



# Co-digestion of *Theobroma cacao* (Cocoa) pod husk and poultry manure for energy generation: Effects of pretreatment methods

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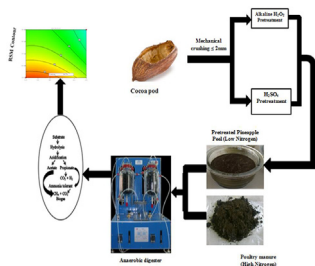
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## GRAPHICAL ABSTRACT



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## ABSTRACT

In this study, biogas was produced from the anaerobic co-digestion of Cocoa pod husk (CPH) and poultry manure. Pretreatment of the CPH was carried out using sulfuric acid and hydrogen peroxide. The physico-chemical, elemental and structural analyses were carried out on the CPH before and after pretreatment. The microbial composition of the fermenting materials were also determined using standard method while the Fourier Transform Infra-red (FTIR) spectroscopy was used to identify the structural changes that took place after pretreatments. Use of alkaline hydrogen peroxide caused high solubilization of the lignin component of the CPH and reduced up to 81% of lignin i.e. initial value of 21.7% m.m<sup>-1</sup> to final value of 4.2% m.m<sup>-1</sup>. Similarly, the alkali reduced the hemicellulose content of the CPH from 27.0% m.m<sup>-1</sup> to 8.5% m.m<sup>-1</sup>. Overall, there was 68% increase in biogas volume from the alkaline pretreated CPH.

## 1. Introduction

Cocoa (*Theobroma cacao*) is primarily and largely cultivated in three tropical regions i.e. Latin America, Southeast Asia and West Africa in which up to 70 percent of global production is from four West African countries (Ivory Coast, Ghana, Nigeria, and Cameroon) (Kaufman and Justeson, 2006; Davison and Howe, 2015). The ten leading world

producers are Cote d'Ivoire, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador, Mexico, Peru and Dominican Republic (FAO, 2017). Ivory Coast and Ghana are by far the two largest producers of cocoa accounting for more than 50 percent of global production in which the former alone produced approximately 1.6 million metric tons of cocoa beans which increased to 1.9 million metric tons during the 2016/2017 crop year. Global production in 2017 was over 4.6 million metric tons

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and as at May 2018, a total of 4.9 million metric tons has been produced. In Nigeria, the average production of cocoa beans is 367,000 metric tons annually (FAO, 2017). With the massive production of cocoa globally, it is obvious that huge biomass is produced from processing of the bean. However, most of these biomass ends up as wastes without any tangible usage whereas, they are a veritable source of renewable energy being lignocelluloses.

Two of the major threats to existence on the planet earth are global warming and the attendant depletion of the ozone layer. The precursors of these occurrences are combustion of fossil fuels leading to pollution menace and the indiscriminate dumping of organic wastes leading to release of anthropogenic gases (Bacenetti et al., 2013). Across the globe, several measures are being taken by many countries and government at various levels to address the issue of global warming and these include: use of combustion devices capable of reducing emissions, adoption of renewable alternative energy and effective management of wastes (Khayum et al., 2018).

The two major methods usually employed in the conversion of organic wastes into energy are thermochemical and biochemical conversions with the latter being the most suitable method used to maximize energy recovery from organic wastes (Li et al., 2017). However, among all the biochemical conversion methods that have been used in literature, anaerobic digestion (AD) is the most proven technology capable of degrading and converting organic matters into biogas using microorganisms in an anaerobic condition.

Several researches have been carried out to investigate the AD of different wastes and biomass in both mono and co-digestion processes. Such includes the anaerobic co-digestion of kitchen waste with cow manure (Zhai et al., 2015), cow slurry with olive pomace and apple pulp (Riggio et al., 2015), cow manure and food waste (Banks et al., 2011), thermophilic anaerobic digestion of cattle manure and pasteurized food wastes (Zarkadas et al., 2015).

In order to enhance the efficiency of AD especially when digesting lignocelluloses, various pre-treatment methods have been designed and employed. Previous authors (Baadhe et al., 2014; Cai et al., 2016; Venturin et al., 2018) employed the use of sulfuric acid for the pre-treatment of corn stalk and obtained almost complete removal of the hemicellulose component of the biomass Song and Zhang (2015) pre-treated wheat straw with hydrogen peroxide and co-digested the pre-treated biomass with cattle manure. The treatment caused a higher yield of biogas than the untreated wheat straw or mono-digestion of the cattle manure. Though not well documented in literature, the use of hydrogen peroxide for pretreatment has been reported in few studies all of which produced slightly different results. Among them, Cai et al. (2016), used hydrogen peroxide to pretreat corn straw and the results showed 19.6, 32.8 and 6.2% reduction in the composition of lignin, cellulose, and hemicellulose respectively. In another study by Sun et al. (2013), the authors obtained a 38.9% reduction in lignin and 31.4 and 33.3% increase in the composition of cellulose and hemicellulose respectively having pretreated corn straw with hydrogen peroxide.

Other recent studies that combined the digestion of chemical pre-treated lignocelluloses with animal manure with improvement in biogas production include *Chromolaena odorata* shoot with poultry manure (Dahunsi et al., 2017a), *Carica papaya*s fruit peels with poultry manure (Dahunsi et al., 2016a), *Arachis hypogaea* hulls with poultry manure (Dahunsi et al., 2017b) and *Telfairia occidentalis* fruit peels with poultry manure (Dahunsi et al., 2018a,b,c). The aim of this study therefore is to evaluate the anaerobic co-digestion of CPH and poultry manure. The study also sought to establish the optimal pretreatment condition for *Theobroma cacao* pod husk prior to co-fermentation with poultry manure. The pretreatments were done using two chemicals i.e. use of sulfuric acid and hydrogen peroxide in order to increase the substrates biodegradability and enhancement of biogas generation.

## 2. Material and methods

### 2.1. Sample collection

The pod husks of *Theobroma cacao* used in this study were sourced from Ile-Ife, one of the major producing localities in South-western Nigeria. The pods were shredded to pieces and sundried until constant weight was achieved. The co-substrate poultry manure, on the other hand, was obtained from the Landmark University Teaching and Research Farms, and kept refrigerated at  $-4^{\circ}\text{C}$  until use. Grinding of the dried CPH to sizes of about 2 mm was carried out with the aid of a knife mill (SOLAB, SL-31, Brazil). This was followed by sieving of a portion of the ground husk with sieves of 0.075 to 1.00 mm mesh sizes in order to determine the most abundant particles that were used in the pretreatment procedures and this was later referred to as the 'sifted' sample while the remaining portion was called the 'not sifted' (Venturin et al., 2018). Afterwards, both the sifted and the not sifted samples were then refrigerated at  $-4^{\circ}\text{C}$  till further usage.

### 2.2. Physicochemical analyses

For the CPH, poultry manure and inoculum, measurements of the concentration of important parameters which include carbon, nitrogen phosphorus, phosphates, sulfates, potassium, sodium, magnesium, calcium, nitrates, ammonium, iron, copper, zinc, aluminum, and manganese were done with the aid of an inductively coupled plasma mass spectrometry. The standard method for the analyses of water and wastewaters was employed for COD determination (APHA, 2012) while that of Volatile fatty acids (VFAs) was done with the use of a gas chromatography (Clarus 580GC, PerkinElmer, USA) to which was attached a flame ionization detector (FID). A standard procedure (Finnish Standard Association, 1990) was used in the determination of total and volatile solids of the substrates. A microtube (Spectroquant, Merck) test was used for determination of total content of phenolics after which a 4-amino antipyrine colorimetric measurement was carried out (Monlau et al., 2012).

### 2.3. Structural analyses

Determination of the concentration of the structural component (lignin, cellulose, and hemicellulose), the fixed and extractive solids were all carried out for the CPH (untreated and treated samples) (Sluiter et al., 2008). In evaluating the extractable components, samples were heated in a Soxhlet apparatus for 6 h while the same samples were burnt in a muffle furnace for fixed solids determination (Sluiter et al., 2008). In order to evaluate the concentration of total lignin, cellulose and hemicellulose, the dried CPH sample (0.3 g) was heated in a thermostatic bath with 3 mL of 72% sulfuric acid ( $\text{v.v}^{-1}$ ) at  $30^{\circ}\text{C}$  for 1 h. The filtrate was then used in determining the carbohydrate contents (Sluiter et al., 2012). For determining the concentration of sugars and acetic acid, the liquid chromatography method was employed using the LC-MS mass spectrometer (SHIMADZU, Japan) with mobile phase being  $0.005\text{ mol.L}^{-1}$  sulfuric acid in an isocratic mode, at  $45^{\circ}\text{C}$  with injection volume of  $20\ \mu\text{L}$  and flow of  $0.6\ \text{mL.min}^{-1}$ . Thereafter, the calibration curves with corresponding LC-MS standards (Sigma-Aldrich) was used in the determination of the concentration of each compound (Bazoti et al., 2017). The determination of the concentrations of furfural and hydroxymethylfurfural (HMF) was carried out using same methods with appropriate modification according to the earlier reported method (Bazoti et al., 2017).

### 2.4. Experimental design of pretreatments

The design of the experiment was done using the Response Surface Methodology (RSM) due to its versatility in experimental design and optimization studies. For the acidic pretreatment, sulfuric acid ( $\text{H}_2\text{SO}_4$ )

was used in humid steam in an autoclave with the input variables Exposure time (min), Temperature (°C), H<sub>2</sub>SO<sub>4</sub> concentration (%) and Dry mass (g). In the same way, the alkaline pretreatment was carried out with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) with variables: Exposure time (min), Temperature (°C), Agitation (rpm), H<sub>2</sub>O<sub>2</sub> concentration (%) and Dry mass (g). In both designs, the evaluated responses were the residual or percentage lignin, cellulose and hemicellulose composition in m.m<sup>-1</sup> in the pretreated CPH. All the experimental runs generated by the designs with their corresponding variables were tested in anaerobic digestions experiments in order to determine the optimum values for each pretreatment.

### 2.5. Pretreatment with H<sub>2</sub>SO<sub>4</sub>

This was carried out in the presence of sulfuric acid using the humid steam of an autoclave. Four important variables were evaluated in order to optimize the acidic pretreatment and they are as follow: exposure time of 5, 15, 25, 35 and 45 min), autoclave temperature of 80, 90, 100, 110 and 120 °C), H<sub>2</sub>SO<sub>4</sub> concentration of 0, 0.5, 1, 1.5 and 2% (v.v<sup>-1</sup>), and dry mass of 2, 4, 6, 8 and 10 g which were all experimented as a modification to earlier methods (Baadhe et al., 2014; Venturin et al., 2018).

### 2.6. Pretreatment with H<sub>2</sub>O<sub>2</sub>

The alkaline pretreatment procedure was carried out with hydrogen peroxide using an orbital shaker. As done for the acidic treatment, values were chosen based on the modification of earlier methods and considering the biomass in question (Rabelo et al., 2011; Venturin et al., 2018). In doing this, exposure time of 50, 60, 70, 80 and 90 min were considered while the rest were orbital shaker temperature of 30, 38, 46, 54 and 62 °C, agitation of 130, 140, 150, 160 and 170 rpm, dry mass of 1.5, 3, 4.5, 6 and 7.5 g and H<sub>2</sub>O<sub>2</sub> concentration of 3, 6, 9, 12 and 15% (v.v<sup>-1</sup>). An antifoam agent was added so as to reduce foaming.

### 2.7. Determination of functional groups

The functional groups present in the pretreated CPH which is an indication of the chemical changes that have taken place as a result of the pretreatment application as well as the functional groups in the untreated CPH were determined using the IR tracer-100 Fourier Transform infrared spectroscopy (FTIR) (SHIMADZU, Japan). The procedure of Zhao et al. (2018) was employed in doing this.

### 2.8. Biogas potential (BP) test

In order to quantify the maximum biogas that the substrates (CPH + poultry manure) could produce under constant temperature (37 °C) and pressure for a 30-day retention period, the Biogas potential test was carried out using 250 mL capacity mini batch digesters connected to 500 mL eudiometer tubes with 10% (m.v<sup>-1</sup>) volatile solids following standard procedures (Angelidaki et al., 2009). Activity tests were performed on acetate and cellulose in order to check the quality of the used inoculum which has a specific activity of 0.5 g CH<sub>4</sub>-COD/g VSS-d on acetate. Compliance was also done to the VDI 4630 (2006) standard while carrying out the test. The biogas yield was observed to be increasing and stabilized when the daily yield of gas was less or equal to 1% of the total produced gas.

### 2.9. Anaerobic co-digestion

After the pretreatment regimes, both the pretreated and untreated samples of the CPH were anaerobically co-digested with poultry manure which was based on the most suitable responses obtained from the experimental design of the pretreatments. The digestion was carried

out using the Computer controlled anaerobic digester (EDIBON). The digester is a paired system with two double jacketed anaerobic chambers with electronic sensors for regulating parameters such as water flow, temperature, mixing rate, gas production etc. The inoculum which made up 10% of digesters total volume was obtained from a running mesophilic digester treating cattle manure (Angelidaki et al., 2009). Produced biogas was collected in the water displacement trough attached to the digester while comparison was made between the results from the pretreated samples and the untreated (sifted and not sifted). Similarly, a comparison was made between the results of the untreated sifted sample and the not sifted one so as to evaluate the effect of sifting as it relates to biogas yield. Biogas composition analysis was carried out by infrared and electrochemical sensors (BIOGASS5000, USA) majorly to determine the values of CH<sub>4</sub>, CO<sub>2</sub>, and H<sub>2</sub>S.

### 2.10. Assessment of economic viability of pretreatments

There is a need to establish the economic viability of pretreatment in order to justify the investment into the procurement of acid and alkali besides the cost of obtaining energy used for the pretreatment. In doing this, the balance between production and usage of energy was done by comparing the cost of obtaining heat energy and chemicals with the extra energy that will be obtained from the additional biogas as a result of the applied pretreatments. The purpose was to evaluate the possibility that the additional biogas obtained would suffice for the cost of heat energy and chemicals. The heat energy required (HER) for pretreating CPH was determined via Eq. (1):

$$HER = \frac{m \times Sh \times (T_{final} - T_{initial})}{3600} \quad (1)$$

where:

$m$  = mass substrate (1000 kg);

$Sh$  = specific heat of water i.e. 4.18 kJ kg<sup>-1</sup> C<sup>-1</sup>

$T$  = Temperature (°C)

3600 = the amount of calories produced by the biomass

The United States costs of hydrogen peroxide and sulfuric acid i.e. USD 400 and 300 respectively was adopted.

### 2.11. Combustion/cooking test for produced gas

The cooking test was conducted in order to evaluate the combustibility of the biogas generated in all four experiments carried out in this study using a biogas burner designed for the purpose. Two commodities (Water and rice) were boiled using the biogas while the time taken for boiling was documented which corresponds to the heating value and combustibility of the biogas in each case (Alfa et al., 2013).

### 2.12. Analysis of microbial community

In order to analyze the microbial community, 45 mL each was taken from all samples, co-substrates, and effluents on days 6, 12, 18, 24 and 30 respectively and stored at -20 °C. The total DNA extraction from all samples was carried out according to the method of Vilchez-Vargas et al. (2013) followed by conventional PCR in order to target the total bacterial population using the total bacterial primers P338f and P518r (Boon et al., 2002). Agarose gel electrophoresis was thereafter used to evaluate the purity of the extracted DNA and PCR products which was followed by Real-time PCR analysis using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The total bacterial population was then analyzed (Desloover et al., 2015). The quality of the Real-time PCR products was checked by examining the different parameters which were obtained with the StepOnePlus software V2.3. Analysis of each sample was done in triplicate and results were presented as copies per gram of wet sludge.

**Table 1**  
Characterization of CPH before and after pretreatments, poultry manure and inoculum.

Parameter	Inoculum	Poultry manure	Cellulose Standard	Pretreated CPH		Untreated CPH	
				H <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> O <sub>2</sub>	Sifted	Not Sifted
pH (Sample + Inoculum)	7.79 ± 0.02	6.92 ± 0.01	7.85 ± 0.10	7.88 ± 0.11	7.90 ± 0.10	7.82 ± 0.10 <sup>a</sup>	7.83 ± 0.10 <sup>a</sup>
Total solids (% m.m <sup>-1</sup> )	4.2 ± 0.02	256.5 ± 9.02	95.1 ± 1.00	88.9 ± 0.11	90.4 ± 0.01	96.5 ± 1.02 <sup>b</sup>	94.1 ± 0.02 <sup>b</sup>
Volatile solids (% m.m <sup>-1</sup> )	3.0 ± 0.01	166.1 ± 7.05	85.3 ± 2.00	75.9 ± 2.01	52.5 ± 1.02	70.6 ± 2.01 <sup>c</sup>	68.4 ± 0.01 <sup>c</sup>
Total Lignin (% m.m <sup>-1</sup> )	29.5 ± 2.01	5.6 ± 0.01	ND	28.6 ± 0.20	4.2 ± 0.02	19.2 ± 0.11 <sup>d</sup>	21.7 ± 0.01 <sup>d</sup>
Cellulose (% m.m <sup>-1</sup> )	3.3 ± 0.01	3.2 ± 0.01	99 ± 1.01	49.0 ± 0.01	39.8 ± 1.01	29.2 ± 0.10	31.7 ± 0.10
Hemicellulose (% m.m <sup>-1</sup> )	20.9 ± 0.01	1.8 ± 0.01	ND	8.5 ± 0.01	8.7 ± 0.11	25.2 ± 0.01	27.0 ± 0.10
Fixed solids (% m.m <sup>-1</sup> )	1.4 ± 0.01	0.6 ± 0.01	0 ± 0.00	1.9 ± 0.01	1.1 ± 0.01	3.8 ± 0.02	3.7 ± 0.10
Extractives (% m.m <sup>-1</sup> )	ND	0.4 ± 0.01	ND	ND	ND	17.9 ± 0.01	16.8 ± 0.02
Solids after pretreatment (% m.m <sup>-1</sup> )	–	–	–	3.8 ± 0.00	2.4 ± 0.02	–	–
Added sample (g)	0 ± 0.00	0 ± 0.00	1 ± 0.10	2.4 ± 0.12	2.4 ± 0.12	2.4 ± 0.12	2.4 ± 0.12
COD (g COD/g VS)	148.08 ± 1.10	229.9 ± 8.05	ND	212.32 ± 1.20	204.62 ± 0.10	206.72 ± 5.00	211.05 ± 0.10
BP (L <sub>N</sub> biogas. kg VS <sub>add</sub> <sup>-1</sup> )	25.6	544.2 ± 8.09	617.4 ± 2.01	203.6 ± 2.10	633.2 ± 3.10	321.1 ± 3.10	382.4 ± 3.00
μ <sub>max</sub> (L <sub>N</sub> biogas. kg VS <sub>add</sub> <sup>-1</sup> ).d <sup>-1</sup>	ND	200.3 ± 4.02	154.0 ± 2.02	222.6 ± 0.01	275.5 ± 2.10	44.5 ± 0.10	52.2 ± 0.10
Day of μ <sub>max</sub>	ND	2–3	3–4	4–6	1–2	4–6	3–4

Values shown in table are means of triplicate analyses with respective standard errors; superscripts with same letters are statistically the same by the Tukey's test at 5%; ND = Not determined; BP = Biogas potential; μ<sub>max</sub> = maximum biogas generation rate.

### 2.13. Statistical analysis of data

A major component of the RSM is the central composite design (CCD) which has been proved to be effective in experimental designs. The CCD was employed in this study to evaluate the different pretreatments. Afterward, the STATISTICA V. 12 software (StatSoft, Tulsa, USA) was employed in analyzing the different responses with a 95% ( $p < 0.05$ ) confidence level while comparison of mean values was done using the Analysis of variance (ANOVA) and the Tukey's test.

## 3. Results and discussion

### 3.1. Characterization of CPH husk before pretreatment

Various sizes ranging: 0.622, 0.401, 0.261, 0.109 and 0.073 mm were obtained from sieving of the ground untreated dried CPH and each of these sizes was obtained from 28.1 ± 1.2, 51.6 ± 1.0, 13.1 ± 1.0, 5.1 ± 1.2 and 2.1 ± 0.5% respectively from the total ground sample. The most abundant sample fraction was the 0.401 mm and this was subsequently used for the pretreatment procedures. As shown in Table 1, results of the structural analysis by chromatography showed the contents of total lignin, cellulose, hemicellulose and fixed solids to be 21.7 ± 0.01, 31.7 ± 0.10, 27.0 ± 0.10 and 3.7 ± 0.10% m.m<sup>-1</sup> respectively for the untreated not sifted sample while for the untreated sifted sample, the values were 19.2 ± 0.11, 29.2 ± 0.10, 25.2 ± 0.01 and 3.8 ± 0.02% m.m<sup>-1</sup> respectively.

Similarly, the composition of lignin, cellulose, hemicellulose, and fixed solids are very close to earlier results (Cai et al., 2016; Venturin et al., 2018) from the structural characterization of different part and whole biomass of corn stalk. The two methods of pretreatment had positive effects on the CPH in terms of structural solubilization but at different rates.

### 3.2. Elemental composition of CPH before pretreatment

As shown in Table 2, the CPH is rich in nutrients elements and minerals that are required for the growth and proliferation of microorganisms in a fermentation medium. Of importance are values of important elements like carbon, nitrogen, phosphorus, potassium, calcium, and magnesium which further reveal the husk as rich in nutrients and elemental composition. Similarly, the CPH is moderately high in soluble sugars which can be utilized and converted to alcohols by microorganisms during the subsequent anaerobic fermentation for the purpose of biogas generation. This is similar to the submission of earlier researches (Dahunsi et al., 2016a,b, 2017a,b,c).

### 3.3. Characterization of pretreated sifted CPH

#### 3.3.1. Sulfuric acid pretreatment

Use of sulfuric acid caused enormous solubilization of the hemicellulose component of the CPH. This solubilization was evident by the breakage of all important chemical bonds (Hydrogen and covalent and Van der Waals forces) in the husk. Similarly, the hemicellulose component of the husk was depolymerized by the hydrolysis of xylose to form monosaccharides while the concentration of both lignin and cellulose after the pretreatment. This shows that the two components were not affected by the acids but on the other hand were strengthened as evident in their increase

The optimal condition for the most efficient acidic pretreatment of the CPH was 2% (w/v) H<sub>2</sub>SO<sub>4</sub>, autoclave temperature of 121 °C and 4.01 g dry mass for 60 min. The outcome of this was reduction in hemicellulose content by 69% (from 27.0 ± 0.01 to 8.5 ± 0.01% m.m<sup>-1</sup>) while total lignin content increased by 24% (from 21.7 ± 0.01 to 28.6 ± 0.20% m.m<sup>-1</sup>) while cellulose also increased by 35% (31.7 ± 0.10 to 49.0 ± 0.01% m.m<sup>-1</sup>). This result agrees with previous studies (Baadhe et al., 2014; Cai et al., 2016; Venturin et al., 2018) where almost complete removal of hemicellulose was reported when corn stalk was pretreated using acids. In this study, however, lesser volume of acid was used to achieve higher hemicellulose solubilization which is a major improvement in the current study. Acidic treatment was observed to also cause the increase in the composition of both total lignin and cellulose and this also agrees with the study by Cai et al. (2016) who achieved 87% increase in hemicellulose content of corn stalk while increasing the composition of lignin and cellulose by 25% each after the application of acidic pretreatment. Other authors obtained similar results after applying acidic pretreatment to different biomass. However, the important chemical groups found in lignin are usually associated with the 1734, 1716, 1633, and 1604 cm<sup>-1</sup> bands. All these bands experienced reduction due to acidic pretreatment application. Though the composition of cellulose increased in this study, it was obviously modified due to the exposure to factors like acid concentration, temperature, and pressure similar to previous findings (Cai et al., 2016; Zhao et al., 2018).

The results obtained for the acidic pretreated biomass in this study strongly agree with previous studies that reported structural composition (lignin) increment characterized by pseudo-lignin formation due to acidic pretreatment coupled with extreme temperature and pressure (Cai et al., 2016). Such an increase in lignin ended up inhibiting the rate and final volume of biogas produced as seen in this study and may sometimes completely inhibit the entire anaerobic digestion process.

As reported in the literature, inhibition of the digestion process is

**Table 2**  
Elemental composition of CPH + poultry manure and inoculum before and after pretreatments.

Parameter	Inoculum	Poultry manure		H <sub>2</sub> SO <sub>4</sub> Pretreated CPH		H <sub>2</sub> O <sub>2</sub> Pretreated CPH		Untreated sifted CPH		Untreated not sifted CPH	
		CPH only	CPH + poultry manure	CPH only	CPH + poultry manure	CPH only	CPH + poultry manure	CPH only	CPH + poultry manure	CPH only	CPH + poultry manure
Ash Content (%)	5.56 ± 1.02	19.56 ± 2.02	20.34 ± 3.01	3.51 ± 1.00	19.06 ± 3.05	4.20 ± 0.01	18.04 ± 3.01	4.71 ± 0.00	17.38 ± 3.01		
Moisture Content (%)	90.48 ± 3.02	68.94 ± 6.01	71.47 ± 7.02	93.21 ± 1.05	88.91 ± 11.03	80.51 ± 1.01	76.56 ± 6.02	87.2 ± 0.02	72.47 ± 4.00		
Total Carbon (g/kg TS)	265.21 ± 0.10	298.41 ± 9.01	897.92 ± 12.05	722.55 ± 5.21	922.64 ± 9.04	436.11 ± 2.05	598.41 ± 9.01	435.04 ± 1.23	594.18 ± 7.04		
Total Nitrogen (g/kg TS)	48.00 ± 2.02	61.12 ± 5.22	44.17 ± 4.02	30.04 ± 0.25	41.68 ± 1.02	21.04 ± 0.02	35.25 ± 2.02	22.43 ± 1.20	31.20 ± 2.02		
C/N	6/1	5/1	20/1	24/1	22/1	21/1	17/1	19/1	19/1		
Acetate (g COD/g VS)	1.04 ± 0.10	2.01 ± 1.01	1.11 ± 0.01	0.10 ± 0.01	1.08 ± 0.01	0.04 ± 0.10	0.07 ± 0.01	0.04 ± 0.10	0.07 ± 0.01		
Propionate (g COD/g VS)	1.07 ± 0.02	1.99 ± 0.02	1.13 ± 0.01	0.12 ± 0.01	1.25 ± 0.05	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.19 ± 0.01		
TVPAs (g COD/g VS)	2.44 ± 0.10	2.61 ± 1.01	2.10 ± 0.01	1.12 ± 0.10	1.74 ± 0.02	0.11 ± 0.01	1.18 ± 0.02	1.02 ± 0.01	1.17 ± 1.00		
Ammonia (mg/g VS)	4.97 ± 1.01	17.12 ± 2.02	14.25 ± 1.02	2.09 ± 0.03	15.02 ± 1.04	1.20 ± 0.01	11.00 ± 1.05	1.07 ± 0.10	12.01 ± 2.01		
Uronic acids (% VS)	1.67 ± 1.11	0.62 ± 0.01	1.95 ± 0.05	2.08 ± 0.10	1.81 ± 0.02	1.61 ± 1.00	1.22 ± 0.01	1.01 ± 0.10	0.61 ± 0.01		
@Soluble sugars (% VS)	4.02 ± 2.10	2.27 ± 1.00	4.45 ± 0.05	7.35 ± 0.10	5.72 ± 1.10	3.04 ± 1.00	2.42 ± 0.03	3.17 ± 0.10	2.28 ± 1.00		
Phenols (mg L <sup>-1</sup> )	4.71 ± 2.10	0.91 ± 0.01	0.01 ± 0.01	0.003 ± 0.01	0.02 ± 0.01	0.001 ± 0.01	0.06 ± 0.01	0.001 ± 0.10	0.01 ± 0.01		
Total Phosphorus (g/kg TS)	6.30 ± 0.02	8.12 ± 1.05	6.27 ± 0.03	5.58 ± 0.01	7.04 ± 1.03	3.00 ± 0.01	5.02 ± 1.02	3.37 ± 0.01	5.11 ± 1.02		
Potassium (g/kg TS)	7.20 ± 0.11	9.27 ± 1.03	9.01 ± 1.01	9.23 ± 0.01	9.17 ± 1.01	3.03 ± 0.01	5.29 ± 1.07	3.25 ± 0.01	4.73 ± 0.02		
Phosphate (g/kg TS)	3.00 ± 0.02	2.85 ± 1.01	2.77 ± 1.00	3.40 ± 0.10	2.88 ± 0.03	1.04 ± 0.01	2.52 ± 1.00	1.20 ± 0.20	1.55 ± 1.01		
Sulphate (g/kg TS)	134 ± 2.00	136.71 ± 9.02	100.00 ± 3.00	111.10 ± 3.01	122.11 ± 4.00	54.00 ± 2.00	86.65 ± 4.00	61.04 ± 1.02	66.14 ± 3.02		
Calcium (g/kg TS)	80.00 ± 0.10	48.81 ± 3.05	523.50 ± 1.42	584.03 ± 5.01	388.88 ± 8.15	333.7 ± 0.22	228.16 ± 7.03	361.30 ± 2.03	211.12 ± 5.01		
Magnesium (g/kg TS)	96.00 ± 0.10	125.11 ± 8.02	47.50 ± 1.02	70.10 ± 1.40	95.82 ± 3.05	35.22 ± 0.02	75.82 ± 2.04	39.40 ± 1.10	75.51 ± 4.01		
Manganese (g/kg TS)	1.18 ± 0.22	0.09 ± 0.01	0.06 ± 0.01	0.020 ± 0.00	0.04 ± 0.01	0.009 ± 0.01	0.04 ± 0.02	0.010 ± 0.10	0.01 ± 0.01		
Iron (g/kg TS)	1.18 ± 0.11	1.77 ± 0.03	1.72 ± 0.01	1.43 ± 0.01	1.55 ± 0.02	0.40 ± 0.01	0.94 ± 0.01	0.46 ± 0.01	0.71 ± 0.01		
Zinc (g/kg TS)	38.00 ± 0.02	56.11 ± 7.01	46.01 ± 2.01	42.04 ± 0.01	46.01 ± 2.01	14.40 ± 0.02	36.01 ± 2.01	18.20 ± 0.01	36.01 ± 2.01		
Aluminium (g/kg TS)	0.80 ± 0.11	0.44 ± 1.02	1.27 ± 0.01	1.43 ± 0.02	0.74 ± 1.02	0.15 ± 0.02	1.04 ± 1.01	0.20 ± 0.10	0.24 ± 1.00		
Copper (g/kg TS)	4.80 ± 0.10	5.67 ± 0.05	3.33 ± 0.11	4.02 ± 0.10	4.71 ± 0.02	2.02 ± 0.10	3.36 ± 0.02	2.31 ± 0.10	3.07 ± 0.02		

N = 120; COD = Chemical Oxygen Demand; TVFAs = Total volatile fatty acids; C/N = Carbon/Nitrogen ratio.

usually caused by different lignin derivatives paramount among which are phenolic lignin, syringyl ring, and acetyl lignin. Phenolic lignin is not easily degraded during digestion (Alfa et al., 2014). The very reason lower volume of gas was produced from the acidic pretreated CPH as shown in this study was the preferential action of the acid causing changes in the lignin structure. However, the action of the alkali was on the entire lignin thereby causing enormous solubilization. For syringyl ring at the  $1329\text{ cm}^{-1}$  band, there was an increase in its composition as a result of alkaline pretreatment over the untreated biomass (Sun et al., 2013; Alfa et al., 2014). For the composition of acetyl lignin at the  $1251\text{ cm}^{-1}$  band, both pretreatment procedures decreased its composition with the alkali after a higher effect with 36.1% (Alfa et al., 2014). On the other hand, the acidic treatment decreased the peak intensity at  $1059\text{ cm}^{-1}$ , an indication of hemicellulose breakdown (Sun et al., 2013). In the result of the analysis, both crystalline and the amorphous cellulose ( $1110\text{ cm}^{-1}/897\text{ cm}^{-1}$ ) were present in the CPH as shown. After pretreatment, the ratio between the two cellulose types revealed 17.2 and 48.2% decrease for acidic and alkaline respectively (Venturin et al., 2018).

Effect analysis with 95% confidence interval was used to evaluate the composition of lignin, cellulose, and hemicellulose after pretreatment as the responses. Results of the analysis showed two of the four variables are significant. These are temperature and time of exposure which leads to the development of mathematical models so as to maximize the cellulose while reducing the lignin and hemicellulose contents in the pretreated CPH. Validation of the three derived models was carried out via analysis of variance (ANOVA) at 99.95% confidence interval. The contour curves of the mathematical models and their respective 3-D plots were constructed to show the lignin, cellulosic and hemicellulosic compositions as shown in Fig. 1. The models were shown to have good statistical abilities evident by their coefficient of determination ( $R^2$ ) values which are 0.9633, 0.9835 and 0.9771 for lignin, cellulose, and hemicellulose respectively.

### 3.3.2. Hydrogen peroxide pretreatment

As seen in this study, the most efficient condition for the alkaline pretreatment of the CPH was: 7.5% (w/v)  $\text{H}_2\text{O}_2$ , orbital shaker temperature of  $30^\circ\text{C}$ , agitation at 130 rpm for 75 min using 3 g dry mass of the ground CPH. With this condition, total lignin content was reduced by 71.34% (from initial 17.8 to  $5.1\text{ m.m}^{-1}$ ), cellulose increased by 39% (from initial 26.6 to  $43.3\text{ m.m}^{-1}$ ) while hemicellulose decreased by 61% (22.8 to  $8.8\text{ m.m}^{-1}$ ). Contrary to the effects of acidic pretreatment on the CPH, use of alkaline hydrogen peroxide caused the higher lignin solubilization while that of hemicellulose was partial with an increase in the composition of cellulose.

The most visible effect of pretreatment was observed in the alkaline pretreated CPH in which enormous breaking of lignin bonds occurred. All the important bands/peaks associated with lignin i.e. 1734, 1716, 1633, 1604 and  $1516\text{ cm}^{-1}$  were completely flattened, ruptured and have even disappeared in some cases after the alkaline pretreatment thus causing enormous solubilization of lignin. This agrees with previous studies on the use of different alkalis ( $\text{H}_2\text{O}_2$ , NaOH, and KOH) in biomass pretreatment prior to anaerobic digestion process (Dahunsi et al., 2016a,b, 2017a).

Though not well documented, pretreatment with hydrogen peroxide has been reported in few studies in which slightly different result was obtained. Cai et al. (2016), used hydrogen peroxide to pretreat corn straw and obtained 19.6, 32.8 and 6.2% reduction in the composition of lignin, cellulose, and hemicellulose respectively. Similarly, Sun et al. (2013) obtained a 38.9% reduction in lignin and 31.4 and 33.3% increase in the composition of cellulose and hemicellulose respectively after pretreating corn straw with hydrogen peroxide.

Alkaline hydrogen peroxide treatment of CPH as seen in this study enhanced the decrease in the lignin and cellulosic ratio by over 75% whereas there was an increase with acidic pretreatment using sulfuric acid. Among the five variables used in the statistical effect evaluation for both lignin and hemicellulose compositions, only three (exposure time, shaker temperature and  $\text{H}_2\text{O}_2$  concentration) were significant on the pretreated biomass with 95% confidence interval. Only one of them

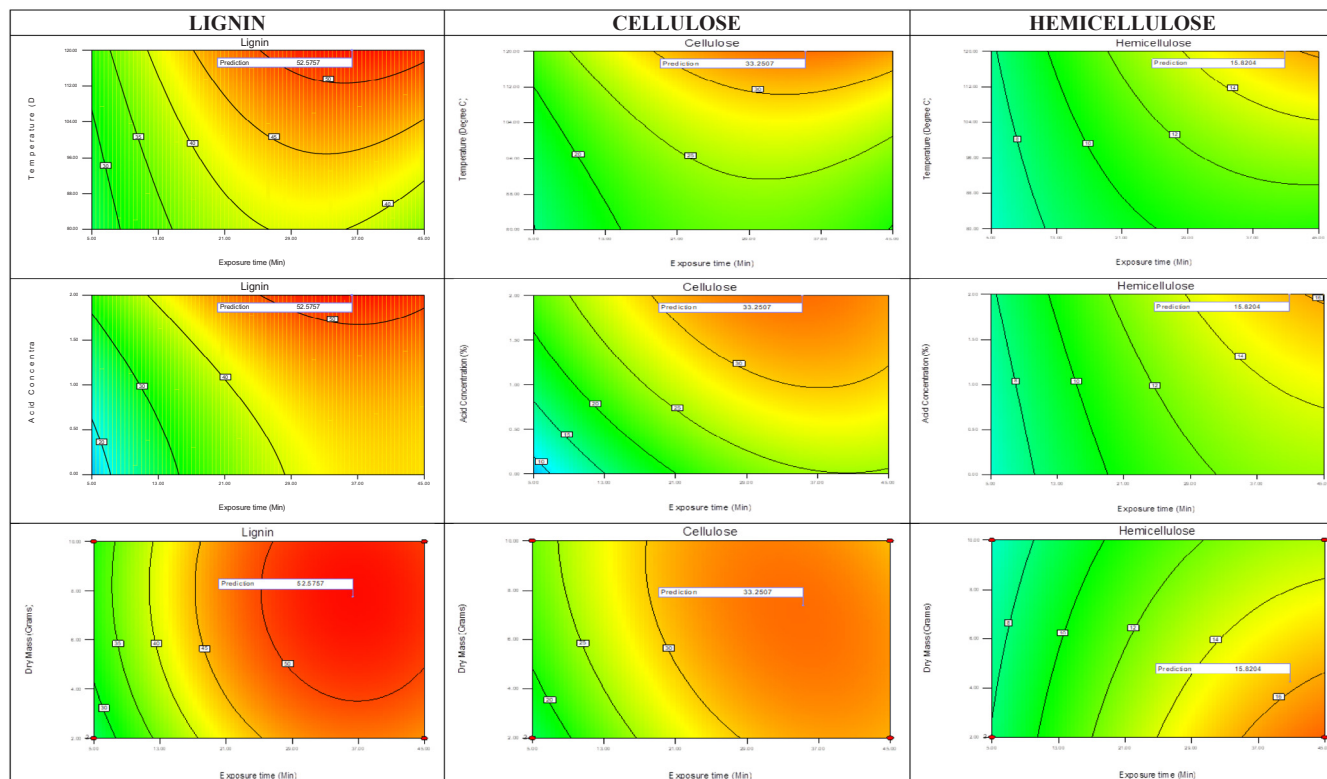


Fig. 1. Contour plots for the optimization of acidic pretreatment for CPH.

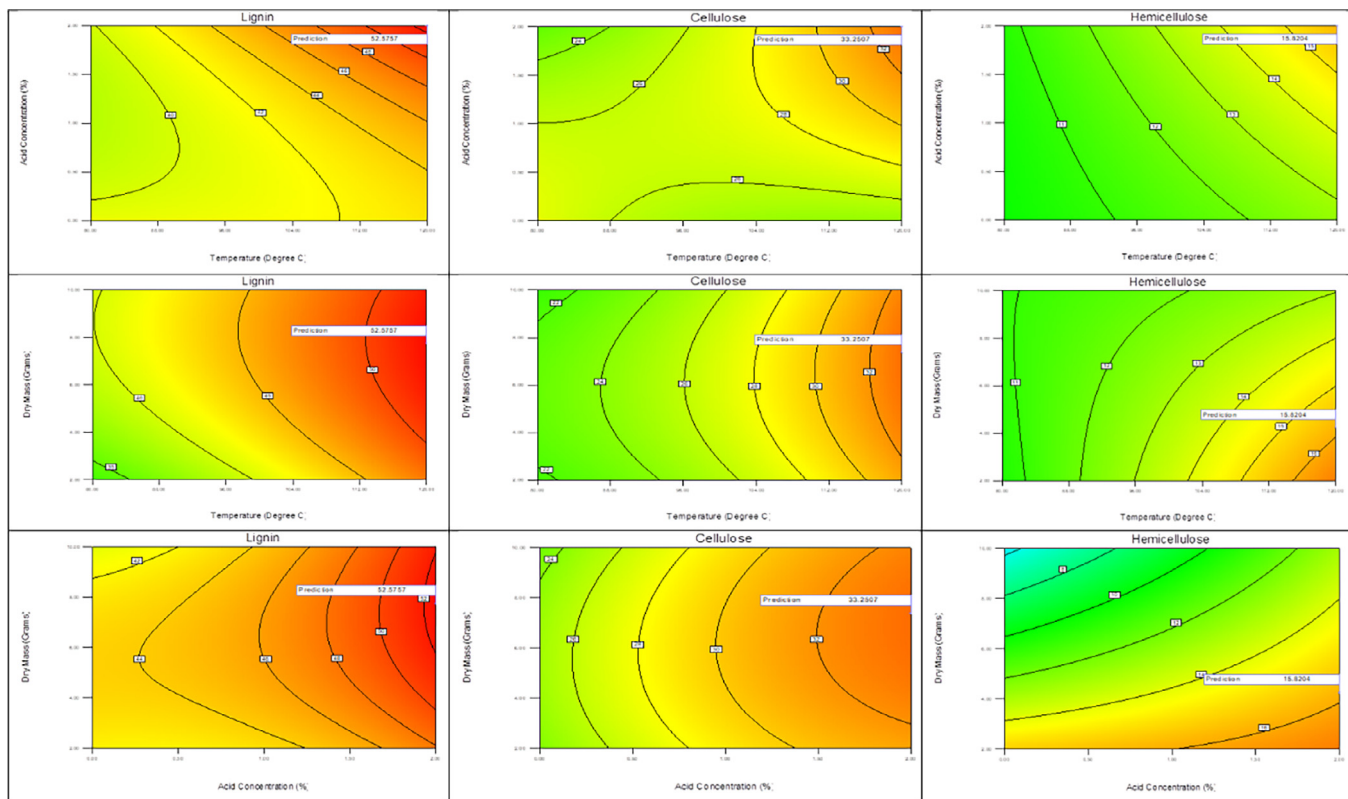


Fig. 1. (continued)

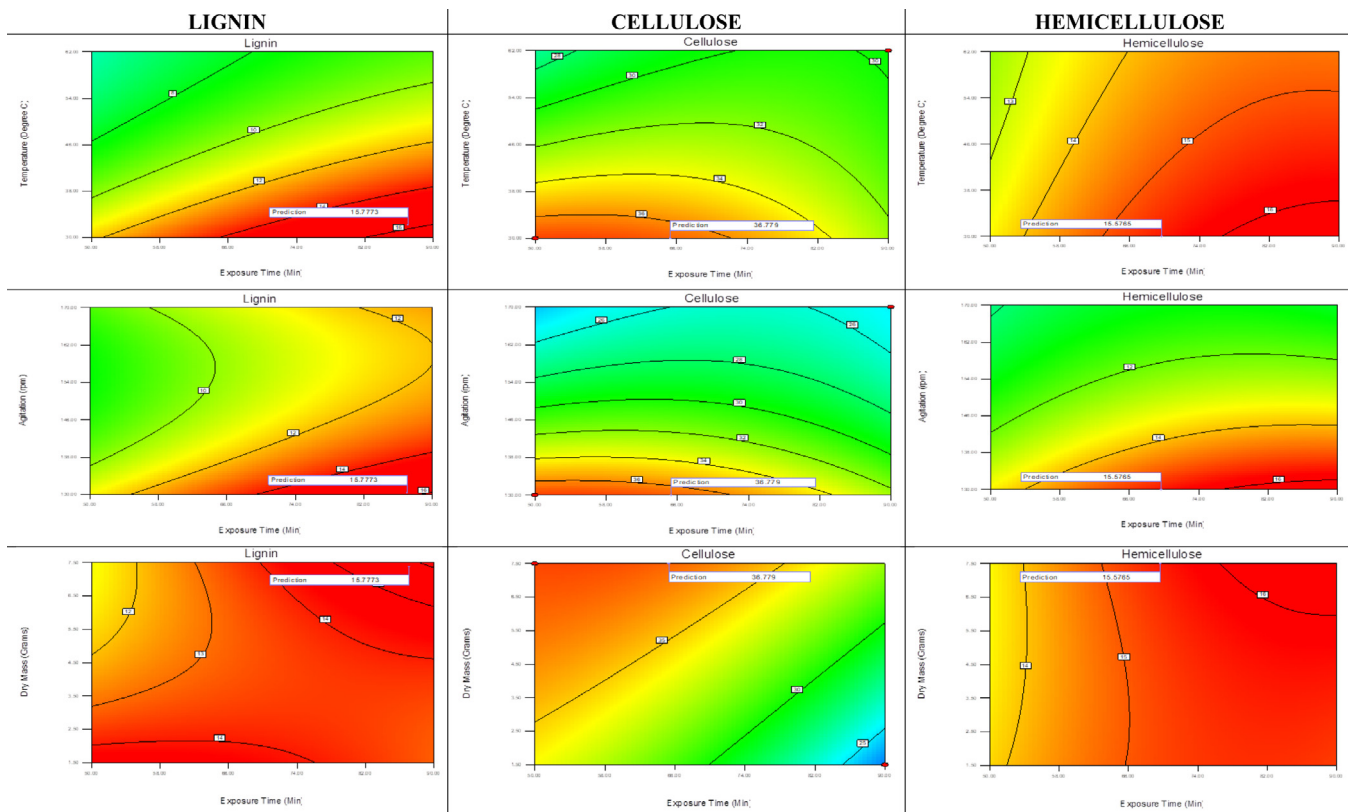


Fig. 2. Contour plots for the optimization of alkaline pretreatment for CPH.

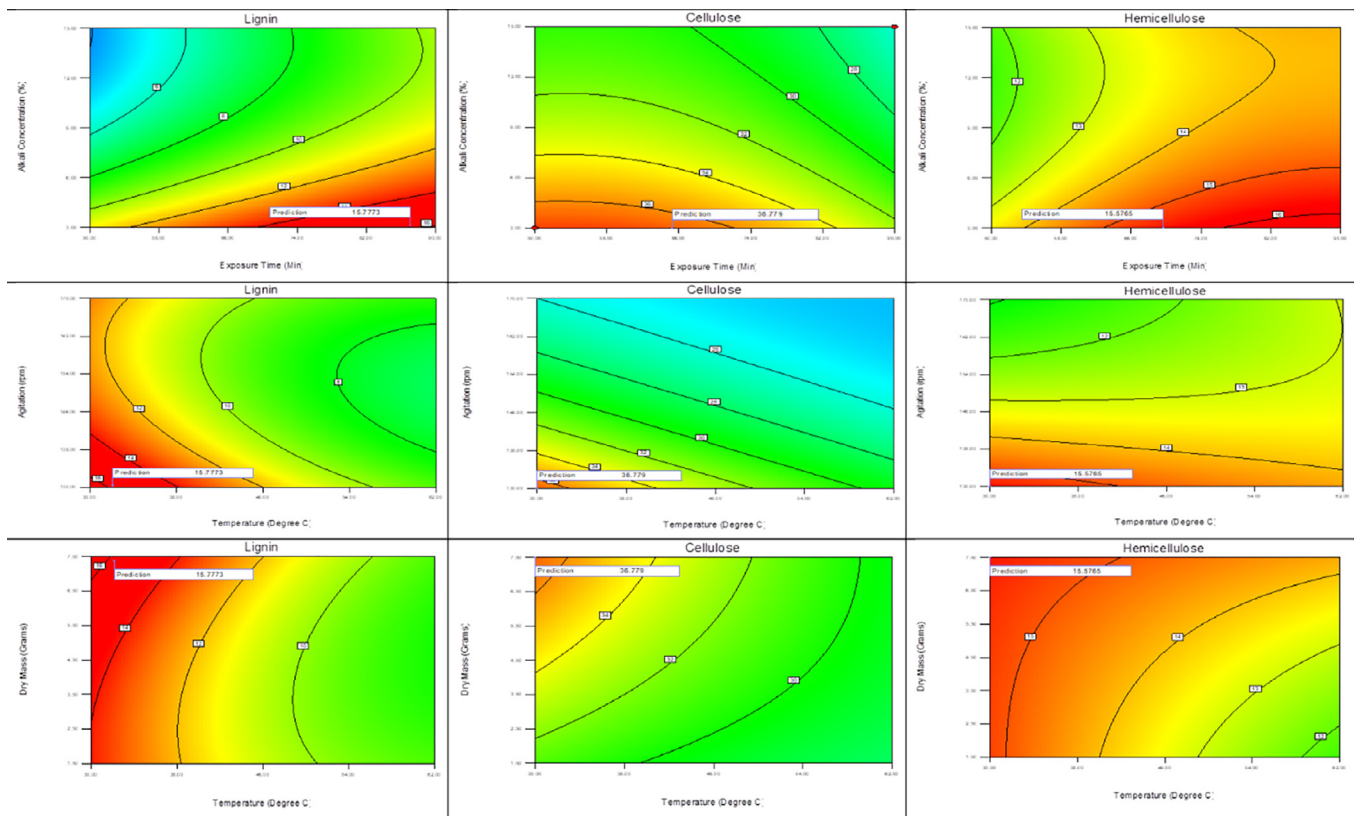


Fig. 2. (continued)

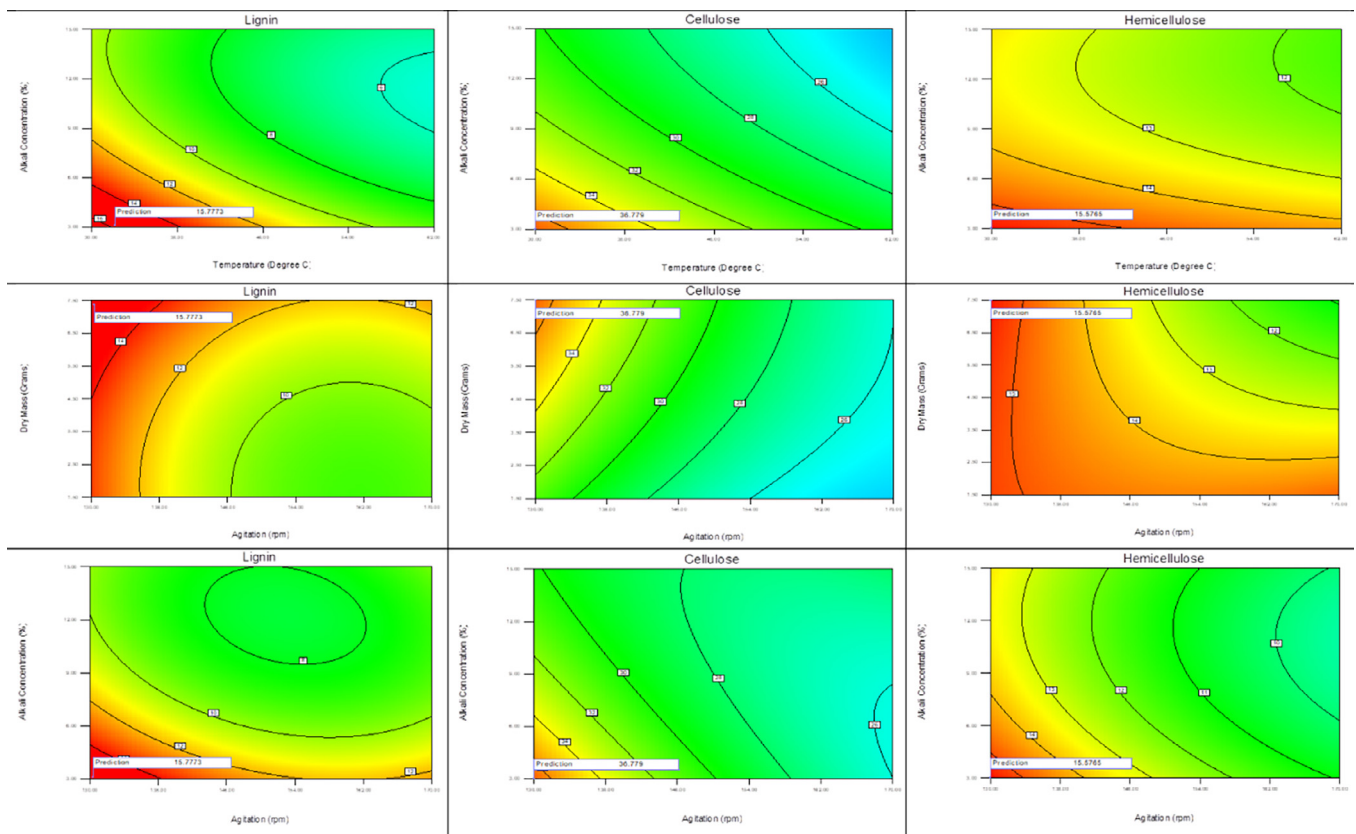


Fig. 2. (continued)



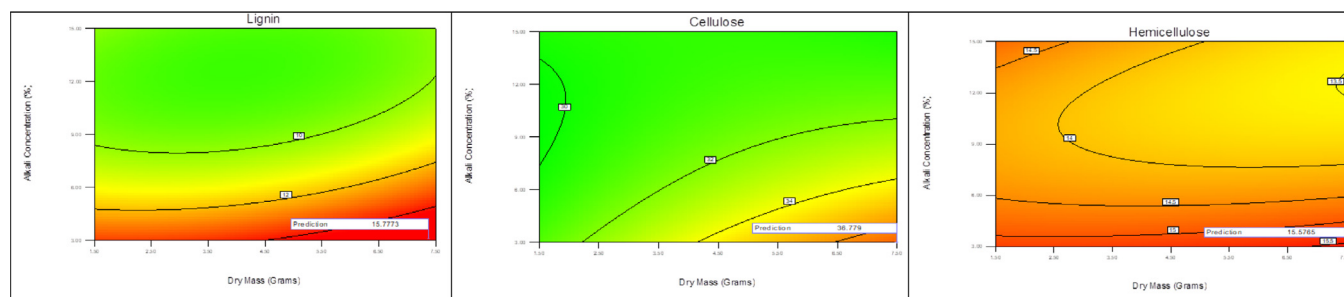


Fig. 2. (continued)

Table 3

Wave lengths that correspond to a given functional group and respond to infrared spectroscopy, and their respective relative values for H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> pretreatments tested for CPH.

Wavelength (cm <sup>-1</sup> )	Assignment	Untreated	H <sub>2</sub> SO <sub>4</sub> Pretreated		H <sub>2</sub> O <sub>2</sub> Pretreated	
		Absorbance/Ratio	Absorbance/Ratio	Variation (%)	Absorbance/Ratio	Variation (%)
3348	O–H stretch (Hydrogen cellulose connections bond)	0.3261	0.2516	22.8	0.4156	–27.4
2900	C–H stretch (Methyl/methylene cellulose group)	0.1134	0.1021	10.0	0.1653	–45.8
1734	Carbonyl bonds (Associated with removal of lignin side chain)	0.1024	0.1004	2.0	0.0192	81.3
1716	Carboxylic acids/ester groups	0.1141	0.0682	40.2	0.0665	41.7
1633	Aromatic ring stretch (Associated with lignin removal)	0.2015	0.0305	84.9	0.1012	49.8
1604	Aromatic ring stretch (Changes in lignin structure)	0.1931	0.1133	41.3	0.1057	45.3
1516	Generic lignin	0.1421	0.1191	16.2	0.0636	55.2
1516/897	Lignin/cellulose ratio	2.9	3.5	–20.7	0.7	75.9
1373	Phenolic O–H stretch (Changes in lignin structure)	0.2222	0.1423	36.0	0.1810	18.5
1319	Syringyl ring stretch (Changes in the lignin monomer)	0.2062	0.1128	45.3	0.1650	20.0
1251	C–O absorption (Result of acetyl-lignin groups cleavage)	0.1419	0.1405	1.0	0.1061	25.2
1110	Crystalline cellulose	0.4504	0.1452	67.8	0.2334	48.2
1059	C–O–C stretch (Cellulose and hemicellulose)	0.3752	0.2755	26.6	0.4720	–25.8
897	Amorphous cellulose	0.1054	0.0429	35.7	0.0678	35.7
1110/897	Crystalline/amorphous cellulose ratio	9.0	6.8	24.4	4.7	47.8
833	C–H flexion of syringyl	0.1208	0.0135	88.8	0.0110	90.9
771	Crystalline cellulose (I $\alpha$ )	0.0188	0.0115	38.8	0.0280	–48.9
719	Crystalline cellulose (I $\beta$ )	0.0344	0.0271	21.2	0.0372	–8.1
771/719	Ratio of crystalline cellulose polymorphs (I $\alpha$ /I $\beta$ )	0.3	0.2	33.3	0.4	–33.3

ND = Not determined; All positive values indicates decrease.

(H<sub>2</sub>O<sub>2</sub> concentration) was however significant at 99.95% in order to maximize the cellulose component (Fig. 2).

### 3.3.3. Assessment of untreated biomass

The comparison was done between both untreated samples of the untreated CPH i.e. sifted and not sifted so as to evaluate the possible effect of sieving on the biomass using the Tukey test. The results revealed that the two samples were statistically insignificant in the composition which means that sieving brought about no significant difference between the samples.

### 3.3.4. Structural changes in the CPH after pretreatments

Table 3 shows the results of structural/molecular changes in the CPH which was caused by the pretreatments as well as those of the untreated biomass. The percentage relative variation was computed using Eq. (2)

$$RV = 100 * (U - P) / U \quad (2)$$

where:

RV = Relative variation (%)

U = Absorbance of untreated CPH

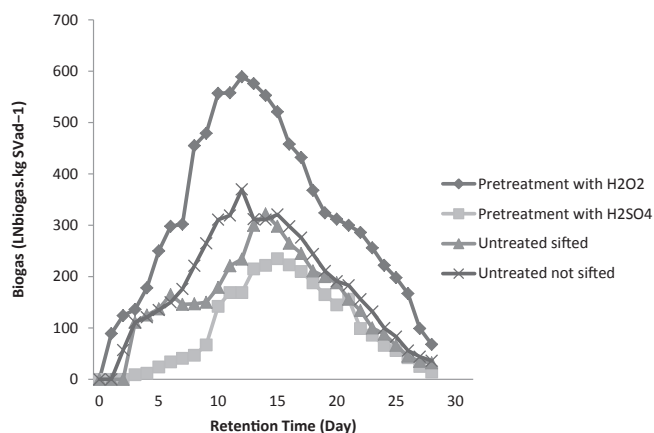
P = Absorbance of pretreated CPH

All the bands revealed by the FTIR spectra were between the 3348 and 2900 cm<sup>-1</sup> indicating the presence of chemical bonds of cellulosic. At the end of hydrogen peroxide treatment, the cellulose content of the pod increased also causing an increase in absorbance to the tune of 20.8 and 52.5% for both pretreated and untreated samples. An increase at the 1373 cm<sup>-1</sup> band was also observed. A different result was obtained

showing reduction of the cellulosic O–H bonds after the acidic pretreatment and this further resulted in absorbance reduction in the 3448 cm<sup>-1</sup> band beside phenolic lignin decrease at the 1373 cm<sup>-1</sup> peak.

### 3.4. Effect of anaerobic digestion on biomass structure

The anaerobic digestion process further conferred more structural breakdown to the CPH in terms of reduction in all three structural component i.e. lignin, cellulose and hemicellulose as well as the fixed solids as evident in the digestates. There were 23, 40, 26 and 13%; 45, 21, 26, 14%; 20, 14, 8 and 15% and 11, 18, 27 and 21% reductions in the composition of lignin, cellulose, hemicellulose and fixed solids respectively after the anaerobic digestions involving the acidic pretreated, alkaline pretreated, not sifted untreated and sifted untreated CPH respectively. Similarly, the concentration of uronic acids was reduced by 33.12, 41.11, 17 and 26% in the four experiments respectively while those of soluble sugars increased by 37.4, 46.7, 22.1 and 26.3% for the four experiments. Most of the chemical parameters in the CPH were also affected by the AD process in which increase in value were recorded for these parameters due to enormous degradation that have taken place as evident in the digestates. Only parameters such as ash content, moisture, carbon and calcium were not affected. There was also significant reduction in COD across all digestions by average values of 48.9, 60.19, 40.23 and 48.3% for the acidic pretreated, alkaline pretreated, not sifted untreated and sifted untreated CPH respectively.



**Fig. 3.** Daily biogas production per kilogram of added volatile solids for H<sub>2</sub>S<sub>2</sub> pretreated CPH + poultry manure, H<sub>2</sub>SO<sub>4</sub> pretreated CPH + poultry manure, untreated sifted CPH + poultry manure and untreated not sifted CPH + poultry manure.

### 3.5. Biogas potential (BP)

As shown in this study, the inoculum produced a minimal quantity of biogas which was less than 10% of total generation from all the pretreated and untreated substrates and was also lower than the potential of the microcrystalline cellulose used as a standard. Production from the latter was higher by over 70% than the value obtained from the reference 650 LNbiogas.kg VSad<sup>-1</sup> produced by following the VDI standards 4630 (2006).

As shown in Fig. 3, biogas generation from the anaerobic co-digestion of the hydrogen peroxide-treated CPH + poultry manure was high. On the other hand, the digestion of the sulfuric acid pretreated biomass + poultry manure produced about 68% LNbiogas.kg VSad<sup>-1</sup> lower biogas volume than the alkaline pretreated CPH + poultry manure. The second highest in terms of gas generation was the untreated not sifted sample + poultry manure which was also followed by the untreated sifted sample + poultry manure while the acidic pretreated sample + poultry manure produced the least quantity of gas. In comparison, the alkaline pretreated CPH + poultry manure produced more biogas (52%) than the untreated samples. Using the ANOVA, there was a significant difference between the values obtained for the two untreated samples and to further confirm this, the post-hoc test was carried out using Tukey's test with F and p values of 0.69 and 0.0005 respectively.

The overall volume of biogas produced from the four digestion setups i.e acidic pretreated + poultry manure, alkaline pretreated + poultry manure, untreated sifted + poultry manure and not sifted sample + poultry manure are 203.6 ± 5.1, 633.2 ± 6.2, 321.1 ± 2.1 and 382.4 ± 2.3 respectively. The highest biogas generation was obtained from the alkaline pretreated CPH + poultry

manure which was achieved 2 days before. The maximum biogas generation rate was calculated using the equation below:

$$\text{Maximum biogas generation rate} ((L_{N\text{biogas}} \cdot \text{kg VS}_{\text{ad}}^{-1}) \cdot \text{d}^{-1}) \\ = 0.05 \text{m}^3 \text{Total biogas} \quad (3)$$

The alkaline hydrogen peroxide-pretreated CPH + poultry manure produced the highest volume of biogas as seen in this study and was followed by the not sifted untreated sample + poultry manure. Fig. 3 shows a 58% more biogas produced by the alkaline pretreated sample over the untreated one indicating the efficiency of treatment with the alkali. This corroborates the findings of Venturin et al. (2018) who reported increased biogas yield after pretreatment with a low concentration of hydrogen peroxide. The need for biomass pretreatment prior to digestion for further justified in the rate values of maximum biogas generation in LNbiogas.kg VSad<sup>-1</sup>.d<sup>-1</sup> and the day the maximum biogas yield was achieved (Mancini et al., 2018). The highest was obtained from the alkaline treated experiment which reached the peak of production in 11 days. The difference in the time of cooking as observed for each commodity is due to the quality of produced biogas in terms of methane content.

In all, gas production commenced on the 2nd experimental day and reached the peak on the 11th day out of the total of 30 day retention time thus indicating that though at a lower retention time as adopted in this study, higher biogas generation was achieved. The composition of the biogas generated across the four different digestions showed methane to be within 63 ± 1.2 and 66 ± 2.1, carbon dioxide of between 20 ± 1.2 and 28 ± 0.2 and hydrogen sulfide of between 4 ± 0.2 and 7.3 ± 0.2.

### 3.6. Microbial composition, volatile fatty acids (VFAs) dynamics, Stoichiometry and mass balance

The molecular method of isolation and identification used in this study helped to properly identified all the major microbial groups present in the inoculum, fermenting substrates and the effluents after digestion. Aerobes of the genera *Bacillus* dominated the aerobic organisms in all samples with members such as *Bacillus pantothenicus*, *Bacillus licheniformis* and *Bacillus stearothermophilus* while other aerobes include *Serratia ficaria* and *Proteus vulgaris*. For the anaerobic group, members of the genera *Clostridium* were the dominant with members including *Clostridium clostridioforme*, *Clostridium histolytica* and *Clostridium species* while others are *Fusobacterium mortiferum* and *Porphyromonas assacharolyticum*. The identified methanogens include members of the genera *Methanosarcinaceae*, *Methanobacteriales*, *Methanosetaceae* and *Methanomicrobiales*.

The diverse microorganisms and their population brought about robust microbial activities in the digesters and one of the effects is VFA's accumulation due to production of several intermediate acids capable of causing inhibition to the digestion process. These VFAs accumulation largely depend on the balance between their production

**Table 4**  
Stoichiometry and mass balance for one ton of CPH.

Parameter	H <sub>2</sub> SO <sub>4</sub> Pretreated Cocoa pod + Inoculum	H <sub>2</sub> O <sub>2</sub> Pretreated Cocoa pod + Inoculum	Untreated Cocoa pod + Inoculum
<i>Input</i>			
Cocoa pod + Inoculum (kg)	1000	1000	1000
Volatile solids (VS) (kg)	859	705	914
<i>Output</i>			
Methane (CH <sub>4</sub> ) (%)	58.5	65.2	58.2
Carbon dioxide (CO <sub>2</sub> ) (%)	23.5	22.6	21.4
Digestate (kg VS)	561	341	535
Sum	643	428.8	614.6
Mass balance*	0.25	0.39	0.33
% Volatile solids (VS) removal	35	52	41

\* Input-output)/input (%).

**Table 5**  
Energy and economic evaluation for the digestion of CPH.

Energy parameters	H <sub>2</sub> O <sub>2</sub> pretreated	H <sub>2</sub> SO <sub>4</sub> pretreated	Not Sifted (Untreated)	Sifted (Untreated)
Produced electrical and heat energy from combined heat and power (CHP)	3315	1011	1298	1203
Produced heat energy (kWh t <sup>-1</sup> TS)	2236	877	1013	910
Produced electrical energy (kWh t <sup>-1</sup> TS)	1079	534	685	676
<i>Heat balance</i>				
Heat energy gain (kWh t <sup>-1</sup> TS) <sup>*</sup>	1223	-136	-	-
Heat energy requirement (kWh t <sup>-1</sup> TS)	1055	1436	-	-
Heat energy requirement with 80% of heat recovery (kWh t <sup>-1</sup> TS)	211	287	-	-
Net heat energy (kWh t <sup>-1</sup> TS) <sup>#</sup>	168	-1300	-	-
Net heat energy with 80% of heat recovery (kWh t <sup>-1</sup> TS)	134	-1040	-	-
<i>Electrical balance</i>				
Electrical energy gain <sup>§</sup>	394	-252	-	-
Energy for mixing during pretreatment	218	-554	-	-
Net electrical energy	176	-806	-	-
<i>Economic evaluation</i>				
Cost of H <sub>2</sub> O <sub>2</sub> and H <sub>2</sub> SO <sub>4</sub> (€ t <sup>-1</sup> TS)				

\* Difference of heat energies produced by the pretreated experiment minus the untreated.

# Difference between the heat energy gain and the heat energy required for the pretreatment.

§ Difference of electricity energy produced by pretreated experiment minus the untreated.

and consumption by the digester's bacterial community. Two prominent VFAs were implicated in this study i.e. acetate and propionate whose accumulation was very minimal from the commencement of the process through to the middle between the 13th 15th days when their concentrations were at the peak. This shows a gross imbalance between the hydrolysis-acidogenesis and the acetogenesis-methanogenesis combined stages of the digestions. This agrees with previous submissions. The high population of members of the genera *Clostridia* which are facultative anaerobes brought about pronounced acetogenesis and methanogenesis stages in the digesters. This group of organism breaks down amino-acids into acetic and propionic acids with ammonia as the end-product.

In order to evaluate the volatile solids consumption/removal in all the experiments, the mass balance was evaluated as shown in Table 4. These showed a high consumption of volatile solids as a result of the high microbial population and diversity which was more pronounced in the alkaline pretreated biomass + poultry manure.

### 3.7. Results of the cooking test

In the cooking test carried out to confirm the flammability/combustibility of the produced biogas, the gas produced from the alkaline pretreated sample showed higher combustibility/cooking rate than the one produced from the acidic pretreated biomass. The gas from the alkaline pretreated sample boiled both water and rice at 0.08 L/min and 0.0039 kg/min respectively whereas the gas from the acidic pretreated biomass boiled the same commodities at lower cooking rates i.e. 0.14 L/min and 0.0045 kg/min respectively. These values were also higher than those obtained when cooking was done with the biogas from the untreated samples which recorded cooking rates of 0.16 L/min and 0.0049 kg/min and 0.18 L/min and 0.0051 kg/min respectively. Considering the design of the used biogas stove, biogas flow rates of 0.0057 m<sup>3</sup>/min was obtained.

### 3.8. The composition of digestate after digestion

Analyses of the digestates from all the four digestions regimes showed that the materials were rich in nutrients, elemental compositions, and microbial biomass. Of importance is the increase in the values of all major and minor elements over their initial concentration prior to anaerobic digestion. In terms of structural composition, there was an increase in the lignin to cellulose-hemicellulose complex ratio with values of 0.4–0.7, 0.2–1.4, 0.3–1.0 and 0.3–1.0 for the acidic

pretreated, alkaline pretreated, untreated sifted and untreated not sifted CPH respectively. The sulfuric acid pretreated sample had the lowest lignin to cellulose-hemicellulose complex ratio and equally produced the least volume of biogas.

As also seen in this study, the anaerobic co-digestion process had profound effects on the biodegradability of all the substrates used as further solubilization of the components of the pretreated CPH was caused by the anaerobic digestion process. The value of most elements was seen to have increased after the digestion except for carbon and calcium which must have taken up by the digester's microflora and used for metabolism and cell wall synthesis. This phenomenon is similar to the results of some previous studies on the effect of fermentation on substrates digestibility (Dahunsi et al., 2016a,b, 2017c,d,e, 2018a,b,c, 2019).

Analysis of the digestates from all experiments showed elevated levels of all major and minor elements and richness in the nutrient. This further shows that such digestate could be reused as biofertilizer to enhance soil fertility improvement and crop yield. However, there was inefficiency in the utilization of the bulk carbohydrate content in the digesters. This further corroborates the submission of Croce et al. (2016).

### 3.9. Energy and economic balance

The computation of the energy balance for this study was done using standard methods with the combined heat and power (CHP) system (Dahunsi et al., 2016a,b, 2017c,d,e) as shown in Table 5 while heat loss was neglected (Dahunsi et al., 2016a,b, 2017c,d). For the alkaline treated experiments, the 1223 kWh t<sup>-1</sup> TS heat energy gain exceeded the 1055 kWh t<sup>-1</sup> TS used in the pretreatment thus giving a net heat energy of 168 kWh t<sup>-1</sup> TS. The possibility is there that this net energy can be increased via the use of heat exchanger during the pretreatment. Heat exchangers have been previously employed to increase the recovery of thermal energy up to about 80% (Dahunsi et al., 2017e). Perhaps, full integration of heat energy is another prominent method for assessing the economic feasibility in this study as earlier reported (Dahunsi et al., 2016a,b, 2017d,e). For the acidic pretreated sample of CPH, the investment into acid purchase, equipment usage and time seemed fruitless because the -136 kWh t<sup>-1</sup> TS heat energy gain was far below the 1436 kWh t<sup>-1</sup> TS heat energy used in pretreatment with a net heat energy of -1300 kWh t<sup>-1</sup> TS.

In electrical energy assessment, an account was only taken for the energy consumed during substrate mixing while neglecting that used

during mechanical grinding since the same grinding was done for all experiments following earlier submissions (Dahunsi et al., 2017a,b,c,d). For the alkaline treated experiment, the net electrical energy of 394 kWh t<sup>-1</sup> TS far outweighs the -252 kWh t<sup>-1</sup> TS obtained from the acidic pretreated CPH. This further confirms that alkaline pretreatment is profitable as against the use of acids which on the other hand will lead to loss of time, energy and other investments. The net heat and electrical energies obtained can be sold directly to consumers or injected into the energy grid adhering to existing environmental and governmental regulations.

#### 4. Conclusions

Use of alkaline hydrogen peroxide as a pretreatment agent in this study caused high solubilization of the lignin component of the CPH and reduced up to 81% of lignin. Similarly, the alkali reduced the hemicellulose content of the pod by approximately 69%. A major achievement in this study is the upsurge in biogas volume from the alkaline pretreated CPH which was 68% higher than the acidic pretreated biomass and 40% more than the untreated biomass. Further usage of hydrogen peroxide for biomass pretreatment is therefore advocated in the pretreatment of CPH prior to anaerobic digestion.

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#### Conflict of interest

The author declares no conflict of interest whatsoever

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2019.03.093>.

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