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Research paper

Biogas generation from *Sorghum bicolor* stalk: Effect of pretreatment methods and economic feasibility

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ABSTRACT

In this study, biogas was produced from the anaerobic digestion of *Sorghum bicolor* stalk. Pretreatment of the biomass was carried out prior to the digestion using sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). The physicochemical, elemental and structural analyses were carried out on the biomass 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 were used to quantify the structural changes that took place after pretreatments. Results showed enormous reduction of hemicellulose and partial solubilization of cellulose with the application of H_2SO_4 for pretreatment with obvious breakdown of all important bonds in the biomass. The most suitable condition for the most efficient acidic pretreatment of the *Sorghum bicolor* stalk was using H_2SO_4 concentration of 0.75% (v.v^{-1}), autoclave temperature of 118 ° C and biomass dry mass of 3.7 g for 52 min. However, the use of H_2O_2 caused huge solubilization of lignin while partial dissolution of hemicellulose took place. The most suitable condition that gave the best result in this pretreatment procedure was H_2O_2 concentration of 6.8% (v.v $^{-1}$), shaker temperature of 28 ° C, agitation at 126 rpm and 3 g of biomass for 85 min. Overall, the use of the H_2O_2 showed reduction of lignin and hemicellulose by 73 and 42% respectively while also increasing the concentration of cellulose by 23%. The acid and alkaline pretreated biomass produced a total of 312.3 and 607.1 LNbiogas.kg VSad $^{-1}$respectively. In comparison, the biomass pretreated with H_2O_2 produced 65% more LNbiogas.kg VSad^{-1} than the other and equally reduced the production time by 5 days. For the alkaline treated biomass, the 1422 kWh t^{-1} TS thermal energy gain exceeded the 945 kWh t^{-1} TS used in the pretreatment thus giving a net thermal energy of 477 kWh t^{-1} TS. However, the acidic pretreatment of *Sorghum bicolor* stalk is not profitable because the -131 kWh t^{-1} TS thermal energy gain was far below the 1025 kWh t^{-1} TS thermal energy used in pretreatment with a net thermal energy of -761 kWh t^{-1} TS. Till now, use of low-cost H_2O_2 for biomass pretreatment is unpopular while the uses of other strong alkali and acids are well studies. However, hydrogen peroxide gave better product yield. Therefore, use of this alkali pose a novel biotechnological means for generating biogas.

1. Introduction

Environmental management and adequate utilization of resources have over the last decades gained a lot of attention in research and developmental issues the world over. Also, the generation and use of renewable energies from available resources is a major concern to stakeholders in the energy industry (Chen et al., 2017). This is more important as the need arose to find alternatives to fuels from fossil origin which are not renewable and whose usage comes with attendant problems of environmental degradation, climate change, global warming among others (Dahunsi et al., 2017a,b). One of such renewable energy is biogas which is usually obtained from the microbial degradation of different biomass in an anaerobic system (Alfa et al., 2014; Lizasoain et al., 2017) whose final effluent or digestates can also be used as biofertilizers due to its richness in nutrients.

The aforementioned environmental challenges have spurred researches in the field of energy and sustainability to search for new, novel and sustainable energy resources which are potent to impact on the development of the society as well as providing economic stability (Mirmohamadsadeghi et al., 2016). In order

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to achieve this, the use of lignocelluloses for the production of clean energy has been proposed in many researches especially in the last decades (Dahunsi et al., 2018b,c). These materials are usually obtained from four major sources: (i) agricultural wastes/residues e.g. corn stover, rice straw, sorghum stalk etc. (ii) forest waste/residues e.g. leaf biomass, woods and chippings, branches, foliage etc.), (iii) energy crops (sunflower, giant cane, switch grass, yellow poplar etc.), (iv) cellulosic materials e.g. food wastes, grasses, pulp and paper residues, organic fraction of municipal solid wastes etc. They are usually abundant in many localities globally and are cost-effective in usage besides having huge potentials for bioconversion into biofuels and other high value chemicals (Xue et al., 2016).

However, most lignocellulosic biomass end up as wastes or pollutants in the environments, burnt off directly and their inherent values lost (Gómez et al., 2014). This is majorly due to the composition of these biomass in terms of polysaccharide celluloses, hemicelluloses, phenolaldehyde polymer lignin, and a host of soluble polar and non-polar substances and this complexity has seriously hindered the success of the conversion of most lignocelluloses to energy considering that it is cost ineffective (Zhang et al., 2016). In order to overcome this phenomenon, the application of pretreatments to these lignocelluloses is imperative (Dahunsi et al., 2017a,b). It should however be noted that the choice of a pretreatment method should be dependent on the different compositions of each lignocellulose so as to achieve effectiveness of usage and cost reductions (Dahunsi et al., 2017c). Currently, most pretreatment approaches focus on the identification, evaluation, development and demonstration of the potential for enzymatic hydrolysis which will shorten the bioconversion period in the long run besides the reduction in enzyme consumption (Kim et al., 2016).

Sorghum bicolor is a major cereal crop cultivated globally and occupies the fifth position among the world's most known cereals (Ayoola et al., 2012). It is characterized by ability to withstand drought and grows in most agricultural systems of the tropics and temperate regions (Nikzad et al., 2017). Virtually all the parts of *Sorghum* are useful including the grains, stems and leaves (Romli et al., 2015). The stalk of this crop fits into the category of lignocelluloses due to its high carbohydrates content and has over the years been used as a potential feedstock for ethanol production but with limitations because of the high lignocellulosic content of the stalk (Theuretzbacher et al., 2013). The high sugar content of this stalk if properly harnessed could serve as suitable feedstock for the hydrolysis stage of anaerobic digestion thereby giving high yield of the final product i.e. biogas. There is therefore the need to assess the potential of this abundant biomass in anaerobic digestion for the purpose of biogas and digestate biofertilizer production. However, appropriate pretreatment procedures must be employed in order to make it biodegradable for anaerobic microorganisms (Ang et al., 2012). This study is therefore aimed at the generation of biogas from the anaerobic mono-digestion of *Sorghum bicolor* stalk through the application of two pretreatments methods i.e. use of sulfuric acid and alkaline hydrogen peroxide and to study their responses in terms of biomass deconstruction and biogas yield. The study also evaluated the economic viability of pretreatment application in order to document the most appropriate and economically feasible pretreatment method for *Sorghum bicolor* stalk and this will also serve as a baseline for subsequent studies on the anaerobic digestion of the biomass.

2. Material and methods

2.1. Collection of raw material

Dried stalk of *Sorghum bicolor* was sourced after harvesting of the grains from a domestic farm within the Landmark University, Omu-Aran, Kwara State, Nigeria. The biomass was further dried to constant weight and was thereafter ground using a knife mill (SOLAB, SL-31, Brazil). After grinding, sieving of the ground biomass was carried out using a set of Tyler test sieves with mesh size of 200–4. This was done so as to determine all the particle sizes in the biomass and to determine the most abundant and appropriate for the pretreatment procedures. After this, all the samples kept in tightly-sealed polythene nylon in order to avoid absorption of moisture and were then refrigerated at 4 °C prior to further use.

2.2. Inoculum preparation

The inoculum used in this study was obtained from the digestate of a running mesophilic biogas digester within the campus. After collection, it was immediately transported to the Microbiology laboratory and carefully sorted in order to get rid of particles which exceed 10 mm in size and was thereafter incubated for 10 days at mesophilic temperature so as to remove background methane production. Analysis of the inoculum showed the physicochemical constituent to be: pH of 7.98, total solids of 21.9 ± 0.2 g/L, volatile solids of 14.2 ± 0.1 g/L, total Kjeldahl Nitrogen of 4.4 ± 0.1 g-TKN/L, ammonium nitrogen of 3.3 ± 0.1 g NH_4^+ -N/L, and total volatile fatty acids of 0.8 ± 0.1 g/L.

2.3. Experimental design of pretreatments

Among the several tools for experimental designs, the Response Surface Methodology (RSM) has been proved to be reliable and efficient in the design of bioprocessing experiments. In this study, the Central Composite Design was used for the designs of two different pretreatments i.e. acidic (using sulfuric acid (H_2SO_4) in humid steam using an autoclave) and alkaline (using hydrogen peroxide (H_2O_2) in an orbital shaker) respectively. The input variables used in the acidic pretreatment were: Exposure time (Min), Temperature (°C), H_2SO_4 concentration (%) and Dry mass (g). in the alkaline pretreatment, the same variables were employed except that the Agitation (rpm) was also evaluated having carried out the experiment in an orbital shaker. In all, the evaluated response was the percentage composition of structural components (lignin, cellulose and hemicellulose in $m.m^{-1}$) after the pretreatments according to the method of Venturin et al. (2018).

2.4. Pretreatments

In the design of the acidic pretreatment, the values used for each of the parameters is exposure time of 5, 15, 25, 35 and 45 min, autoclave temperature of 80, 90, 100, 110 and 120 °C, H_2SO_4 concentration of 0, 0.5, 1, 1.5 and 2% ($v.v^{-1}$), and dry mass of 2, 4, 6, 8 and 10 g as a modification to earlier protocols (Li et al., 2016). For the alkaline pretreatment, the values employed for the variables is: exposure time of 50, 60, 70, 80 and 90 min, orbital shaker temperature of 30, 38, 46, 54 and 62 °C, agitation/rotation of 130, 140, 150, 160 and 170 rpm, dry mass of 1.5, 3, 4.5, 6 and 7.5 g and H_2O_2 concentration of 3, 6, 9, 12 and 15% ($v.v^{-1}$). These were done in modification to the earlier methods in determining H_2O_2 concentration with the addition of an antifoaming agent (Biocane FC 500, Brazil) (Dahunsi et al., 2019b).

2.5. Assessment of economic viability of pretreatments

The use of low-cost hydrogen peroxide for biomass pretreatment is relatively new in the bioenergy literature. As such, there is need to validate the economic viability of using this pretreatment method in order to justify the investment into the procurement of acid and alkali as well as the cost of obtaining energy for pretreatment as seen in this study. This study compared

the cost of obtaining thermal energy and chemicals with the additional energy that will be obtained from the surplus biogas due to pretreatment application and this helped to ascertain the balance between generation and utilization of energy from the anaerobic digestion of *Sorghum bicolor* stalk. The thermal energy requirement (TER) for the pretreatment of *Sorghum bicolor* stalk was determined using Eq. (1) below:

$$TER = \frac{m \times Sh * (Tfinal - Tinitial)}{3600} \quad (1)$$

where:
m = mass substrate (1000 kg);
Sh = specific heat of water (4.18 kJ kg^{-1} $°C^{-1}$)
T = Temperature (°C)

2.6. Proximate characterization of biomass

The standard method for analysis of water and wastewaters (A.P.H.A, 2012) was used in determining the concentrations of total and volatile solids in all samples of *Sorghum bicolor* stalk (acidic pretreated, alkaline pretreated, untreated sifted and the untreated not sifted). For the measurement of pH, 1 g of each sample was dissolved in 20 cm^3 of water and was incubated at 25 °C for a period of 30 min and the supernatant was used for pH measurement using the HI 2211 pH/ORP Meter electrode (Hanna Instruments, Germany). A TOC analyzer (SSM-5000A Shimadzu, Japan) was employed in the determination of total organic carbon (TOC) content of all samples while the total Kjeldahl nitrogen (TN) was determined by using a standard Kjeldahl method. Simple phenolic compounds were determined using an ultra-high performance liquid chromatography (Nexera XR, Shimadzu, Japan) as described by Panjičko et al. (2017). Concentrations of volatile fatty acids (VFAs) were determined using standard method earlier described (Planinić et al., 2016).

2.7. Structural characterization of biomass

The structural composition of all pretreated and untreated samples of *Sorghum bicolor* stalk was determined. In doing this, the content of major structural components such as total lignin, cellulose and hemicellulose were determined using a standard method (Sluiter et al., 2012). Similarly, the extractable materials in the different samples of the biomass were determined via extraction in a Soxhlet extractor for 6 h whereas; samples were burnt in a muffle furnace in order to determine the concentration of fixed solids following the method of Sluiter et al. (2012).

In determining the contents of the structural materials, 0.3 g of the dried *Sorghum bicolor* stalk was treated with 3 mL of 72% sulfuric acid ($v.v^{-1}$) in a thermostatic bath at a temperature of 30 °C for 1 h and the resulting filtrate was used for the determination of carbohydrate (Sluiter et al., 2008). The liquid chromatography coupled with mass spectrometer (LC–MS) (SHIMADZU, Japan) operated with AMINEX® BIORAD HPX87H column in refractive index detector (DIR-10A) was used to analyze carbohydrates such as glucose, xylose, cellobiose, arabinose and acetic acid. The mobile phase in this analysis was 0.005 $mol.L^{-1}$ sulfuric acid in an isocratic mode, at 45 °C, with an injection volume of 20 μL and flow of 0.6 $mL.min^{-1}$. Calibration curves were used to finally determine the concentrations of the compounds using specific LC–MS standards (Bazoti et al., 2017).

For the concentration of furfural and hydroxymethylfurfural, the same LC–MS described above was used but in this case was equipped with a diode array detector (DAD) and operated with C18 column. In this, the mobile phase was 1:8 acetonitrile/water to which 1% acetic acid was added, and the experiment carried out at an oven temperature of 30 °C in an isocratic mode with an injection volume of 20 μL and flow of 0.8 $mL.min^{-1}$. Calibration curves were subsequently used to determine the concentration of the compounds. All samples were analyzed in triplicates. In order to further determine the type and extent of chemical changes that have taken place in *Sorghum bicolor* stalk after pretreatments, the Fourier Transform Infra-Red spectrometer (FTIR) (IRTracer-100, SHIMADZU, Japan) of both pretreated and untreated biomass were obtained (Zhao et al., 2016).

2.8. Digestion experiment and biogas biochemical potential

Based on the best responses from the experimental designs, the anaerobic mono-digestion of the acidic pretreated, alkaline pretreated, untreated sifted and the untreated not sifted *Sorghum bicolor* stalk was carried out. The Computer controlled anaerobic pilot digesters were used and seeded appropriately with the inoculum already described (Steinmetz et al., 2016). Similarly, the biochemical biogas potential (BBP) was carried out based on the best responses from the pretreatments of the biomass. The BBP tests were carried out using 250 mL batch reactors attached to 500 mL eudiometer tubes in triplicates for each sample with 10% ($m.v^{-1}$) volatile solids at mesophilic temperature i.e. 37 °C as following the VDI standards 4630 (2006). Record of biogas production was taken until stability was achieved when the daily biogas yield was $\leq$ 1% of the total biogas production in the experiment.

2.9. Measurement of biogas production

Measurement of the produced biogas volume was carried out on daily basis. The composition of methane CH_4, carbon dioxide CO_2, nitrogen N_2, and H_2 were determined using a SP-2100A gas chromatograph (GC) (Beifenruili Analytical Instrument Co., Ltd, Beijing, China) coupled with a molecular sieve packed stainless-steel column (TDX-01, length × diameter of 2.0 m × 3.0 mm) and a thermal conductivity detector. The temperature of the detector was set at 100 °C while those of the injector and column were set at 50 °C each and 1.0 mL of sample was injected to the column for analysis. Prior to the analysis, calibration of the GC was done using a standard gas with the composition of 54.14, 33.25, 4.88 and 9.99% (v/v) for CH_4, CO_2, N_2 and H_2 respectively.

2.10. Bacteria identification

Analyses of the microbial communities in the inoculum, fermenting substrates and all the digestates were carried out using the 16S rRNA molecular method. In doing this, 1 mL of each sample was collected and kept at −20 °C prior analysis. The DNA extraction from these samples was done with the aid of a Fast DNA SPIN kit for soil analysis. After this, the quantity and integrity of the extracted DNA were checked using an Infinite 200 PRO NanoQuant spectrophotometry (Tecan Group Ltd., Männedorf, Switzerland). The V4–V5 regions of the 16S rRNA genes with over 30 amplification cycles were applied at an annealing temperature of 65 °C) were amplified using the primer pairs: 515-532U and 909-928U and their respective linkers. These pair of primer were used because they target both bacterial and archaeal 16S rRNA genes and is able to capture most of their diversity as earlier reported (Muyzer et al., 1993; Vilchez-Vargas et al., 2013). A total volume of 50 mL PCR mixture containing: 0.5 units of Pfu Turbo DNA polymerase (Stratagene), the corresponding buffer, each deoxynucleotide at 200 mM, each primer at 0.5 mM and 10 ng of genomic DNA. This was used for the PCR procedure using an Eppendorf Mastercycler thermal cycler following the cycles: after 94 °C for 2 min, 35 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 10 min. The resulting products were purified and analyzed using the Illumina MiSeq cartridge (v3 chemistry) for sequencing of paired 300 bp (Boon et al., 2002).

2.11. Statistical analysis

A central composite design (CCD) was used to evaluate the different pretreatments through statistical planning. Afterwards, the software STATISTICA V. 12 (Statsoft, Tulsa, USA) was used for the statistical analysis of the different responses obtained during the study at a 95% ($p < 0.05$) confidence level while the analysis of variance (ANOVA) followed by Tukey's test was used for comparison of mean values.

3. Results and discussion

3.1. Characteristics of untreated biomass

From the grinding of the dried sample of *Sorghum bicolor* stalk, the most abundant particle size was 0.614 mm which constitutes 60.4 ± 2.1% of the total sample while other fractions include 0.863, 0.427, 0.335, 0.218 and 0.111 mm with corresponding 12.3 ± 1.2, 10.2 ± 1.0, 11.6 ± 0.2 5.5 ± 0.4% respectively. Being the most abundant sieved fraction, the 0.614 mm was used for all pretreatment studies while others were discarded. From the analysis carried out as shown in Table 1, the concentration of total lignin, cellulose, hemicellulose and fixed solids were 33.5 ± 0.03, 29.9 ± 0.10, 30.0 ± 0.11 and 4.2 ± 1.01 respectively for the untreated not sifted sample of *Sorghum bicolor* stalk while for the values of 22.3 ± 0.01, 26.7 ± 0.10, 26.2 ± 0.21 and 3.4 ± 0.01 for the untreated sifted sample respectively. These values agree much with previous findings by Su et al. (2006).

However, other researchers obtained different values for the structural components such as Cai et al. (2016), who reported values of the values of 21.4, 43.4 and 19.5% for lignin, cellulose and hemicellulose for the composition of all parts of corn stalk. In another study by Li et al. (2016), the structural composition of different parts of corn stalk was carried out and the composition was 20, 34 and 24% for lignin, cellulose and hemicellulose respectively. Judging by these results, there is no statistical significant difference at $p > 0.05$ between the two untreated samples of *Sorghum bicolor* stalk i.e. the sifted and not sifted.

3.2. Elemental composition of sorghum bicolor stalk

The *Sorghum bicolor* stalk used in this study is composed of moderate nutrients and minerals elements usually required for efficiency of a microbial fermentation medium which includes the anaerobic digestion process. Nutrient elements such as carbon, nitrogen, phosphorus, potassium, calcium, magnesium and sugars are all present in the stalk at appreciable concentrations (Table 2). This composition is similar to the report of previous researches (Li et al., 2010; Song et al., 2014; Fernández-Cegrí et al., 2013).

3.3. Pretreated biomass characterization

3.3.1. Acidic pretreatment with diluted sulfuric acid

There was enormous reduction in the concentration of hemicellulose in all experimental conditions carried with the application of sulfuric acid for pretreatment. There was obvious breakdown of all important bonds which includes the covalent and hydrogen bonds as well as Van der Waals in the biomass which aided the hemicellulose solubilization and partial solubilization of cellulose which correlated to an earlier finding (Baadhe et al., 2014a). The possibility is high that the addition of the acid hydrolyzed the xylose component of the *Sorghum bicolor* stalk to monosaccharides which further account for the high depolymerization of the hemicellulose as seen in this study. In a previous finding, Song and Zhang (2015) reported the enormous loss of hemicellulose and partial cellulose reduction from corn stalk after the application of acid pretreatment. Similarly, Sun et al. (2015) reported huge hemicellulose dissolution by using sulfuric acid to pretreat sunflower oil residue.

By using effect analysis at 95% confidence interval for the percentage composition of lignin, cellulose and hemicellulose after acid pretreatment, only two of the variable i.e. temperature and sulfuric acid concentration were significant for altering the composition of lignin and cellulose in the biomass after the pretreatment process. Usually, lignin and hemicellulose are the two structural components posing the greatest interference to the effective dissolution of lignocelluloses. Judging by the result of the experimental design in this study, a mathematical model can be deduced to maximize the concentration of cellulose while at the same time reduce those of lignin and hemicellulose from a biomass.

The most suitable condition for the most efficient acidic pretreatment of the *Sorghum bicolor* stalk was using sulfuric acid concentration of 0.75% ($v.v^{-1}$), autoclave temperature of 118 °C and biomass dry mass of 3.7 g for 52 min (Table 3a). This means that the cellulosic component of *Sorghum bicolor* stalk can be increased by 19% i.e. from initial 29.9 to 36.7 (% $m.m^{-1}$) and also increase the lignin content by 20% i.e. from 33.5 to 41.8 (% $m.m^{-1}$). Using the same condition, the hemicellulose content of the biomass can be reduced by 65% i.e. from initial 30 to 10.5 (% $m.m^{-1}$). This result can be very much compared with the findings of Li et al. (2016) and Cai et al. (2016) who also used less concentration of sulfuric acid with less experimental time. However, Baadhe et al. (2014b) experimented at an autoclave temperature of 116 °C while using same acidic concentration as used in this study (0.75%), with a higher dry mass (5.5 g) for 30 min. In the study of Li et al. (2016), almost complete removal of hemicellulose was achieved when corn stalk was subjected to acid pretreatment while the concentrations of both lignin and cellulose increased. In the study of Cai et al. (2016) on the acidic pretreatment of corn straw using diluted sulfuric acid, the hemicellulose content was reported to have decreased from 19.5 to 2.5% while the duo of lignin and cellulose increased from 21.4 to 28.6% and 43.4 to 57.8% respectively using the experimental conditions of 2% ($m.v^{-1}$) of sulfuric acid, autoclave temperature of 120 °C and 1:10 ($m.v^{-1}$) solid liquid ratio for a period of 60 min. In a very recent study by Dahunsi et al. (2017d), the hemicellulose component of corn stalk reduced from 24 to 2% while lignin and cellulose both increased 20 to 34% and 34 to 52% respectively by using sulfuric acid concentration for pretreatment.

3.3.2. Alkaline pretreatment with hydrogen peroxide

The use of alkaline hydrogen peroxide for the pretreatment of *Sorghum bicolor* stalk resulted in an entirely different outcome from that of acidic pretreatment discussed above. The alkali brought about the huge solubilization of lignin while partial dissolution of hemicellulose took place. The most suitable condition that gave the best result in this pretreatment procedure was hydrogen peroxide concentration of 6.8% ($v.v^{-1}$), shaker temperature of 28 °C, agitation at 126 rpm and 3 g of biomass for 85 min (Table 3b). This results implies that it is possible to decrease the concentrations of lignin by 73% i.e. from initial 33.5 to 9.2 (% $m.m^{-1}$) and hemicellulose by 42% i.e. from initial 30 to 17.5 (% $m.m^{-1}$). It is equally possible to increase the concentration of cellulose by 23% i.e. from initial 29.9 to 38.9 (% $m.m^{-1}$). The result of this study is far better than the findings of Cai et al. (2016) who used hydrogen peroxide to pretreat corn straw and obtained lignin and hemicellulose content reduction of 19.6 and 6.2% respectively while cellulose increased by 32.8%.

In another study, Dahunsi et al. (2017e) achieved increase in cellulose and hemicellulose respectively while the concentration of lignin decreased when corn straw was pretreated using hydrogen peroxide. In the current study, only one variable i.e. alkali

Table 1
Characterization of *Sorghum bicolor* stalk and inoculum before and after pretreatments.

Parameter	Inoculum	Cellulose standard	Pretreated *Sorghum bicolor* stalk		Untreated *Sorghum bicolor* stalk	
			H_2SO_4	H_2O_2	Sifted	Not sifted
pH (Sample + Inoculum)	7.98 ± 0.01	7.92 ± 0.10	7.82 ± 0.10	7.90 ± 0.10	7.54 ± 0.10[a]	7.64 ± 0.10[a]
Total solids (% m.m^{-1})	21.9 ± 0.01	95.1 ± 3.01	92.4 ± 0.62	86.5 ± 4.01	93.7 ± 2.01[b]	92.5 ± 0.01[b]
Volatile solids (% m.m^{-1})	14.2 ± 0.01	95.1 ± 2.01	77.3 ± 5.01	53.6 ± 4.01	80.4 ± 2.01[c]	76.4 ± 4.11[c]
Total lignin (% m.m^{-1})	29.5 ± 2.01	ND	41.8 ± 1.30	9.2 ± 2.01	27.8 ± 0.11[d]	33.5 ± 0.21[d]
Cellulose (% m.m^{-1})	3.1 ± 0.01	99 ± 3.01	36.7 ± 0.01	38.9 ± 2.01	24.2 ± 0.50	29.9 ± 0.11
Hemicellulose (% m.m^{-1})	24.1 ± 0.01	ND	10.5 ± 0.01	17.5 ± 0.11	225.8 ± 1.01	30.0 ± 0.10
Fixed solids (% m.m^{-1})	1.6 ± 0.01	0 ± 0.00	2.9 ± 0.05	1.5 ± 0.01	3.6 ± 0.02	4.2 ± 0.10
Extractives (% m.m^{-1})	ND	ND	ND	ND	14.3 ± 0.01	16.6 ± 0.02
Solids after pretreatment (% m.m^{-1})	–	–	3.0 ± 0.01	1.8 ± 0.01	–	–
Added sample (g)	0 ± 0.00	1 ± 0.10	2.4 ± 0.11	2.1 ± 0.10	2.5 ± 0.10	2.4 ± 0.10
COD (g COD/g VS)	144.2 ± 1.02	ND	92.6 ± 1.40	81.9 ± 0.14	126.1 ± 3.00	1349.8 ± 0.10
BP ($L_{Nbiogas.}$ kg VS_{ad}^{-1})	26.3	642.4 ± 3.02	212.3 ± 6.10	607.1 ± 1.51	267.3 ± 3.10[f]	295.2 ± 2.10
μmax ($L_{Nbiogas.}$ kg VS_{ad}^{-1}). d^{-1}	ND	161.2 ± 2.05	26.2 ± 0.01	215.4 ± 2.10	42.2 ± 0.10[g]	51.0 ± 0.10
Day of μmax	ND	3–4	5–6	1–2	4–6	3–5

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; μmax = maximum biogas generation rate.

Table 2
Elemental composition of *Sorghum bicolor* stalk and inoculum before and after pretreatments.

Parameter	Inoculum	Pretreated *Sorghum bicolor* stalk		Untreated *Sorghum bicolor* stalk	
		H_2SO_4	H_2O_2	Sifted	Not sifted
Ash content (%)	4.36 ± 1.02	6.0 ± 0.01	5.2 ± 1.02	4.2 ± 0.01	4.7 ± 1.00
Moisture content (%)	91.4 ± 5.02	83.5 ± 2.01	92.2 ± 2.02	80.2 ± 1.01	87.3 ± 0.02
Total Carbon (g/kg TS)	342.2 ± 0.10	521.5 ± 5.22	668.5 ± 2.25	391.3 ± 6.02	412.4 ± 1.20
Total Nitrogen (g/kg TS)	29.4 ± 1.02	29.8 ± 0.22	30.6 ± 0.21	21.2 ± 0.02	23.3 ± 0.20
C/N	12/1	18/1	22/1	18/1	18/1
Acetate (g COD/g VS)	1.03 ± 0.10	0.09 ± 1.10	0.08 ± 0.01	0.04 ± 0.10	0.05 ± 0.10
Propionate (g COD/g VS)	1.05 ± 0.02	0.13 ± 0.03	0.11 ± 0.02	0.06 ± 0.11	0.10 ± 0.10
TVFAs (g COD/g VS)	2.6 ± 0.10	1.16 ± 0.10	1.04 ± 0.10	0.12 ± 0.10	0.14 ± 0.11
Ammonia (mg/g VS)	4.8 ± 0.01	2.0 ± 1.10	2.3 ± 0.02	1.21 ± 0.02	1.17 ± 0.10
Uronic acids (% VS)	1.7 ± 1.11	1.2 ± 1.10	1.8 ± 0.10	1.2 ± 1.10	1.4 ± 0.10
[@]Soluble sugars (% VS)	3.9 ± 2.10	8.1 ± 0.11	8.3 ± 1.10	4.1 ± 1.02	4.7 ± 0.10
Phenols (mg L^{-1})	4.2 ± 2.10	0.005 ± 0.01	0.004 ± 0.10	0.001 ± 0.01	0.001 ± 0.10
Total Phosphorus (g/kg TS)	5.60 ± 0.02	4.2 ± 0.12	5.4 ± 0.11	3.1 ± 0.11	3.5 ± 0.01
Potassium (g/kg TS)	6.0 ± 0.11	5.7 ± 0.11	6.9 ± 0.02	4.2 ± 0.01	5.55 ± 0.01
Phosphate (g/g TS)	1.1 ± 0.02	3.5 ± 0.11	4.2 ± 0.10	2.01 ± 0.01	2.10 ± 0.20
Sulfate (g/kg TS)	84 ± 2.00	91.0 ± 6.10	101.1 ± 2.02	46.2 ± 2.00	56.4 ± 1.02
Calcium (g/kg TS)	84.3 ± 0.10	487.2 ± 1.42	528.5 ± 4.00	321.4 ± 0.42	381.8 ± 2.03
Magnesium (g/kg TS)	68.2 ± 0.10	56.8 ± 2.02	72.4 ± 1.40	33.6 ± 0.02	46.2 ± 1.10
Manganese (g/kg TS)	1.8 ± 0.21	0.013 ± 0.04	0.014 ± 0.10	0.011 ± 0.01	0.014 ± 0.10
Iron (g/kg TS)	1.8 ± 0.01	1.5 ± 0.03	1.5 ± 0.01	0.25 ± 0.01	0.35 ± 0.01
Zinc (g/kg TS)	31.00 ± 0.02	22.2 ± 0.03	24.1 ± 0.01	15.9 ± 0.02	17.3 ± 0.01
Aluminum (g/kg TS)	0.8 ± 0.11	1.3 ± 0.10	1.6 ± 0.02	0.3 ± 0.02	0.3 ± 0.12
Copper (g/kg TS)	4.6 ± 0.10	3.5 ± 0.02	4.2 ± 0.11	2.1 ± 0.10	2.8 ± 0.11

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

concentration had a significant effect at 95% confidence interval for both lignin and hemicellulose contents of *Sorghum bicolor* stalk whereas, for the cellulose content, alkali concentration and exposure time were both significant for the increase of this structural material with 95% confidence interval. From the experimental design used in this study, the significant variables at $p < 0.05$ needed to reduce the concentration of both lignin and hemicellulose while maximizing the content of cellulose can be identified. In the study of Dahunsi et al. (2017f), alkali concentration and experimental time were the only variables found to be significant.

3.4. Evaluation of the effect of sifting on pretreated biomass

After the pretreatment procedures, statistical comparison was done to evaluate the possible effect of sifting on the structural changes of both sifted and not sifted biomass using the Tukey test. Results showed almost equal composition of all structural components i.e. lignin, cellulose and hemicellulose which showed that sieving did not really have any effect on the biomass susceptibility to solubilization after application of acid and alkali pretreatments. This implies that the sieving procedure could be eliminated in subsequent studies as it has no significant effect on the structural changes in *Sorghum bicolor* stalk as well as on the biogas producing potential of the biomass in both acidic and alkaline pretreated biomass. A similar result was obtained by Venturin et al. (2018).

3.5. Structural changes in Sorghum bicolor stalk

As shown in Table 4, the structural composition of both pretreated and untreated arrangement of the molecules that make up the pretreated and untreated *Sorghum bicolor* stalk were evaluated by FTIR spectra. The spectra showed that the biomass is a cellulosic material because all the bands were positioned around the 3448 and 2900 cm^{-1} which are major bands of cellulose and whose absorbance increased by 21.0 and 50.1% after alkaline pretreatment and was evident in the increase in cellulose concentration of the biomass after pretreatment. The decrease in cellulose concentration reported in the acidic pretreated biomass was as a result of the decrease in the cellulosic O-H bonds which also caused a reduction in absorbance at the 3448 cm^{-1} band. This phenomenon had been reported severally for other biomass

Table 3a
Structural composition of crushed and sifted *Sorghum bicolor* stalk after H_2SO_4 pretreatment and actual values of the independent variables.

Run	Exposure time (min)	Temperature (°C)	H_2SO_4 concentration (%)	Dry mass (g)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
1	52.00	118.00	0.75	3.70	41.00	36.70	10.50
2	56.34	119.99	1.80	3.01	41.51	36.16	15.70
3	46.35	119.19	0.70	3.07	43.66	38.57	15.71
4	53.26	117.30	0.50	4.12	40.51	38.48	12.73
5	60.28	121.00	1.70	3.46	40.30	39.49	11.76
6	50.52	120.00	1.80	3.10	40.33	36.64	15.53
7	51.53	122.04	0.70	4.14	40.33	37.39	15.84
8	61.25	114.00	0.50	3.11	40.48	37.54	14.77
9	50.72	115.03	1.99	3.13	40.48	38.50	12.77
10	50.80	118.05	1.02	3.73	39.92	37.30	12.05
11	49.42	119.00	1.00	3.21	40.11	39.26	11.41
12	40.64	121.04	0.66	3.43	39.41	38.19	13.16
13	45.28	121.06	0.60	3.48	39.44	37.08	13.27
14	50.47	113.00	0.44	2.33	41.40	36.74	12.23
15	50.48	120.05	1.08	4.56	40.62	37.56	12.62
16	54.53	121.03	1.11	5.22	41.01	37.55	13.42
17	41.33	121.02	0.60	5.30	41.16	37.57	13.41
18	39.08	119.04	0.43	5.90	41.91	38.19	13.86
19	56.52	121.03	0.60	2.87	48.83	39.89	12.43
20	52.41	119.98	0.90	2.93	48.33	34.62	13.65
21	45.19	122.00	0.04	4.08	44.23	36.77	12.25
22	38.55	120.00	1.00	6.38	42.20	34.05	13.10
23	44.25	120.23	1.01	2.87	48.03	35.45	13.71
24	49.23	119.30	1.07	5.12	45.97	37.58	12.10
25	44.08	120.31	1.03	2.02	47.43	36.32	12.49
26	59.81	119.34	0.70	3.44	43.65	37.11	12.52
27	55.35	115.00	0.43	3.40	45.79	38.26	13.46
28	55.32	116.04	1.01	4.51	39.08	37.75	12.81
29	52.38	114.48	0.80	2.00	36.68	37.81	13.88
30	49.36	116.00	0.09	7.80	42.19	38.56	13.09

Table 3b
Structural composition of crushed and sifted *Sorghum bicolor* stalk after H_2O_2 pretreatment and actual values of the independent variables.

Run	Exposure time (min)	Temperature (°C)	Agitation (rpm)	H_2O_2 concentration (%)	Dry mass (g)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
1	85.00	28.00	126.00	6.80	3.00	09.20	38.90	17.50
2	86.66	30.00	130.03	7.50	3.21	09.47	36.63	18.34
3	80.67	30.00	130.44	7.43	3.30	10.85	35.98	17.58
4	81.05	31.21	130.00	7.50	3.40	09.57	35.79	18.61
5	91.66	30.06	131.13	7.50	3.00	10.81	35.63	18.54
6	89.39	30.00	130.00	7.24	3.73	11.02	33.97	19.78
7	81.27	30.00	133.33	7.50	3.00	10.25	34.89	18.17
8	87.65	30.02	130.01	5.55	3.20	09.08	34.93	19.21
9	81.83	32.00	120.22	6.50	3.10	09.46	35.89	19.07
10	83.46	32.00	130.00	7.51	3.30	10.33	36.07	18.03
11	87.60	31.14	130.00	6.50	3.20	9.67	35.27	19.14
12	85.82	31.44	131.98	7.50	2.10	10.06	36.23	18.97
13	83.50	30.00	130.00	6.84	4.01	11.81	34.38	17.30
14	84.94	30.00	130.00	7.50	3.74	09.78	33.08	16.99
15	88.27	32.14	131.08	7.50	3.76	10.30	33.31	17.54
16	86.21	32.00	129.21	5.50	4.83	09.22	35.26	18.93
17	87.67	30.00	130.01	5.73	3.00	10.18	36.25	17.38
18	86.04	30.54	130.00	7.50	3.74	09.94	35.43	16.01
19	91.66	32.00	129.93	7.60	4.84	11.72	33.92	18.99
20	85.50	31.17	129.51	6.50	3.28	10.52	35.39	17.71
21	85.05	30.14	130.00	7.52	3.10	09.11	33.61	17.09
22	85.38	30.00	131.51	7.07	3.30	10.30	33.38	16.11
23	87.28	30.00	130.00	7.53	3.35	09.76	32.79	15.37
24	85.01	30.00	131.49	7.50	3.11	10.68	32.77	16.21
25	89.20	33.15	130.02	8.50	2.49	11.60	34.19	15.00
26	87.01	34.53	130.00	8.50	3.10	10.99	33.58	15.28
27	86.86	32.16	130.00	7.50	2.85	10.60	33.48	15.24
28	85.33	30.21	129.99	8.50	3.00	10.31	32.95	15.39
29	85.75	30.17	130.00	7.51	3.91	10.35	32.91	17.37
30	85.80	31.11	130.01	7.20	2.17	10.29	32.87	18.41

after acidic pretreatment (Li et al., 2016; Zhao et al., 2016; Cai et al., 2016). Another observation was the modification of the structure of the cellulose in the pretreated biomass since it was exposed environmental conditions such as elevated temperature, pressure and the acid concentration.

In evaluating the changes that occurred to the lignin component of *Sorghum bicolor* stalk, bands around 1734, 1716, 1633, 1604 and 1516 cm^{-1} were detected all of which are strictly connected lignin structural chemical groups. It was observed that all these peaks were seriously affected by the alkaline treatment because they were seen to have either disappeared or completely flattened which must have been caused by the action of hydroxyl radicals (Venturin et al., 2018). However, the effects of sulfuric acid on these lignin peaks were not as severe as those of hydrogen peroxide. Sulfuric acid only caused their reduction safe for the 1516 cm^{-1} alone because of its very tight association with lignin.

Table 4
Wave lengths that correspond to a given functional group and respond to infrared spectroscopy, and their respective relative values for H_2SO_4 and H_2O_2 pretreatments tested for *Sorghum bicolor* stalk.

Wavelength (cm^{-1})	Assignment	Untreated	H_2SO_4 pretreated		H_2O_2 pretreated	
		Absorbance/Ratio	Absorbance/Ratio	Variation (%)	Absorbance/Ratio	Variation (%)
3348	O-H stretch (Hydrogen cellulose connections bond)	0.3340	0.4617	−38.2	0.3233	3.2
2900	C-H stretch (Methyl/methylene cellulose group)	0.1204	0.1641	−36.3	0.1733	−43.9
1734	Carbonyl bonds (Associated with removal of lignin side chain)	0.1001	0.0464	53.6	0.0141	85.9
1716	Carboxylic acids/ester groups	0.0812	0.0612	24.6	0.0338	58.4
1633	Aromatic ring stretch (Associated with lignin removal)	0.1413	0.1102	22.0	0.1110	21.4
1604	Aromatic ring stretch (Changes in lignin structure)	0.1432	0.1113	22.3	0.0755	47.3
1516	Generic lignin	0.0824	0.1041	−26.3	0.0434	47.3
1516/897	Lignin/cellulose ratio	3.4	3.1	8.8	0.6	82.4
1373	Phenolic O-H stretch (Changes in lignin structure)	0.2312	0.1223	47.1	0.1712	26.0
1319	Syringyl ring stretch (Changes in the lignin monomer)	0.1271	0.1133	10.9	0.1341	−5.5
1251	C-O absorption (Result of acetyl-lignin groups cleavage)	0.1514	0.1102	27.2	0.1085	28.3
1110	Crystalline cellulose	0.3404	0.2162	36.5	0.3024	11.1
1059	C-O-C stretch (Cellulose and hemicellulose)	0.4954	0.4559	8.0	0.4110	17.0
897	Amorphous cellulose	0.0444	0.0331	25.5	0.0298	32.9
1110/897	Crystalline/amorphous cellulose ratio	6.7	7.4	−10.4	4.4	34.3
833	C-H flexion of syringyl	0.1105	0.0135	87.8	0.0100	91.0
771	Crystalline cellulose ($I\alpha$)	0.0144	0.0114	20.8	0.0106	26.4
719	Crystalline cellulose ($I\beta$)	0.0222	0.0343	−54.5	0.0272	−22.5
771/719	Ratio of crystalline cellulose polymorphs ($I\alpha/I\beta$)	0.2	0.3	−50	0.2	0

ND = Not determined; % Relative variation = 100 ∗ (Absorbance of untreated Sorghum bicolor stalk − Absorbance of pretreated Sorghum bicolor stalk)/Absorbance of untreated Sorghum bicolor stalk; All positive values indicates decrease.

This explains why the acidic pretreatment caused an increase in lignin composition which has also been previously reported (Li et al., 2016; Yao et al., 2018).

The partial actions of sulfuric acid on the lignin component of *Sorghum bicolor* stalk resulted in the formation of pseudo lignin such as earlier reported by Lizasoain et al. (2017) and Yao et al. (2018) by pretreating corn and wheat stalk respectively. The aftermath of these formations is the reduction in the final yield of biogas or complete inhibition of the anaerobic digestion process. One of such lignin derivative is phenolic lignin at detectable at the 1373 cm^{-1} peak which decreased after sulfuric acid pretreatment but increased in the hydrogen peroxide pretreatment since the action of the acid on this lignin type was preferential while that of the alkali was not thereby making the lignin to remain in the *Sorghum bicolor* biomass after alkaline pretreatment (Zhao et al., 2016). Similar result was observed for yet another type of lignin i.e. the syringyl lignin detectable at the 1329 cm^{-1} band (Zhao et al., 2016; Yao et al., 2018). The third type of lignin found in the biomass studied is the acetyl lignin detectable at the 1251 cm^{-1} band and whose composition decreased after both pretreatment procedures which was more pronounced in the alkaline pretreatment (Zhao et al., 2016).

In evaluating the changes in hemicellulose composition of *Sorghum bicolor* stalk, there was an observed decrease at the 1059 cm^{-1} which has a strong association with hemicellulose. This was absolutely responsible for the high solubilization of hemicellulose reported in this study after acidic pretreatment (Zhao et al., 2016).

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 pantothenticus, 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 *Methanosaetaceae, Methanomicrobiales* and *Methanosarcinaceae*. These arrays of organisms have been implicated in previous anaerobic digestion processes (Dahunsi et al., 2017a,c). These diverse microorganisms and their population brought about robust microbial activities in the digesters and one of the effects is volatile fatty acid'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 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 (Riggio et al., 2017). 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 in all the experiments, the mass balance was evaluated as shown in Table 5. 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.

Table 5
Stoichiometry and mass balance for one ton of *Sorghum bicolor* stalk.

Parameter	H_2SO_4 Pretreated *Sorghum bicolor* stalk + Inoculum	H_2O_2 Pretreated *Sorghum bicolor* stalk + Inoculum	Untreated *Sorghum bicolor* stalk + Inoculum
Input			
Sorghum bicolor stalk + Inoculum (kg)	1000	1000	1000
Volatile solids (VS) (kg)	773	536	764
Output			
Methane (CH_4) (%)	54.5	61.5	55
Carbon dioxide (CO_2) (%)	23.5	20.5	23
Digestate (kg VS)	586	321	550
Sum	664	403	628
Mass balance[a]	0.14	0.25	0.18
% Volatile solids (VS) removal	24	40	28

[a](Input − output)/input (%).

3.7. Biogas potential (BP)

As shown in Table 1, the inoculum used in this study had a low biogas potential as it produced less than 10% of total biogas generation in all experiments. The inoculum also produced less biogas than the microcrystalline cellulose standard whose biogas generation capacity was 80% higher than the 650 LNbiogas.kg $VSad^{-1}$ reference values of the VDI standards 4630 (2006). Interestingly, the biogas produced by the microcrystalline cellulose standard compared favorably with that from the alkaline pretreated biomass which gave the highest yield of biogas in all experiments. The acid pretreated biomass produced a total of 312.3 LNbiogas.kg $VSad^{-1}$ while the alkaline pretreated biomass produced 607.1 LNbiogas.kg $VSad^{-1}$ (Fig. 1). In comparing production from the two pretreated biomass, the biomass pretreated with hydrogen peroxide produced 65% more LNbiogas.kg $VSad^{-1}$ when than the other. Total production from the two untreated samples was 287.3 and 354.2 LNbiogas.kg $VSad^{-1}$ for the untreated sifted and the untreated not sifted samples respectively. This showed that the alkaline pretreated biomass produced 56 and 51% more biogas than both untreated samples respectively. This result contradicts the findings of Sun et al. (2015) who reported reduction in biogas yield with the application of alkaline hydrogen peroxide pretreatment simply due to usage of low alkali concentration. However, Lizasoain et al. (2017) reported that the presence of lignin was responsible for low gas production after the pretreatment of corn stalk.

Biogas production from the two untreated samples of *Sorghum bicolor* stalk further confirms that sifting had no tangible effect on the structural composition and biogas yield of the biomass as there is no statistically difference between the productions from the two samples according to Tukey test. The slight increase in biogas production in the not sifted sample over the sifted one could be due to the advantage of having diverse particulate size over the other which has only the smaller sieved particles whose contribution to total biogas generation cannot compare with contribution from the larger particles in the other sample (Croce et al., 2016).

The over 50% more biogas production from the alkaline pretreated biomass over the untreated ones goes further to justify the need for pretreatment of lignocellulosic biomass prior to anaerobic digestion (Zhao et al., 2018). Also considering the biogas generation rate, it is obvious that the alkaline pretreated biomass produced and reached peak of production faster than other experiments. A rate of over 323% was observed in the alkaline pretreated biomass over the acidic pretreated one which was also achieved 5 days ahead as the peak of production was achieved on the 12th experimental day in the alkaline pretreated biomass while it was between the 17th and 21st days in all other experiments.

According to the analysis carried out, all the biogas produced in all experiments contains fairly high methane and carbon dioxide composition whereas, the hydrogen sulfide content was low (Mancini et al., 2018). The acidic pretreatment in this study impaired the biogas production kinetics whereas; alkaline pretreatment enhanced the rate and final volume of biogas produced. The composition of the biogas generated across the four different digestions showed methane to be within 54.5 ± 1.2 and 61.5 ± 2.1, carbon dioxide of between 20.5 ± 1.2 and 23.5 ± 0.2 and hydrogen sulfide of between 3.3 ± 0.2 and 9.1 ± 0.2. Analysis of all the digestates in this study shows elevated levels of nutrient as against their initial composition in the biomass prior to anaerobic digestion. This means that further solubilization of the pretreated *Sorghum bicolor* stalk took place during the digestion process (Dahunsi et al., 2018a, 2019a; Dahunsi, 2019; Dahunsi et al., 2019c). Similarly, there was increase in the lignin:cellulose–hemicellulose complex in all digestates with the lowest recorded in the digestate from the acidic pretreated biomass which also produced the least biogas volume.

As shown in Table 6, the energy balance in this study was computed using the combined heat and power (CHP) system while neglecting heat loss. For the alkaline treated biomass, the 1422 kWh t^{-1} TS thermal energy gain exceeded the 945 kWh t^{-1} TS used in the pretreatment thus giving a net thermal energy of 477 kWh t^{-1} 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., 2017c). Perhaps, full integration of thermal energy is another prominent method for assessing the economic feasibility in this study as earlier reported (Dahunsi et al., 2017d). For the acidic pretreated sample of *Sorghum bicolor* stalk, the investment into acid purchase, equipment usage and time seemed fruitless because the −131 kWh t^{-1} TS thermal energy gain was far below the 1025 kWh t^{-1} TS thermal energy used in pretreatment with a net thermal energy of −761 kWh t^{-1} 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., 2017b). For the alkaline treated experiment, the net electrical energy of 140 kWh t^{-1} TS far outweigh the −120 kWh t^{-1} TS obtained from the acidic pretreated *Sorghum bicolor* stalk. 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

As shown in this study, pretreatment with hydrogen peroxide removed 73 and 42% of lignin and hemicellulose respectively

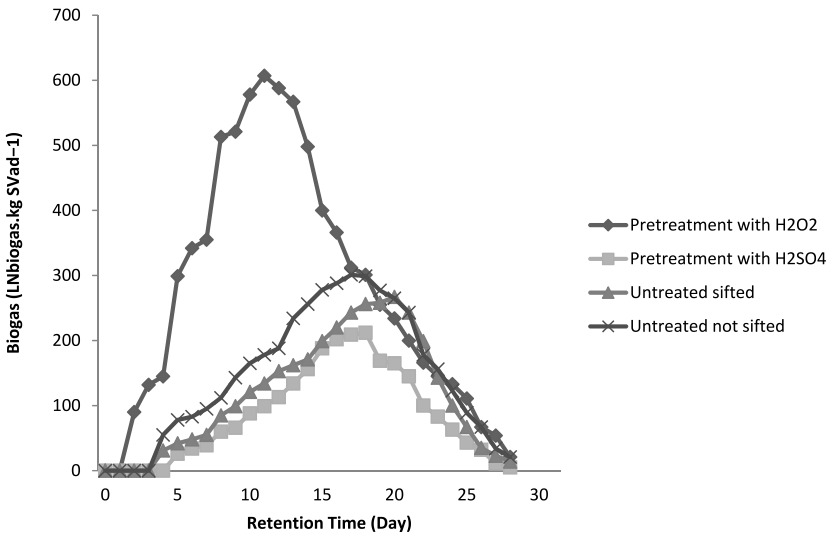

Fig. 1. Cumulative biogas production per kilogram of added volatile solids for H_2S_2 pretreated, H_2SO_4 pretreated, untreated sifted and untreated not sifted *Sorghum bicolor* stalk.

Table 6
Energy and economic evaluation for the digestion of *Sorghum bicolor* stalk.

Energy parameters	H_2O_2 pretreated	H_2SO_4 pretreated	Not sifted (untreated)	Sifted (untreated)
Produced electrical and thermal energy from combined heat and power (CHP)	3278	1166	1400	1197
Produced thermal energy (kWh t^{-1} TS)	2575	1031	1153	1162
Produced electrical energy (kWh t^{-1} TS)	1413	642	891	762
Thermal balance				
Thermal energy gain (kWh t^{-1} TS)[a]	1422	−131	–	–
Thermal energy requirement (kWh t^{-1} TS)	945	1025	–	–
Thermal energy requirement with 80% of heat recovery (kWh t^{-1} TS)	189	205	–	–
Net thermal energy (kWh t^{-1} TS)[b]	477	−951	–	–
Net thermal energy with 80% of heat recovery (kWh t^{-1} TS)	382	−761	–	–
Electrical balance				
Electrical energy gain[c]	522	−120	–	–
Energy for mixing during pretreatment	382	387	–	–
Net electrical energy	140	−753		
Economic evaluation				
Cost of H_2O_2 and H_2SO_4 (€ t^{-1} TS)				

[a]Difference of thermal energies produced by the pretreated experiment minus the untreated.
[b]Difference between the thermal energy gain and the thermal energy requirement for the pretreatment.
[c]Difference of electricity energies produced by pretreated experiment minus the untreated.

and increased cellulosic content of *Sorghum bicolor* stalk by 23%. Application of this alkali also increased the final volume of biogas by 65% over the acidic pretreated sample and equally reduced the production time by 5 days. Till now, use of low-cost hydrogen peroxide for biomass pretreatment is unpopular while the uses of other strong alkali and acids are well studies. However, hydrogen peroxide gave better product yield. Therefore, use of this alkali pose a novel biotechnological means for generating biogas.

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Conflict of interest

The authors declare that there is no conflict of interest in this paper.

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