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Research Paper

Biogas Production from Optimized Alkaline Pretreated Rice Straw Codigested With Animal Manure in a Batch System Bioreactor

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ABSTRACT
The Presence of lignin in lignocellulosic substrates greatly limits anaerobic digestion for production of biogas. The need for initial pretreatment to sufficiently remove it became inevitable. Optimized alkaline pretreatment method was used to pretreat rice straw samples for application in biogas production in a batch bioreactor. The pretreated rice straw was co-digested with 1:1, 2:1 and 3:1 ratios of cow dung, pig waste and poultry droppings respectively, as amendments. Proximate composition, lignin, cellulose and hemicellulose content of the feedstock were determined by standard methods. Results obtained revealed that lignin concentration reduced from 17.4% to 7.3% after alkaline pretreatment. There was a significant difference ($P \leq 0.05$) in biogas yield in allalkaline pretreated rice straw (APRS) amended with animal manure compared to untreated rice straw (URS) alone. Alkaline pretreated rice straw (APRS/CD) 1:1 ratio of rice straw to cow dung showed the highest yield of biogas followed by alkaline pretreated Rice Straw codigested with poultry dropping (APRS/PD) 2:1 with a cumulative biogas yield of 27.05dm^3 and 22.47dm^3 respectively. Composition of components gases in biogas produced by rice straw/cow dung 1:1 which produced highest volume of biogas in this study, were CO 1.15%, CO_2 33.56% and CH_4 64.96%Proximate characteristics of digestate, such as nitrogen, phosphorus and potassium increased, while others including carbon, total solids and total volatile solid content decreased after anaerobic digestion. Findings from this study have shown that optimization of Alkaline pretreatment of lignocellulosic waste (Rice Straw) enhanced biogas production.

Keywords: *Alkaline pretreatment, Lignocellulosic waste, animal manure and Biogas*

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I. INTRODUCTION:

Over the past century, the rapid rise in dependence on petroleum and its allied energy industries has contributed to rapid rise in the world economy. It has been under looked that the underlying energy resource in the form of fossil fuel can deplete, and therefore is a limited form of energy. It cannot be overemphasized also that combustion of these products contributes to emissions of greenhouse gases (e.g. fossil fuel-derived carbon dioxide (CO_2) emissions) which have become a global concern, since about 88% of global energy consumption is derived from fossil fuels (Achinas, 2017). Therefore, developing and developed countries have made efforts towards overcoming the environmental challenges and overdependence on fossil fuels by increasing their proportion of renewable energy to between 20-40% by 2020 (Karmellos, 2016).

Among other renewable energy sources, biogas is seen as one of the most promising renewable energy resources that can replace part of the fossil fuel-based energy used today.It shows great potential and many advantages, including both climate and economic benefits (Meyer-Aurich,2016). Considering its accessibility to all, a biogas process can be implemented in small or large scale by the wealthy or poor, which is important when designing flexible and sustainable energy solutions in both industrialized and developing countries (Holm-Nielsen, 2009) as opposed to fossil fuels. Substrates or raw materials used in the production of biogas can be sourced by rural dwellers which include but not limited tovarious types of waste products, such as manure, straw, municipal wastewater, food waste etc., and dedicated energy crops (Appels,2011; Vasco-Correa, 2018). This implies that if the technology is implemented, it can be a source of employment therefore reducing the poverty index of Nigeria which is currently on the increase (Ogujiuba, 2015). Among the substrates used in production of biogas, lignocellulosic materials, such as agricultural residues, are of great interest due to their high abundance and potential for biogas production (Azman, 2015).Biogas production is a rich technology that employs complete diversification of organic waste into energy, economically and environmentally rich products.

The biogas produced, containing energy carrier methane, can be used for production of heat, electricity and vehicle fuel after upgrading (removal of carbon dioxide and trace gases) (Holm-Nielsen, 2009). The residues left after biogas production are rich in mineral nutrients and can be used as fertilizer during crop production to replace fossil energy-requiring mineral fertilizers. Thisenables recycling of nutrients between urban and rural areas (Holm-Nielsen 2009; Weiland, 2010;Möller& Müller, 2012; Vasco-Correa, 2018).

When plant-based materials (*e.g.* agricultural residues) are used for biogas production, the first step of microbiological process, hydrolysis, becomes rate-limiting. This is the major limitation of use of cellulolytic materials since the crystalline structure of lignocelluloses obstructs degradation in the initial step, thus, slowing downhydrolysis of these insoluble compounds into lower polymers (Lynd,2002; Mulat& Horn, 2018;). However, several efforts have been made to overcome the obstacle associated with the degradation of lignocellulosic and similar materials through adoption of several pretreatment methods that would expose the material and increase its accessibility to biological attack (Martínez-Gutiérrez, 2018).This study therefore seeks to employ mechanisms that would contribute to exposure of lignocelluloses to microbial attack through the adoption of several physicochemical methods and evaluate the effect of alkaline pretreatment of rice straw on biogas production in a batch bioreactor system.

II. MATERIALS AND METHODS

Collection and Processing of sample of Rice Straw (RS), Nitrogenous Wastes and Cow Rumen Liquor.

Rice straw was collected from a rice farm at Abakiliki in Ebonyi state and were transported to the laboratory for processing. The sample was sorted and washed then sundried after which they were milled to fine reduced sizes, using a crushing machine and subsequently stored in air tight polyethene bags.

Cow dung, pig waste and poultry droppings were used as amendments to co-digest the substrate in order to improve their biodegradability and nutritional composition. The cow dung was collected from an abattoir located around 34 Artillery Brigade, Obinze, in Owerri West Local Government Area of Imo State, Nigeria. Pig waste and poultry droppings were collected from piggery and poultry farms in Ihiagwa, respectively. All samples were sun-dried and ground into fine powder before storing in an air tight polyethylene bag.

Cow rumen liquor was used as inoculum in all the anaerobic digesters. Fresh cow rumen waste was collected as soon as the cow was slaughtered using 10 liter air tight buckets to preserve the anaerobes. The cow rumen sample was strained with white cloth filter and the filtrate (liquor) was used immediately to inoculate the processed substrates.

Design of Optimization of Alkaline Pretreatment of Rice Straw

Optimization of conditions for alkaline pretreatment of Rice straw was carried out using Box Behnken Design; a type of Response Surface Model, in Minitab 17version. The 3 factors, 3 level analysis of the parameters to determine the optimum conditions for the alkaline pretreatment of the substrate was adopted. This produced 15 runs with different values for each factor in each run, in other to determine the optimum condition which was used to pretreat the substrate for lignin degradation.

Table1: Box Benken design for optimization of alkaline pretreatment of substrate

StdOrder	RunOrder	PtType	Blocks	NaOH %/ Concentration	Concentration of substrate (g)	Time (h)
1	1	2	1	2	10	48
2	2	2	1	6	10	48
3	3	2	1	2	14	48
4	4	2	1	6	14	48
5	5	2	1	2	12	24
6	6	2	1	6	12	24
7	7	2	1	2	12	72
8	8	2	1	6	12	72
9	9	2	1	4	10	24
10	10	2	1	4	14	24
11	11	2	1	4	10	72
12	12	2	1	4	14	72

13	13	0	1	4	12	48
14	14	0	1	4	12	48
15	15	0	1	4	12	48

Using the values for each factor as specified in Table 1 for each run, rice straw samples were separately pretreated, and concentration of lignin obtained after pretreatment was used as the response.

Alkaline Pretreatment of Rice Straw Samples

Sodium hydroxide was used to pretreat each substrate at ambient temperature to improve their biodegradability, and their potential for anaerobic biogas production. Optimum conditions obtained from the optimization study were used according to the method of Shetty (2016**)** with few modificationSix grams (6g) of NaOH dissolved in 100m/l of water was used to pretreat fourteen grams (14g) of the substrate for 40 hours after which the substrates were washed until the pH is brought to 7.0 and sun-dried. This process was repeated until the required mass of the substrate needed for anaerobic digestion was obtained.

Optimum values obtained from Response Optimizer were applied in the pretreatment process and the concentrations of lignin remaining in each case, after pretreatment was determined and recorded.

Determination of Lignin,Cellulose,Hemicellulose Contents of Rice Straw

Lignin, cellulose and hemicellulose contents of each substrate were measured before and during and after pretreatment at 3 intervals (i.e. before pretreatment, after 15 days and after 30days of pretreatment). These were done according to methods described by Lin (2010)

Hemicellulose determination

Some 1g of sample (rice straw and water hyacinth was weighed into a flask and 150 ml of 0.5 M NaOHwas added and boiled for 3hours with distilled water .the mixture was filtered after cooling before washing to neutrality, finally the residue is dried to constant weight at 105^{0}C.

Hemicellulose =weight before treatment- weight after treatment

Lignin determination

Some 0.3g of dried sample (rice straw and water hyacinth) was weighed into a test tube and 3ml of 72%H_2SO_4 and kept at room temperature for 2hours and shaken at 30minutes interval for hydrolysis to take place, thereafter the mixture is autoclaved for 1 hour at 121^{0}C for second hydrolysis to occur and cooled and filtered. Residue is dried at 105^{0}Cto get the acid insoluble lignin thereafter the acid soluble lignin is determined by ashing the hydrolyzed sample at 575^{0}Cin a muffle furnace and measuring the absorbance of the acid hydrolyzed sample at 320nm.

Lignin content = Acid insoluble lignin + Acid soluble lignin

Then,

Cellulose content determination = Lignin content - Hemicellulose content.

3.6. Co- digestion of Substrates with Nitrogenenous Sources (Amendment)

Cow dung, pig waste and poultry droppings which were sun-dried, were mixed in three ratios of 1:1, 2:1 and 3:1 with each of the primary substrates. The composites were loaded into the digesters after thorough mixing with water. Then 1.6 liters of freshly strained cow rumen waste which was used as source of inoculum was added to each composite and properly mixed. Each of these inoculated composites was charged into the biodigester which was designed and operated as described in subsection 3.6.1 below.

Bioreactor design, set up and operation

Plastic containers of 10 L capacity each were used to construct the bio digesters. Two holes were bored on the cover of the plastic container, one for gas outlet and the other for thermometer. A three quarter inch gas hose was installed in one of the holes and held tight with an epoxy glue to ensure that there is no gas leakage. In the other hole, a thermometer was installed and also made air tight with epoxy glue. The other hose from the 10 liter container was connected to a calibrated water filled inverted 3 liter mini bucket (container). The bioreactor was set up in triplicates. The gas collection was by water displacement method (Aragaw, 2013), pH and temperature changes during the course of anaerobicdigestion was monitored with a digital hand held pH meter and the installed thermometer respectively.

Anaerobic digestion of the substrates was carried out under controlled and reproducible conditions using 10 liters capacity digester. Five hundred and twenty grams (520g) of each ratio of the mixed composites, as well as the control experiment, which was set up without amendment, was weighed separately with an electronic weighing balance and mixed thoroughly with 1.2 liters of water for optimum gas production. This was loaded in the bioreactors to about ¾ of its volume (Ojolo, 2008). Fresh cow rumen waste was strained and 1.6 liters of the inoculum was used to pitch the loaded substrate in the bio digester and made up to eight (8) liter mark. The digesters were made air tight to exclude oxygen. The hose from the digesters outlet was connected to the inverted water filled mini containers, and properly labeled. The digesters were periodically shaken to avoid stratification of the substrate and also ensure thorough mixing of the digester content, while maintaining intimate contact between the microorganisms and the substrates in order to enhance complete digestion of the substrates.

Anaerobic digestion of the substrates lasted for 35 days hydraulic retention time, during which daily ambient temperature remained around 28-32^{o}C. The daily volumes biogas yields from the bioreactors were monitored by water displacement method using an inverted calibrated water filled mini container as container by Aragaw,(2013). Here volume of biogas yield is equivalent to the mean value of water displaced from the mini container after 24hours or before then if empty.

3.8 Analysis of Composition of Biogas Produced

Analysis of composition and percentages of each component of biogas produced in the study was carried out using Gas Chromatograph (GC). This was carried out in triplicates and their means computed. The GC used in this study was manufactured by Buck Scientific (M910, USA) equipped with FID detector and capillary column (Elite-5, 30m*0.25mm*0.25μm). The workstation was Total chrom Navigator used for data processing. The temperature for column chamber, inlet chamber and detector were 150 ^{o}C, 200 ^{o}C and 250 ^{o}C, respectively. High purity nitrogen was used for carrier gas in this study, and the flow rate for nitrogen was 2.0 ml/min. The split ratio of gas sample in inlet chamber was 20:1, which is used to control the amount of biogas flew into column, and prevent the unconventional peak, such as flat peak, trailing peak. The flow rate was 450mL/min for air produced by automatic air source (BCHP, SPB-300, China) and 45mL/min for hydrogen produced by hydrogen generator. The temperature programme used is as follows;

Table 3.3: Temperature programme

Initial temp	Hold	Ramp	Final temp
50.00	5.000	10.000	180.00
180.00	2.000	5.000	220.00
220.00	0.000	5.000	310.00

3.9 Proximate Analysis of Slurry

Physicochemical analysis of the slurries was carried out before and after anaerobic digestionusing standard methods. The following parameters were determined; Total solid content,Volatile solid content, Total moisture content, Total Carbon content, Total Nitrogen content, Total potassium content and Total phosphorus content.

RESULTS AND DISCUSSION:

Optimizationof factors affecting alkaline (NaOHOopretreatment of rice straw (RS)

From the results obtained using rice straw, main effects plot of interactions of the factors affecting the pretreatment process is as shown in Figure 1.

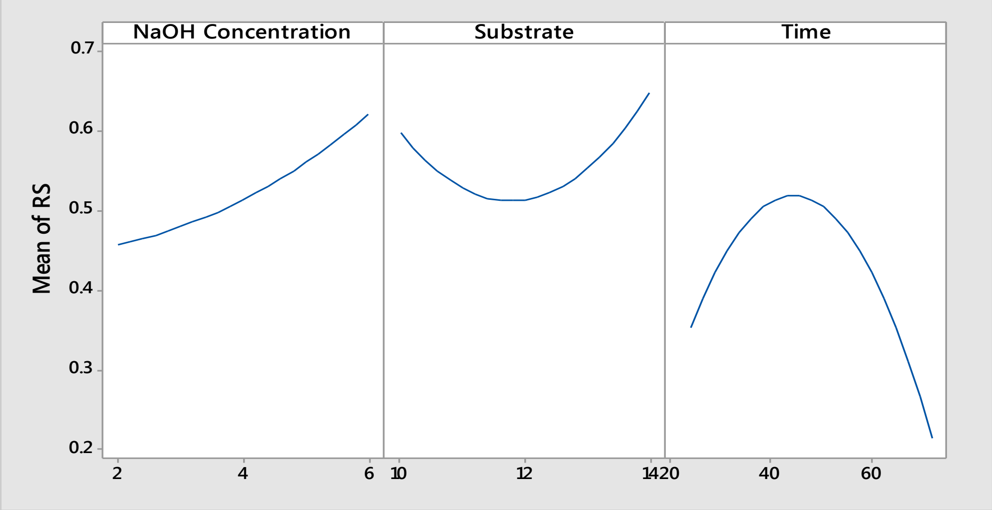

Figure 1: Main effect plot for the preliminary pretreatment of Rice Straw

Figure 1 showed that increase in concentration of alkaline (NaOH) from 2% to 4% resulted in corresponding increase in degradation of the biomass. This also continued when the concentration increased to 6%. Initial increase in concentration of biomass from 10g to 12 g caused a reduction in pretreatment output. However, when the concentration of rice straw was increased to 12.2g, there was a sudden rise in rate of pretreatment, which continued up to when concentration was 14g. Time required for pretreatment increased proportionally with rate of pretreatment from 20h to 40h and then plateaued off. Further extension of time brought about a drastic fall in rate of reduction. This could imply that at constant biomass concentration using 6% concentration of NaOH and 14g of rice straw (RS), maximum of about 40h will suffice for pretreatment. The surface plots illustrating these interactions between the factors affecting lignocellulosic biomass pretreatment are as shown in Figure 2.

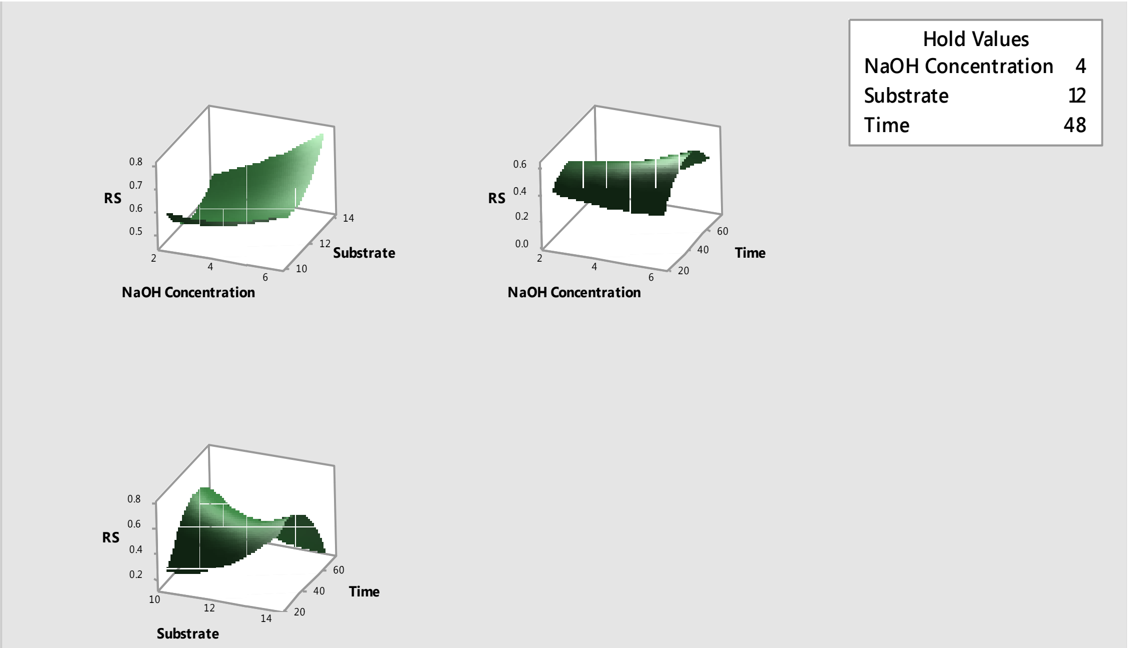

Figure 2: Surface plot for the preliminary pretreatment of Rice Straw

Using Response Optimizer (Minitab 17®) to analyze the concentrations of lignin remaining after pretreatment in each of the solutions from the 15 runs, it was observed that the optimum conditions were; 6% of NaOH, 14g of rice straw at 39.52 h of pretreatment. With these optimum conditions, the predicted minimum concentration of lignin that will remain after pretreatment is 0.83 g, as shown in Figure 3.

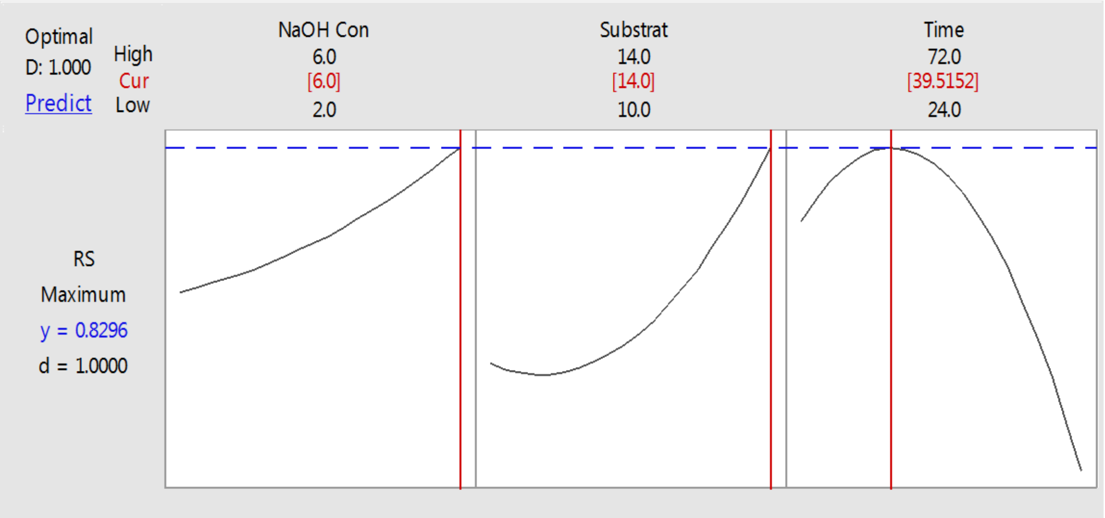

Figure 3: Optimization plot for preliminary alkaline pretreatment of Rice straw.

Table 2: Maximum Cumulative Biogas Yield (dm^3) from the Alkaline Pre-treated Rice Straw and the amendments

Treatments	Biogas Yield(dm^3)
APRS	8.08
(URS)	7.08
APRS/CD 1:1	18.87
APRS/CD 2:1	22.51
APRS/CD 3:1	17.44
APRS/PD 1:1	14.66
APRS/PD 2:1	13.03
APRS/PD 3:1	15.29
APRS/PW 1:1	17.78
APRS/PW 2:1	18.45
APRS/PW 3:1	12.21

Legend: APRS = Alkaline Pretreated Rice Straw
URS = Untreated Rice Straw

From table 2 above it can be seen that alkaline pre-treatment enhanced biogas production by 12.38% though there was no statistical difference in biogas yield in APRS compared to the URS, there was a significant difference ($P \leq 0.05$) in biogas yield in all the APRS amended with animal manure compare to APRS and URS alone. The APRS co-digested with different animal manure, APRS/CD 2:1 showed the highest yield in biogas followed by APRS/CD 1:1, with a cumulative biogas yield of 22.51 and 18.87dm^3, respectively.

Analysis of composition of biogas

Results obtained from Gas chromatography analysis of biogas sample collected from biodigester that gave highest yield in this study indicated the percentage composition as follows; 1.149 of CO, 33.556% of CO_2 and 64.960% of Methane as shown in table3

Table 3.: % Composition of biogas

Component gas	%composition
CO	1.15
CO_2	33.56
CH_4	64.96

Proximate composition of slurry before and after anaerobic digestion

The physicochemical parameters of the samples before and after digestion were determined to ascertain if there was an increase or decrease in the parameters before and after digestion and whether the difference were significant. This was done for each digestion and the efficiency of digestion was obtained by comparing the nitrogen, phosphorus and potassium (NPK) ratio of the digestate (used slurry).

proximate Composition of Rice Straw and Cow Dung Before and After Digestion

Figure 4 shows that RS/CD (3:1) recorded the highest carbon content of 30.97 and 28.38; while RS had the least carbon content of 23.37 and 18.18 before and after digestion respectively. Similarly, results showed that Nitrogen content of 1.87 and 2.52; and Potassium concentration of 0.89 and 0.9 were recorded for RS/CD 2:1; while RS also recorded least Nitrogen values of 0.9 and 0.23; and least potassium values of 0.75 and 0.32 before and after digestion respectively. On the other hand, values (before digestion, after digestion) of phosphorus for the RS/CD 1:1 were (0.066 and 1.05) for RS values were (0.05 and 1.1) indicated that RS/CD 1:1 had the highest phosphorus content before digestion, contrary to TS values for RS/CD 1:1 were (98.2 and 93.67) and RS (89.22 and 82.15) showed that the RS/CD (1:1) had highest values while RS had least values before and after digestion.

Before digestion, RS/CD (1:1) had the highest volatile solids content (93.12) while RS had the least content (88.15); RS had the highest moisture content (16.52) while RS/CD (3:1) had the least content of (10.3); and RS/CD 3:1 had the highest NPK ratio of (34.67) while After digestion, RS/CD (1:1) had the highest volatile solids content of (83.31) while RS/CD 3:1 had the least (81.1); RS had the highest moisture content (18.89) while RS/CD 3:1 had the least content of (13.5) and RS/CD (2:1) had the highest NPK ratio of (2.62) while RS recorded the least value of (0.65).

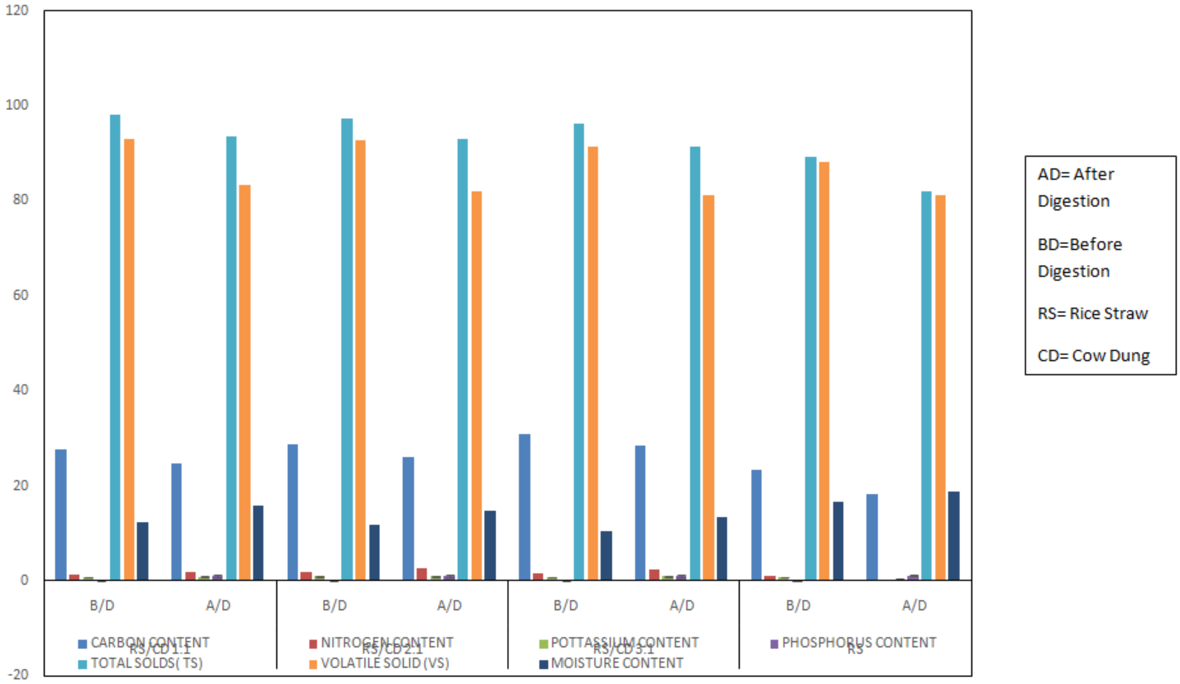

Figure 4: The proximate composition of the slurry before and after digestion for the pretreated rice straw amended cow dung.

Proximate Composition of Mixture of Rice Straw and Poultry Droppings Before and After Digestion

Physicochemical composition of for rice straw and Poultry Droppings before and after digestion combination is shown in Figure 5. it can be seen that before digestion, RS/PD (3:1) had the highest carbon content (30.98) while RS had the least (23.37). After digestion, RS/PD (2:1) had the highest carbon content (27.04) while RS had the least (18.18). Also, before digestion, RS/PD (2:1) had the highest nitrogen content (1.77) while RS had the least (0.9). After digestion, RS/PD 2:1 had the highest nitrogen content (2.44) while RS had the least (0.23).

Before digestion, RS had the highest potassium content (0.76) while RS/PD (3:1) had the least (0.51). After digestion, RS/CD (1:1) had the highest potassium content (0.77), while RS had the least (0.32). Also, RS/PD 1:1 had the highest phosphorus content (0.065), while RS/PD (3:1) and RS had the least values (0.05). After digestion, RS had the highest phosphorus content (1.1) while RS/PD 3:1 had the least (1.042).

RS/PD (1:1) had the highest Total Solids (98.2), while RS had the least (89.22). After digestion, RS/PD (1:1) had the highest Total Solids (93.66) while RS had the least (82.15). Similarly, RS/PD (1:1) had the highest volatile solid (92.11) while RS had the least (88.15). After digestion, RS/PD (1:1) had the highest volatile solid (82.21) while RS/PD (3:1) had the least (80).

Furthermore, before digestion, RS had the highest moisture content (16.52) while RS/PD (3:1) had the least (10.8). After digestion, RS had the highest moisture content (18.89) while RS/PD (3:1) had the least (13.6). Finally, it was deduced that before digestion, RS/PD (3:1) had the highest NPK ratio (64.71) while RS had the least (23.68). After digestion, RS/PD (2:1) had the highest NPK ratio (3.82) while RS had the least (0.65).

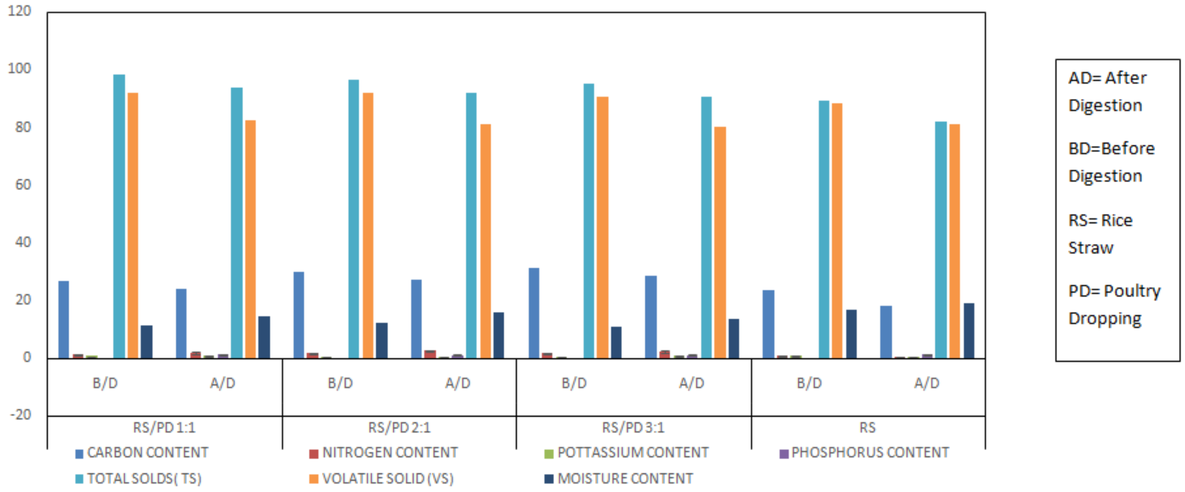

Figure 5:Proximate composition of the slurry before and after digestion for the pretreated rice straw amended poultry dropping

Proximate Composition of Rice Straw and Pig Waste Before and After Digestion

Physicochemical composition of the slurry before and after digestion for Rice Straw and Poultry Droppings combination is shown in Figure 6. Results show that RS/PW (3:1) had the highest carbon content (29.18), while RS had the least (23.37). After digestion, RS/PW (3:1) had the highest carbon content (27.98), while RS had the least (18.18). Similarly, before digestion, RS/PW 3:1 had the highest nitrogen content (2.23) while RS had the least (0.9). After digestion, RS/PW (2:1) had the highest nitrogen content (1.67), while RS had the least (0.23).

Consequently, before digestion, RS/PW (1:1) had the highest potassium content (0.85), while RS/PW (2:1) had the least (0.52). After digestion, RS/PW 3:1 had the highest potassium content (0.53) while RS/PW (1:1) had the least (0.25). Also, before digestion, RS/PW (2:1) had the highest potassium content (0.062) while RS/PW (1:1) had the least (0.023). After digestion, RS had the highest potassium content (1.1) while RS/PW 1:1 had the least (1.015). RS/PW (1:1) had the highest Total Solids (97.1) before digestion while RS had the least (89.22). After digestion, RS/PW (1:1) had the highest Total Solids (92.65) while RS had the least (82.15).

Before digestion, RS/PW 1:1 had the highest volatile solid (91.21) while RS had the least (88.15). After digestion, RS/PW (1:1) had the highest volatile solid (89.12) while RS/ PW (3:1) had the least (80.21). Also, RS had the highest moisture content (16.52) before digestion while RS/PW (1:1) had the least (10.21). After digestion, RS had the highest moisture content (18.89) while RS/PW (3:1) had the least (13.6). It was deduced that before digestion, RS/PW (1:1) had the highest NPK ratio (79.28) while RS had the least (0.65). After digestion, RS/PW 1:1 had the highest NPK ratio (4.34) while RS had the least (0.65).

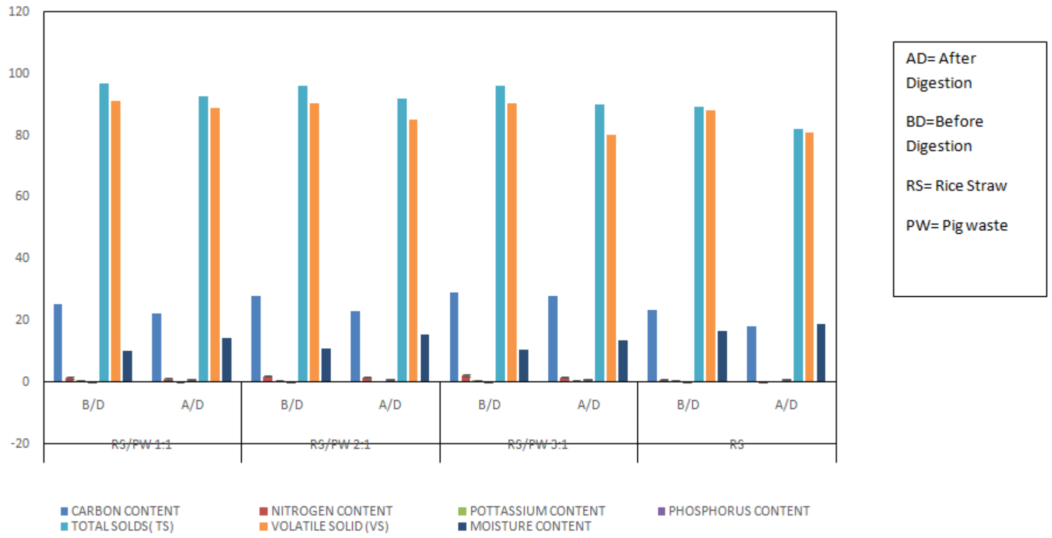

Figure 6: Proximate composition of the slurry before and after digestion for the pretreated rice straw amended pig waste

III. Conclusion

Findings from this study indicated that initial optimized chemical pretreatment enhanced biogas production.
The model used to predict the optimum conditions of chemical pretreatment of substrate for biogas production exhibited a favorable fit with the experimental value which led to increased biogas yield from the pretreated substrate hence suitable for predicting initial alkaline pretreatment of substrate for biogas production process.

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