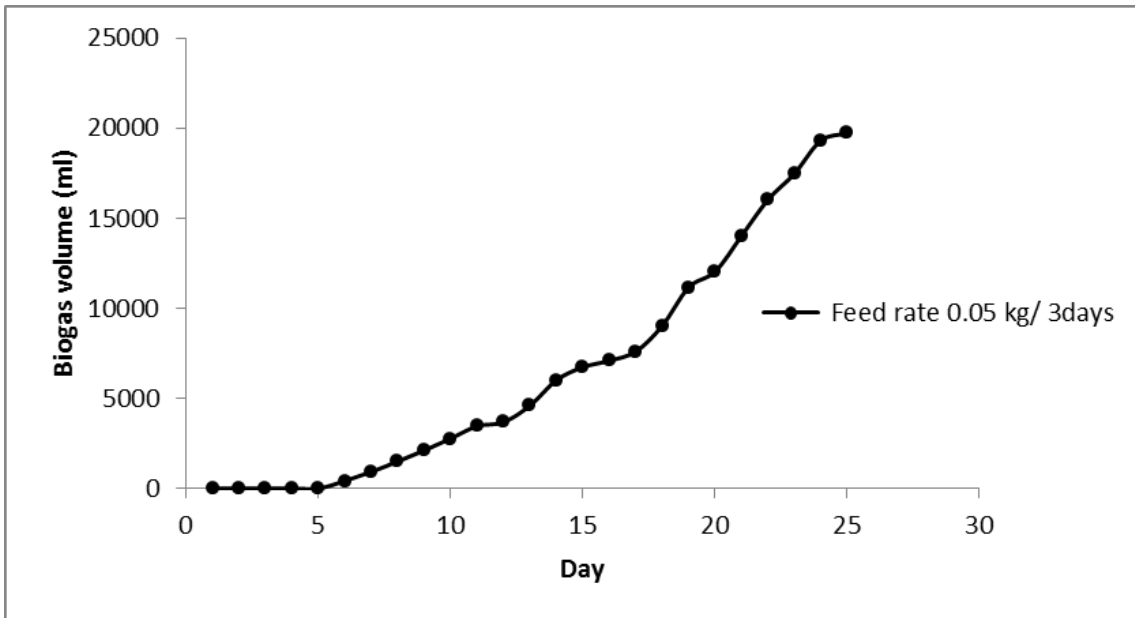**Fig. 1. Schematic of the plug flow digester**

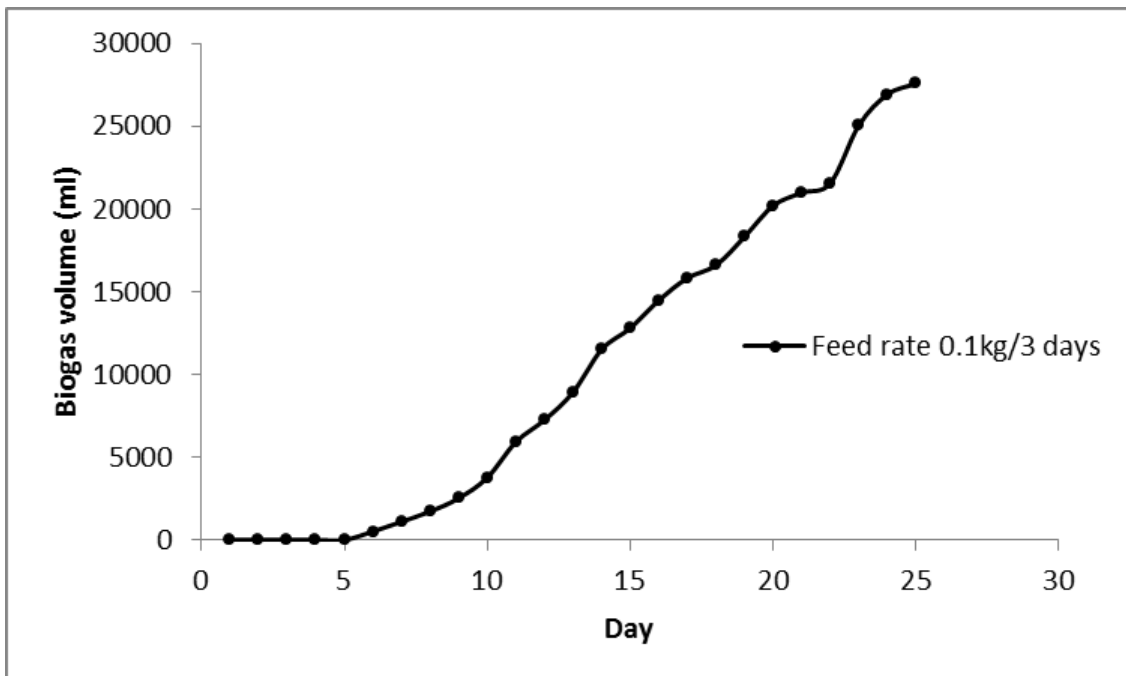**Fig. 2. Biogas production at 0.05 kg/3 day feed rate**

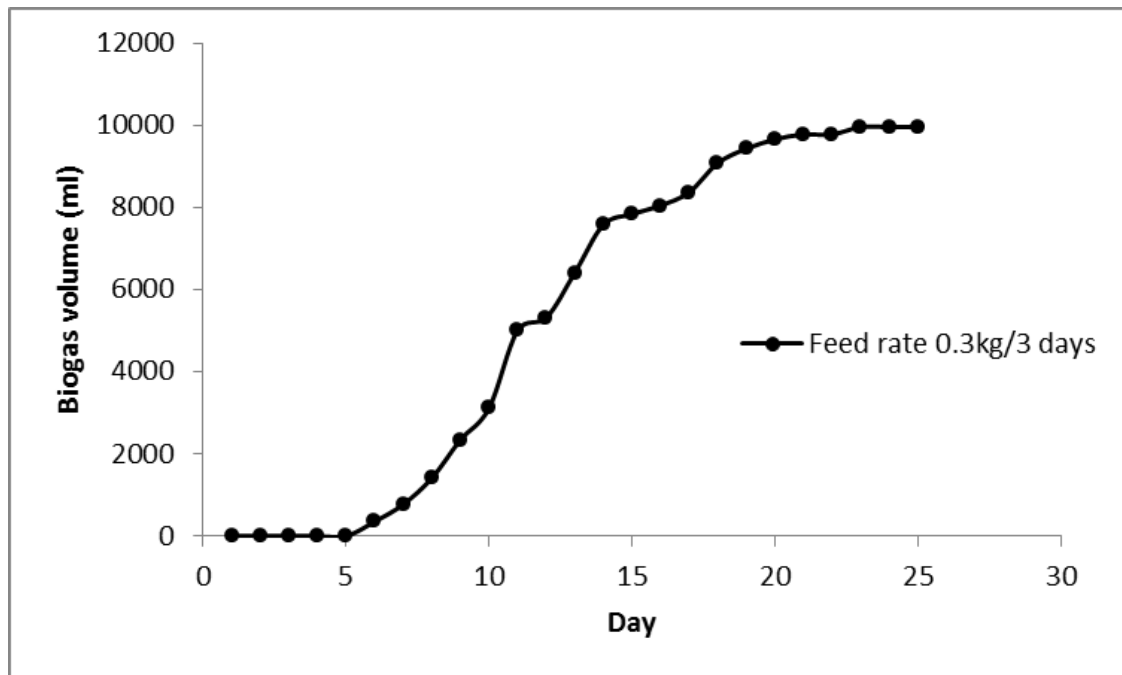**Fig. 3. Biogas production at 0.1 kg/3 days feed rate**

Fig. 4. Biogas production at 0.3 kg/3 days feed rate

Figs. 2 through 4 show that digester 2 generated the maximum cumulative amount of biogas, totaling 27,575 ml, at a feeding rate of 0.1 kilogram every three days. The digester 1 that came after it, which had a feeding rate of 0.05 kg of substrate every three days, produced a total of 19,720 ml of biogas. Digester 3 produced 9,957 ml of biogas cumulatively at a feeding rate of 0.3 kilogram of substrate every three days. The amount of biogas generated varied significantly with incubation time and among the various digesters, according to a two-way analysis of variance ($p<0.05$). While the feeding rate looked to be ideal for substrate utilization and hence biogas generation, digester 2 performed better. As the substrates are not immediately accessible for consumption and subsequent conversion to biogas, a lower feeding rate, as practiced in digester 1, limits bacterial functions. Since there are so many microbial cells in the bioreactor, the substrate usage rate is thus far higher than the substrate available. The microbial cells in digester 3 were insufficient to completely use the supplied substrate through the phases of biogas generation at a higher feeding rate and constant retention time as prior digesters. Organic polymers make up biomass. The bacteria in anaerobic digesters must first break these chains down into smaller component parts such amino acids, simple sugars, and fatty acids. Hydrolysis is the process of severing the chain and dissolving the smaller molecules in the solution.

Methanogens may immediately utilise the hydrogen and acetate released during the hydrolysis. To create molecules that methanogens can utilise, volatile fatty acids (VFAs) with chains longer than acetate must first be catabolized. By means of acidogenic (fermentative) bacteria, the remaining components are further broken down during the process of acidogenesis. VFAs are produced here, along with other byproducts such carbon dioxide, ammonia, and hydrogen sulfide. The third step of anaerobic digestion is acetogenesis. Acetogens continue to break down simple molecules beyond the acidogenesis phase, mostly producing acetic acid, along with hydrogen and carbon dioxide. Anaerobic digestion ends with the biological process of methanogenesis. The bulk of the biogas, which is composed mostly of methane, water, and carbon dioxide, is produced by methanogens using the intermediate products of the phases that came before [18,19]. Underloading and overloading lower the output of biogas [20]. Increased organic loading rate (OLR) will result in higher metabolic activity of microorganisms, which will boost biogas generation. An extremely high OLR value results in VFA buildup and fine particle accumulation, which leads to membrane fouling and reduced biogas production [21]. The primary metabolic intermediaries in anaerobic digestion are VFAs [22]. One of the major problems leading to anaerobic digester instability or even

41

failure, particularly at high organic/solid loading rates, is the buildup of VFAs, which occur from unbalanced rates between hydrolysis/acidogenesis/acedogenesis and methanogenesis. The microbial community participating in the process and, as a result, the biogas output are both influenced by the organic loading rate. After a certain extent, imbalances between the four stages of anaerobic digestion would be anticipated, leading to process inhibition owing to the buildup of VFAs. Increasing the organic loading rate up to that point, however, might boost biogas output. A process failure and permanent acidification might ultimately arise from too high OLRs. Effluent recirculation is a method for addressing the issues brought on by overloading. As a result, the reactor's capacity for producing biogas was limited by the extra substrate present. All digesters showed a lag period from days 1 to 5, which may have been caused by the microorganisms adapting to their new surroundings and diet. For digesters 1 and 2, the exponential phase lasted from day 6 to the final day of operation, but for digester 3, the exponential phase stopped on day 21 and the biogas output remained constant from day 22 until the last day of operation.

3.2 Digester Treatment Efficiency

The effluents COD, TS, and VS were measured in order to assess the digester's efficiency. Table 2 and Fig. 5 include the findings of the study of the COD levels in the digester effluent.

According to Fig. 5, the effluent with the greatest COD, 1860.53 mg/l, was generated at a feeding rate of 0.3 kilogram of substrate every three days. After then, digester 2 (feeding at a rate of 0.1 kg every three days, with an effluent COD value of 1720.94 mg/l) and digester 1 (feeding at a rate of 0.05 kg every three days, with an accomplished COD value of 1250.82 mg/l) come into play. Also, it was found that the effluent COD dropped as the organic loading rate did. Digester

1, 2, and 3 each had a decrease in COD effluent from 1250.82 mg/l to 810.49 mg/l, 1720.94 mg/l to 940.17 mg/l, and 1860.53 mg/l to 987.68 mg/l, respectively. The COD decrease with incubation time and in the various digesters differed significantly, according to a two-way analysis of variance ($p<0.05$). By under loading the digester, the substrate will be used nearly entirely and VFAs will be converted to biogas, lowering the final effluent's COD level. However if the digester is overloaded, VFA will build up and organic materials won't be used, which raises the COD level in the effluent.

The World Health Organization (WHO) permitted limit of 250 mg/l and the Federal Ministry of Environment (FME) legal limit of 120 mg/l for effluent discharge were both reported as not being met by the COD effluent. This is due to the fact that increased COD levels in the effluent indicate the presence of undigested materials due to a short or decreased hydraulic retention period. This suggests that for the desired COD decrease, lengthy retention durations are necessary.

The effluent's total solids content for days 10 and 20 was analyzed in a lab setting. This is seen in Fig. 6.

On day 10, digester 3 found the highest concentration of TS in the effluent, followed by digesters 2 and 1 with values of 42.81%, 10.76%, and 6.24%, respectively. Yet by day 20, there was an 88% drop in TS in digester 3, followed by a 63% reduction in TS in digester 2, and a 70% reduction in TS in digester 1. This suggests that a slower loading rate will significantly decrease the TS content when it is retained in the digester. Yet, digester 2 still provides a near result with the loading rate that generates the best biogas.

Moreover, analyses of the feedstock effluent's volatile solid concentration were done on days 10 and 20, respectively. Fig. 7 shows the calculated value.

Table 2. Effluent COD variation for the feed rates

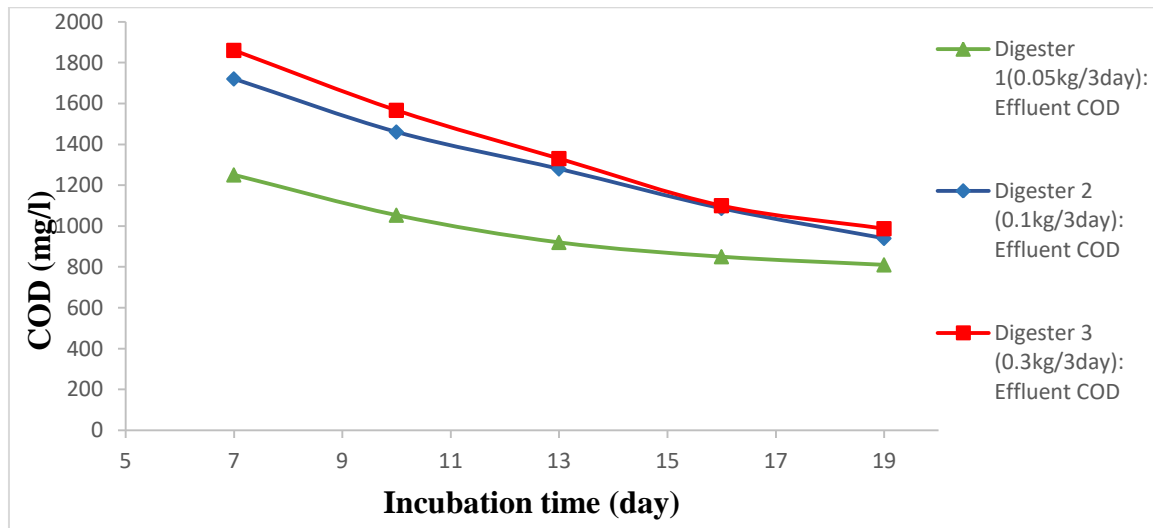| Time (Day)	Effluent COD (mg/l)			Effluent standard	
	Digester 1 (0.05kg/day)	Digester 2 (0.1kg/day)	Digester 3 (0.3kg/day)	WHO (mg/l)	FME (mg/l)
Day 7	1250.82	1720.94	1860.53	250	120
Day 10	1053.69	1460.98	1567.10	250	120
Day 13	920.34	1280.15	1330.94	250	120
Day 16	850.17	1086.92	1100.42	250	120
Day 19	810.49	940.17	987.68	250	120

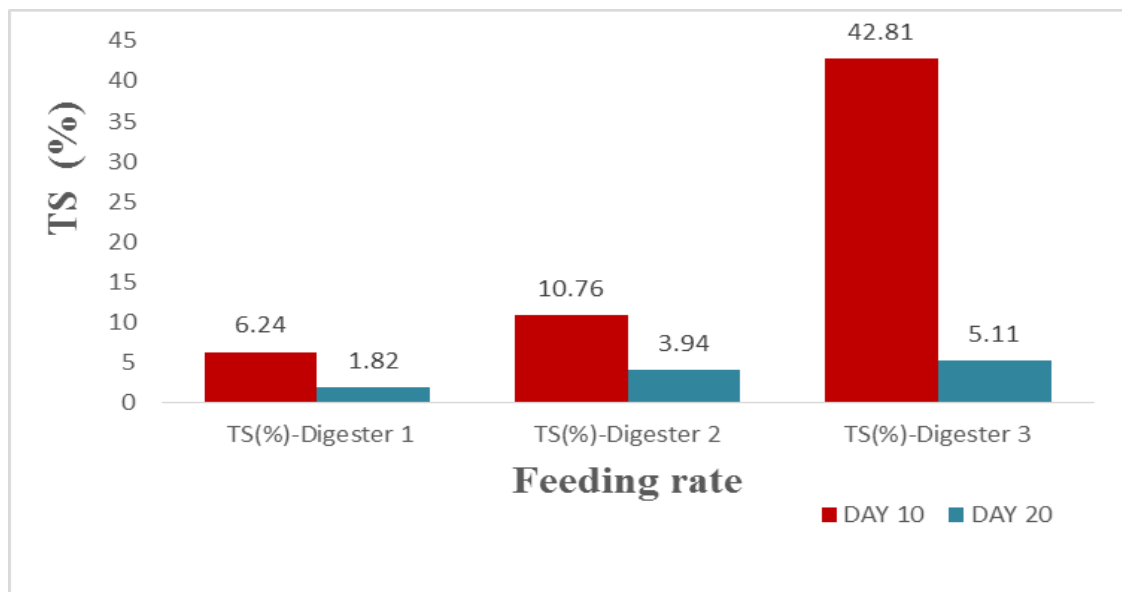**Fig. 5. COD variation of effluent for digester 1 to 3**

Fig. 6. Variation of effluent total solid for digester 1 to 3

Table 3. pH Variation of effluent for three feed rates

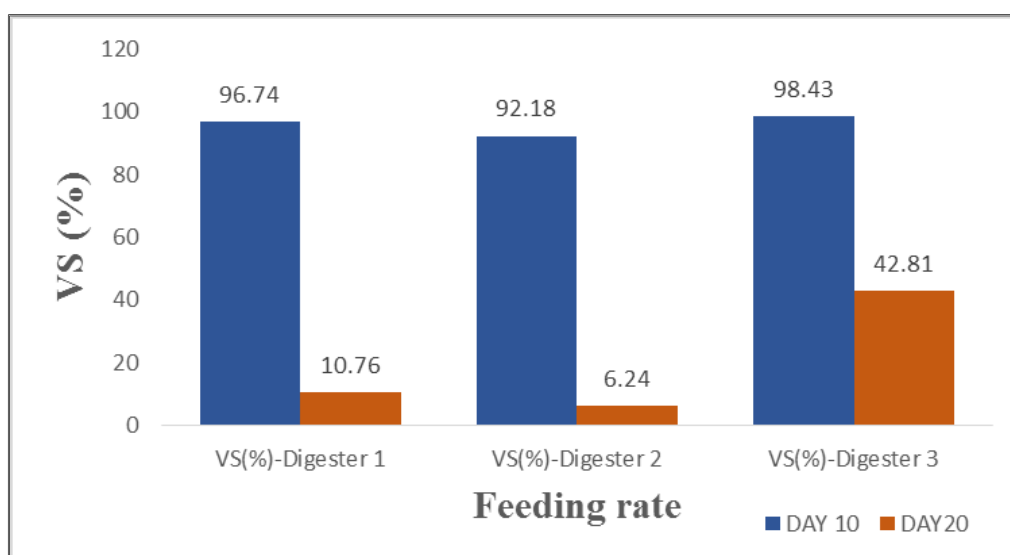| Time (days) | pH$_{out}$- feeding rate 0.05kg/ 3day (Digester1) | pH$_{out}$- feeding rate 0.1kg/3day (Digester 2) | pH$_{out}$-0.3kg/ 3day feeding rate (Digester3) | Standard | | |
				FME	WHO	DWA
7	7.98	8.08	7.85	6.5-8.5	6-9	5.5-9.5
10	8.11	8.82	8.74	6.5-8.5	6-9	5.5-9.5
13	8.10	8.00	7.84	6.5-8.5	6-9	5.5-9.5
16	8.84	8.86	7.85	6.5-8.5	6-9	5.5-9.5
19	8.68	8.60	8.36	6.5-8.5	6-9	5.5-9.5

Fig. 7. Volatile solid variation for three feed rate

The three digesters had different levels of volatile solids, which might be a sign of the solids that could be converted to biogas. Volatile solids in Digester 2 decreased by 93% as incubation time rose. This could be related to the increased biogas output that was observed. Volatile solids decreased by 89 and 56.5% in digesters 1 and 3, respectively.

3.3 Process Stability

Using effluent pH, the stability of the process was tracked for the three digesters. This was done in the lab using effluent that was collected on days 7, 10, 13, 16, and 19. The outcome is shown in Table 3.

The pH of Digester 1 is between 7.98 and 8.11, Digester 2 is between 8 and 8.86, and Digester 3 is between 7.84 and 8.74. The effluent pH did not significantly change with incubation time or across the various digesters, according to a two-way analysis of variance ($p>0.05$). The anaerobic digestion (AD) mechanism may become disturbed by the buildup of VFA. The capacity of the buffer will be diminished and the pH will drop significantly if the VFA content is too high [23]. The pH range that was measured fell within the 6.5–8.5 range that is suitable for anaerobic digesters. This suggests that the system was running consistently.

4. CONCLUSION

The co-digestion of cow dung, *Oryza sativa*, and *Phaseolus vulgaris* in a plug flow digester show

tremendous promise for treatment and biogas generation. The substrate should be fed at a rate of 0.1 kg every three days in order to produce the most biogas. Both under- and over-loading the system had no positive impact on the output of biogas. The digester 1 with the lowest feeding rate provided superior COD and TS reduction, according to the treatment efficiency study looking at COD, TS, and VS. The digester 2 that generated the most biogas had effluent with the lowest VS content and a substantial decrease in COD and TS. The effluent from Digester 3 has significant COD, TS, and VS contents due to its high organic loading rate. The pH of the effluent was used to gauge the stability of the systems, and it was found that there was little variation in pH. This occurrence demonstrated that the system's functioning remained steady during the research period.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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