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Anaerobic Digestion of *Rothmannia longiflora* seed cake for biogas generation: An optimization study.

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Abstract
The need for sustainable energy sources with low greenhouse gas emissions has increases exploration of other options. Considering the future production of biodiesel from oil seeds, efficient use of the seed cake is critical. In this study, an anaerobic digestion on Rothmannia longiflora seed cake was carried in batch system under mesophilic condition (27.42 – 32.88^{o}C) for 35 days. Proximate and ultimate analyses were performed to assess the physical and chemical characteristics of the cake. Proximate analysis revealed a moisture, volatile matter, fixed carbon, and ash of 23.13 %, 56.97 %, 17.96 % and 1.94% respectively and the ultimate results of the seed cake were found to be carbon (37.36 %), hydrogen (4.46 %), oxygen (32.57 %) and 0.19 % sulphur. The seed cake calorific values from the proximate and ultimate data are 15.21 and 15.63MJ respectively. Optimization process to investigate the effect of retention time (RT), feeds to water mixing ratio (MR) and pH using shows a maximum biogas yield of 1980 cm^{3}at1st week of RT, 3:1 mixing ratio and a pH of 7.0. The linear and quadratic effects of the parameters investigated on biogas yield were significant (p-value < 0.05) at 95% confidence level. However, no significant interactive effect was observed between these input variables (p > 0.05) except for retention time with pH.

Keywords: *Optimization, Rothmannia longiflora, Seed cake, Anaerobic digestion.*

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I. Introduction

Depletion of non-renewable energy sources, overpopulation, food security concerns and environmental concerns have accelerated the need for sustainable energy production [1, 2]. Biodiesel is one of the promising sustainable energy sources, however, its processing produces various residues such as; seed cover, de-oil cake, and waste glycerol. Considering the future use of oilseeds for biodiesel production, efficient utilization of the seed cake is required. One of the problems that arise is the disposal of the filter cake after the oil has been drained. Biogas generation from these cakes can be the best solution for its efficient use. Biogas from the cake provides energy and the digested cake can be used for agriculture [3].Using these residues for other purposes will help make the energy recovery process more comprehensive, economical, and environmentally friendly and can increase revenue.

Biogas is a biofuel produced from the anaerobic digestion of organic waste. Biogas can replace fossil fuels in the production of electricity and heat, and can also be used as a gas vehicle fuel [4]. Production of energy crops for biogas production may compete against food production for agricultural land. However, increased cultivation of energy crops has triggered land-use change and monoculture, which often involve environmental adverse factors such as biodiversity loss or soil erosion [5]. Therefore, research efforts focus on the bioenergy deployment from agricultural and farming waste [6].

Anaerobic digestion is a promising method for converting agricultural waste into energy (biogas). It is an alternative fuel production option for bioenergy production; as a biochemical process, it converts organic waste into valuable products [7].Anaerobic digestion (AD) offers the very good advantage that the digestate left after processing can be used as manure or animal feed. However, anaerobic digestion requires less energy, cheap, less sludge production and stable digestate and energy production [8].Anaerobic digestion is affected by a variety of factors, including microbial population, acidity (pH), carbon to nitrogen mass ratio (C/N ratio), operating temperature, substrate particle size, organic loading rate, hydraulic retention time, total solids content and reactor configuration (batch or continuous, single or two-stage) [9]

There have been some studies of biogas production from de-oiled seed cakes of plant seed oils [10, 11, 12, 13, 14]. However, biogas production from de-oiled seed cake of *Rothmannia longiflora* has not been found in the literature. Therefore this study aimed to investigate feasibility of biogas production from *Rothmannia longiflora* seeds cake and to evaluate some of the operating parameters of biogas generation under anaerobic conditions.

II. Materials and Methods

Sample collection

The fruits of *Rothmannia longiflora*were obtained from Wanke village along Lungu road in Shagari local government area of Sokoto state, Nigeria. The seeds were crushed to fine powder and de-oiled using Soxhlet extraction.

Sample pre-treatment

60g of the seeds cake was soaked in water (500 cm^3) and left overnight to remove excess oil and dirt. The oily substances floating on the surface are gently separated from the residual filter cake by sieving. The cake was further subjected to chemical treatment by soaking in H_2SO_4 (1%) and NaOH (1%) in the ratio of 1:1 overnight and then filtered. The filtrate was then washed with distilled water to ensure neutrality. The filter cake was then dried and pulverized to a powder using a pestle and mortar. Samples were kept in polyethylene bags for further analysis.

Analytical methods

Proximate analyses carried out are moisture, ash, volatile matter and fixed carbon. The moisture content of the seed cake sample was determined according to ASTM D4442-16 method while the volatile matter and ash content were determined following ASTM E1755-01 and ASTM E872- 82, respectively. Ultimate analysis was performed in a high frequency induction furnace by weighing 0.015g of the sample into a silica crucible and then 0.4g of iron flux and 1.5g of tungsten flux was added and a gas pressure was set and maintained at 0.18Mpa. The fixed carbon was estimated by difference using equation (1).

$$FC = 100 - (\%\,MC + \%\,Ash + \%\,VM) \quad (1)$$

Where FC = Fixed carbon content, % MC = Percentage moisture content, %Ash = Percentage Ash Content, % VM = Percentage Volatile Matter.

Calorific value of the seed cake was estimated from proximate and ultimate data using equation (2) and (3) as given by [15] and [16].

$$HHV = 0.3536FC + 0.1559VM - 0.0078Ash \quad (2)$$

$$HHV = 0.3259C + 3.4597 \quad (3)$$

Design of Experiment

A Taguchi design was employed to create the design matrix, then later defined in a response surface methodology on Minitab 17 software. The three input parameters investigated are retention time (RT), pH and feed to water mixing ratio (MR).

Slurry preparation

The slurries of defatted cake of *R. longiflora* were mixed with sterilized water in the ratio (1:1, 1:2, 1:3, 1:4 and 1:5). The digesters used were cleaned empty cans. The proposed slurries were adjusted to acidic, neutral and alkaline at pH of (4, 5, 7, 8 and 9). The gas was collected using urine bags with a capacity of 2000 cm3. Digital thermometer is used to measure daily ambient temperature [17].The gas was collected gas within a period of five weeks. The methane content of the biogas was determined using a portable gas analyzer (GA2000 Plus).

Analysis of the Digestate

The digested waste was analyzed to assess the elemental composition for bio-fertilizer quality using XRS – FP. The digestate was crushed and pulverized to 100% passing through 75µm sieve. The pulverized aliquot was analyzed using Genius – IF Xenemetrix.

III. Results and Discussion

Characteristics of the Seed Cake

Table 1: Results of Proximate and Ultimate analysis of the seed cake

Parameters	Values
Moisture Content (%)	23.13 ± 0.015
Ash Content (%)	1.94 ± 0.010
Fixed Carbon (%)	17.96 ± 0.021
Volatile Matter (%)	56.97 ± 0.011
Carbon (%)	37.36 ± 0.015
Hydrogen (%)	4.46 ± 0.031
Oxygen (%)	32.57 ± 0.015
Sulphur (%)	0.19 ± 0.05

Proximate analysis provides information on the properties of a feedstock which extensively gives an insight on how best it could be used as a renewable energy source. Table 1, shows results of proximate analysis of *Rothmannia longiflora* de-oiled seed cake. The cake has moisture content of 23.13%. The moisture content determines the combustion tendency of solid fuels. High moisture (> 10 wt %) may result in production of low quality bio-oil when the substrate is pyrolysed [18]. In another findings, [19] reported that a substrate with a moisture (< 50%) could be suitable for thermal conversion process while higher moisture renders the substrates as potential feedstock for bioconversion like fermentation and anaerobic digestion. [20], reported that feedstock moisture affects anaerobic digestion because it enhances bacterial motility, facilitates nutrient solubilization and transport, and reduces mass transfer limitations for heterogeneous substrates. Therefore, the seed cake could be a good candid for bioethanol and biogas production. The volatile matter of 56.97% was recorded for the cake; the amount of methane produced depends on the amount of volatile matter, which is the amount of solids present in the waste and its degradability [21]. As reported by [22] volatile solids are responsible for biogas production; therefore, this filter cake has good prospects as a feedstock for biogas production.

Ash is a percentage of non-combustible inorganic minerals present in a substance. The seed cake show ash of 1.94% which is lower than 6.2 and 4.8 % reported for Mahua and Neem seed cake [23].High ash content has an inert effect on the calorific value of the fuel and its apparent heat [24].High ash content (25 – 35%) reduces biogas production potential as only organic matters can be converted [25]. Therefore, biomass with higher ash content will be less efficient in biogas production. Fixed carbon (FC) is the solid combustible residue left behind when biomass is heated and volatiles are expelled from [26]. The fixed carbon content of biomass has a positive effect on its energy potential [24]. The percentage fixed carbon of the seed cake was found to be 17.96 %, the value is higher than 4.70 % reported for African star apple seed cake [27] but lower when compared to 18.9, 22.0 and 21.0 % recorded for Mahua, Neem and Jatropha seed cake respectively [23,28]. Calorific value is a measure of amount of heat emitted on combustion of fuel. The calorific value obtained from the cake is comparable to that of some seed cakes reported in the literature (Table 2).

Table 2: Calorific value of the seed cake

Feedstock	Calorific value (MJ)	Reference
Rothmannia longiflora seed cake	15.21[a]	This study
	15.63[b]	This study
Mustard seed cake	15.00	Sakar*et al* (2013)
Jatropha seed cake	15.21	Biradar*et al*(2014)
Mahua de-oiled cake	19.97	Mulimani and Navindgi (2018)
Neem de-oiled cake	22.00	Mulimani and Navindgi (2018)

a = calorific value (from proximate data), b = calorific value (from ultimate data),

Optimization of Biogas Production and Statistical Analysis

Table 3: Analysis of Variance (ANOVA) of Biogas Yield

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	7499641	833293	705.62	0.000
Linear	3	2263932	754644	639.02	0.000
RT	1	952469	952469	806.54	0.000
Mixing ratio	1	36819	36819	31.18	0.000
pH	1	7401	7401	6.27	0.024
Square	3	488506	162835	137.89	0.000
RT*RT	1	120633	120633	102.15	0.000
MR*MR	1	177152	177152	150.01	0.000
pH*Ph	1	381383	381383	322.95	0.000
2-Way Interaction	3	163560	54520	46.17	0.000
RT*Mixing ratio	1	4466	4466	3.78	0.071
RT*pH	1	9246	9246	7.83	0.014
Mixing ratio*pH	1	45	45	0.04	0.848
Error	15	17714	1181		
Total	24	7517355			

R-sq = 98.76% , R-sq(adj) = 98.62% , R-sq(pred) = 96.69%

Analysis of variance (Table 3) was undertaken to investigate the effect of retention time (RT), substrate to water mixing ratio (MR) and pH on the biogas production. Based on the results of the 25 runs created (Table

5) a quadratic model was generated to represent biogas production. Each factor variable was assessed for its statistical significance by comparing probability values (p-value) at 95% confidence. A p-value less than 0.05 ($p < 0.05$) indicate significance of the factor on the biogas yield whereas p-values greater than 0.05 ($p > 0.05$) shows non-significance effect. As shown in Table 3, the model was found to be significant (F-value = 705.62, p-value = 0.000). The fit of the model was also expressed by the coefficient of determination (R^2 value), which was found to be 0.9876.The value reveals that the mathematical model could explain 98.76% variability in the biogas yield. An R^2 value in the range of 0.75 - 1 indicates a good statistical model fit [29]. However, the Pred. R^2 value of 96.69% was in good agreement with the Adj R^2 which indicated good fitness of the model. However, some model terms were found not to be significant ($p > 0.05$), namely; interaction term between mixing ratio (MR) with retention time (RT) and mixing ratio with pH (Table 3). The generated model equations before and after excluding insignificant terms were given in equation (4) and (5) respectively:

Biogas Yield = -4980 + 281.0 RT + 899.0 MR + 1765.3 pH - 111.3 RT*RT - 134.8 MR*MR - 146.07 pH*pH - 40.1 RT*MR + 40.0 RT*pH - 2.8 MR*Ph (4)

Biogas Yield = -4317 + 279.3 RT + 682.4 MR + 1627.9 pH - 113.26 RT*RT - 123.5 MR*MR - 131.28 pH*pH + 24.09 RT*pH (5)

Table 4: Percentage Methane and Carbon dioxide in the Biogas produced

Digesters	% CH_4	% CO_2
A	22.70	72.90
B	23.80	71.40
C	42.00	53.60
D	41.12	54.50
E	37.60	58.20

The percentage methane recorded for the biogas produced according to digesters was in the range of 22.7 – 42.0 % (Table 4). The composition of biogas depends on the source materials, retention time, temperature, and the treatment process. Biogas mainly composed of typically CH_4 (35 – 75%), CO_2 (25 –65%), H_2 (1 - 5%), N_2(0.3 – 3%) and some traces of water vapor, ammonia and hydrogen sulfide [30].

Table 5: Design matrix with the actual biogas Yield

StdOrder	Blocks	PtType	RT(week)	Mixing ratio (MR)	pH	Biogas Yield (cm^3)
1	1	1	1	1	4	746
2	1	1	1	2	5	1731
3	1	1	1	3	7	1980
4	1	1	1	4	8	1478
5	1	1	1	5	9	383
6	1	1	2	1	5	1408
7	1	1	2	2	7	1975
8	1	1	2	3	8	1752
9	1	1	2	4	9	936
10	1	1	2	5	4	839
11	1	1	3	1	7	1523
12	1	1	3	2	8	1579
13	1	1	3	3	9	1042
14	1	1	3	4	4	996
15	1	1	3	5	5	1088
16	1	1	4	1	8	958
17	1	1	4	2	9	701
18	1	1	4	3	4	704
19	1	1	4	4	5	1076
20	1	1	4	5	7	901
21	1	1	5	1	9	86
22	1	1	5	2	4	33
23	1	1	5	3	5	617
24	1	1	5	4	7	759
25	1	1	5	5	8	76

Individual and Interaction effects of study parameters on Biogas Yield

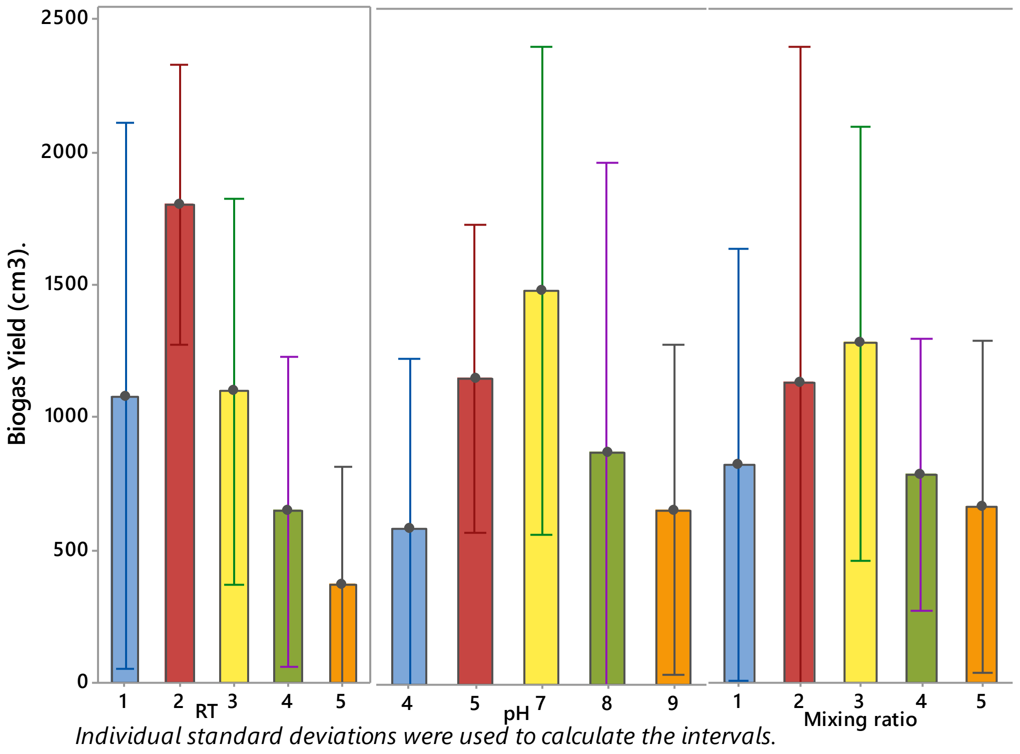

Figure 1: Effects of pH, Retention time (RH) and Mixing ratio on Biogas Yield

The highest mean biogas yield of 1366 cm^3 was observed for 3:1 water-substrate ratio while 5:1 ratio had the least yield of 708 cm^3 (Fig. 1). This means that the feed concentration level significantly affects the digestion process. The biogas yield increases with increase in the mixing ratio from 1:1 – 3:1 after which the gas production declined with further increased in water to substrate ratio. The pH of the substrate was vary from 4-9 to analyze its effect on the biogas yield (Fig. 1). The mean biogas yield obtained by the digester with pH 4, 5, 7, 8 and 9 are 620, 1212, 1558, 916 and 690 cm^3 respectively. This shows that pH 7 resulted in higher biogas yield followed by pH 5, 8, 9, with least been by digester with pH 4. Similar trend was observed by [31].

The gas production at different retention time (in week) is presented in (Fig. 1). The gas production in the second week of retention time was the highest (1804 cm^3). The average volume decreases to 1097 cm^3 in 3rd, 646 cm^3 in the 4th and 372 cm^3 in the 5th week. This result is consistent with the findings of [32], which determined that biogas production from cassava skins and poultry manure peaked during the first 5 to 15 days.

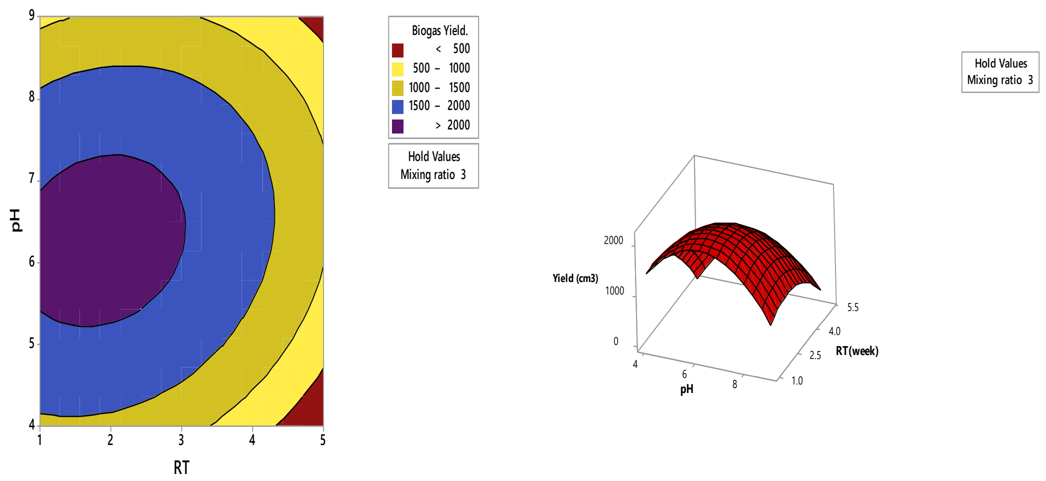

Figure 2: Contour and Surface Plot for Interaction Effect of Retention time and pH

The 3-D surface plot of biogas yield versus the pH and RT is shown in Figure 2. Both retention time and pH significantly affects the biogas yield, as revealed by ANOVA results (Table 2, respective p-value < 0.05). An increase in retention time was observed to increase biogas production. However, subsequent increases in retention time lead to lower biogas production. Likewise, an increase in pH results in a corresponding increase in the amount of accumulated biogas. Increasing pH and RT increased biogas production until cumulative biogas production decreased. The contour plot showing the pH and RT in relation to cumulative biogas yield is shown in Fig.2, as observed from the figure cumulative biogas volume greater than 2000 cm^3 was achieved between pH ranges of 5.2 – 7 and retention time within the first and second week.

Average Daily Ambient Temperature and Biogas Yield

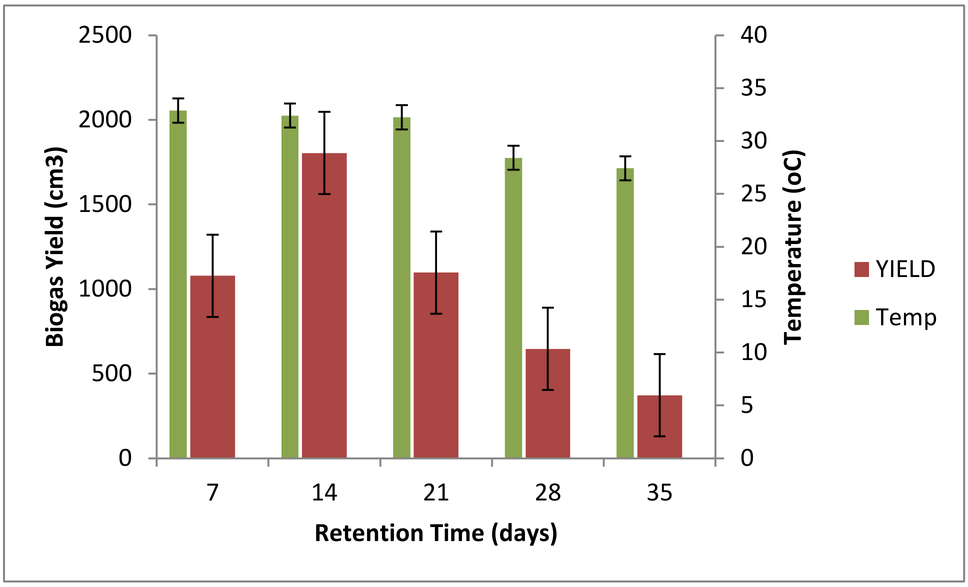

Figure 3: Average Daily Ambient Temperature and Biogas Yield

Figure 3, showed a relation between average daily ambient temperature and average biogas production over 35 days. It was observed that the ambient temperature was in the range of 27.42 – 32.88^oC, which implied that the digesters were within the mesophilic zone which favour methane generation from biomass. Anaerobic processes are divided into three zones based on operating temperature conditions; psychrophilic (10-20 ^oC), mesophilic (20-40 ^oC) and thermophilic (40-60 ^oC) [33]. From 28 – 35 days the mean ambient temperature was recorded to be 28.41and 27.42 ^oC respectively. As evident from the figure, commulative biogas production was high between 32.24 – 32.88^oC. Decreasedin ambient temperature resulted in decreased of biogas yield. Biogas is produced in a biological process that involved group of micros working in an anaerobic condition. The interaction of several factors affects performance of digestion process. Temperature and pH significantly affects digestion process [34].

Mean Daily Biogas Production

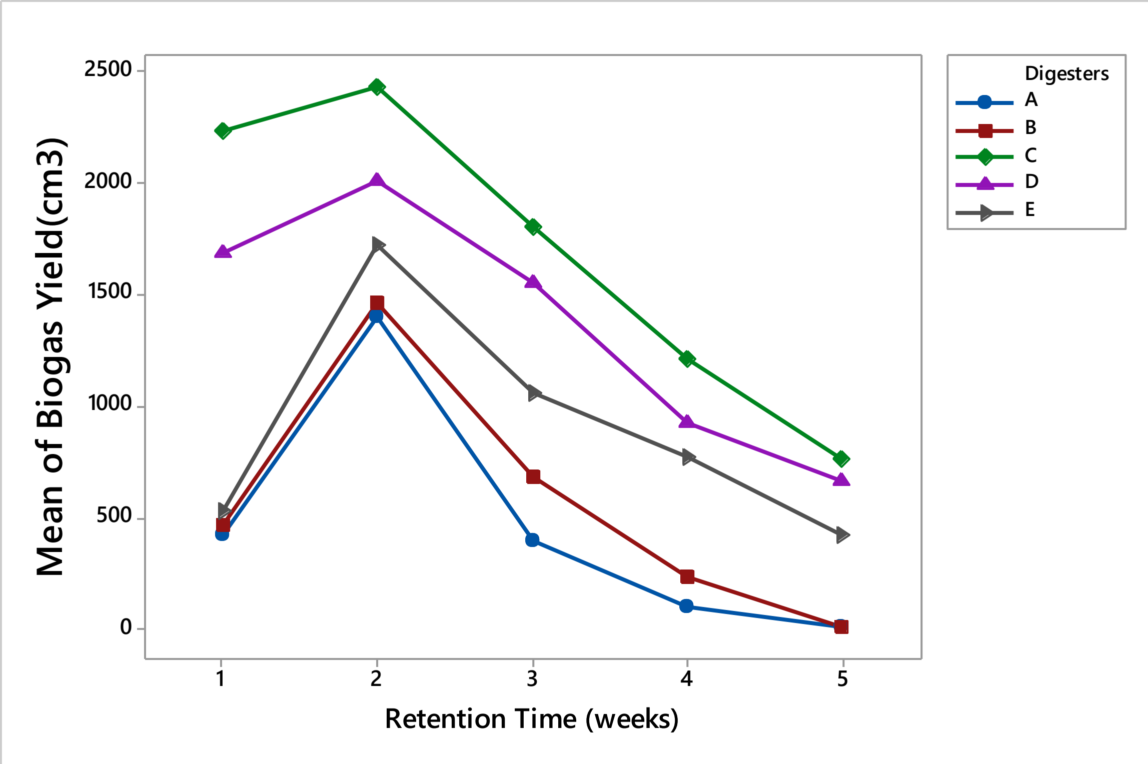

Figure 4: Mean Daily Biogas Yield

Figure 4 shows the daily biogas production of the seed cake as a function of retention time.All the biogas digesters started production within the first week, probably due to the rapid conversion of available organic matter. After the second week of digestion, the daily biogas production gradually decreased.RTs for the first-stage (including hydrolysis, acidogenic, and acetogenic reactions) and second-stage (methanogenesis) processes range from 2 to 4 days and 8 to 10 days, respectively [35]. This could be the reason for better biogas yield within week 1 and 2 as depicted in (Fig. 4)

Cumulative Biogas Production

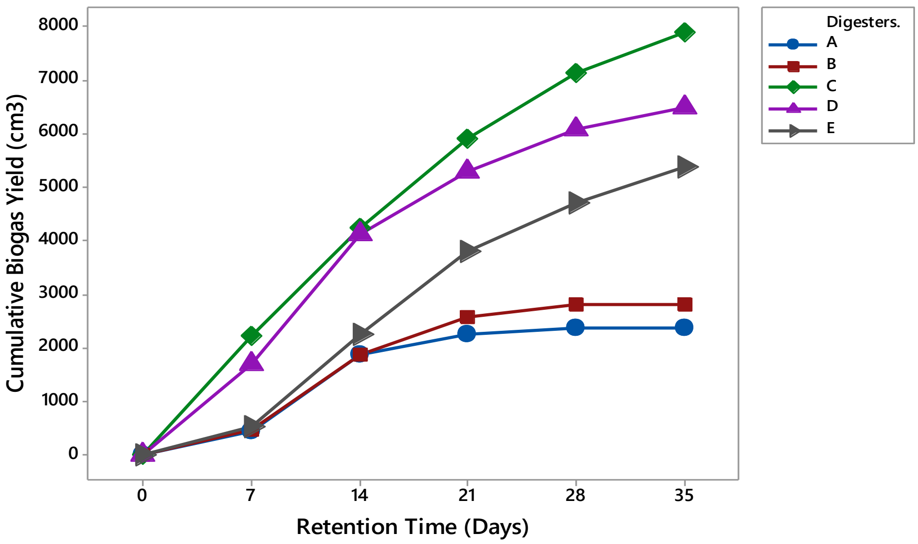

Figure 5: Cumulative Biogas Yield

The results of cumulative biogas production is shown in Figure 5, after 35 days of digestion, the highest accumulative biogas production reached 7890 cm^3 for digester C which was made neutral, followed by digester D having cumulative gas yield of 6490 cm^3 (pH = 5). This could be attributed to the fact that the digesters offered a suitable environment needed for anaerobic microns and enzymes to anaerobically breakdown the cake. The cumulative biogas obtained for the acidic treatments (digesters E and B) were 5380 and 2800 cm^3 respectively. The least cumulative gas volume was obtained by digester A (2370 cm^3). Microbial activity is inhibited when the organism is exposed to low pH levels that hinder the digestive process. Although anaerobic bacteria thrive in the pH range, the fact that methanogens are known to be sensitive at lower pH, resulting in increased VFA concentrations that inhibit methanogenic processes, can lead to methane starvation [36].Biogas production mostly goes through three production periods; the adaptation or initiation period, the transition period and the stabilization period [34].The initiation period lasted 7 days (Fig. 5). During this period, biogas production is low. [37], indicated that the initiation period was between 1 and 3 weeks. The main problem in this period is creation of a balanced population, which is characterized by low biogas production [34].The transition period can be seen after the first week and goes on till closed to 28 day, after which the stabilization period begins.

Mineral Composition of the digestated Seed Cake

For the life cycle assessment of biogas production, the entire process chain needs to be considered [38]. This includes the nutrients in the digestate to the soil where the new plants are to be growing. But before using it as a fertilizer, understanding the nutrient potential of the digestate is important to prevent soil contamination, as anaerobic digestion can alter the chemistry of the initial substrate [29].In addition to macronutrients (P and K) very good amounts of essential nutrient; Ca, Mn, Zn, Fe, and Cu were detected in the digestate (Table 4). Therefore, the use of the digestate as a biofertilizer could help in plant growth.[40], observed that AD processes lead to the conversion of nutrients into a form that is easily used by plants.Organic nitrogen in a feedstock is transformed to inorganic NH_4^+, while organic phosphate to PO_4^{3-}.

Table 4: Elemental Composition of the Seeds Cake Digestate

Elements	Concentration (wt%)
Al	7.642
Si	4.783
P	0.236
K	8.034
Ca	10.191
Fe	19.393
S	3.915
Cu	0.661
Zn	0.284
Mn	0.234
Mg	3.023
Rh	2.941
Sn	1.526

IV. Conclusion

In this work, the anaerobic digestion of deoiled seed cake of *R. longiflora* was optimized to study the effect of retention time, pH and feed-water mixing ratio on biogas. The results showed that the parameters had significant individual effects on biogas yield with retention time having greater significant effect on the biogas yield. Only interaction of retention time with pH shows significant effect on biogas production. The optimum conditions were 7-days (1st week of retention time), 3:1 feed – water ratio and a pH of 7.0. However, the digestate contains essential nutrients required for plants growth. In light of the proximate and ultimate results obtained it reveal that the cake can be a good source for both liquid and gaseous fuel generation.

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