Journal of Cleaner Production 226 (2019) 578–588

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Cleaner energy through liquefaction of Cocoa (*Theobroma cacao*) pod husk: Pretreatment and process optimization

S.O. Dahunsi [a, *], A.T. Adesulu-Dahunsi [b], J.O. Izebere [b]

[a] Sustainable Management of Natural Resources and Environment Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam
[b] Landmark University, Omu-Aran, Kwara State, Nigeria

ARTICLE INFO

Article history:
Received 23 December 2018
Received in revised form
9 April 2019
Accepted 10 April 2019
Available online 13 April 2019

Keywords:
Biogas
Biomass
Energy
Environment
Optimization
Pretreatment

ABSTRACT

This study explored the production of biogas from the mono-fermentation of pretreated Cocoa pod husk. The pretreatment was carried out with sulfuric acid (H_2SO_4) and alkaline hydrogen peroxide (H_2O_2) prepared by adjusting the pH of H_2O_2 to 11.5 by the addition of 5 M NaOH solution. Prior to and after the pretreatments, physicochemical, structural and microbial analyses were carried on the husk using a standard method in each case. In order to determine the changes to the biomass structures after pretreatments, the Fourier Transform Infra-red (FTIR) spectroscopy were used. Average total biogas volume from all the experiments i.e. the acidic pretreated (AcP), alkaline pretreated (AlP), not sifted untreated (NsU) and sifted untreated (SU) was 162.8 ± 5.0, 564.8 ± 5.1, 243.3 ± 4.1 and 220.8 ± 3.3 respectively. This shows that the AlP Cocoa pod husk yielded the highest biogas volume and was followed by the NsU and the SU husk while the lowest was obtained from the AcP biomass. Overall, the AlP biomass produced 71% more total biogas than the AcP one and also produced 57% more biogas than the NsU Cocoa pod husk. The AlP Cocoa pod husk did not only produce the highest biogas volume but also achieved peak of production faster than all the other experimental setups. Total biogas generation was achieved in just 12 days out of the 30-day retention period used in the study in which biogas production started on the 3rd day and climaxed on the 15th experimental day whereas, biogas generation did not commence until after the 5th and 6th days in other experiments and climax was not reached until between the 18th and 21st days. The result of this study has revealed that use of the low cost mild alkali is more efficient in lignin (L) solubilization and subsequent biogas yield improvement. Also, Cocoa pod husk has been shown in this study to be a profound biofuel substrate. Therefore, further use of AlP Cocoa pod husk for biogas and biofertilizer production is hereby advocated especially in major Cocoa producing regions of the world.

1. Introduction

According to the United Nations, (UN, 2017), there are three majors components of sustainable development which are economic development, social inclusion and environmental protection. A subset of the last component is environmental management which has been shown to impart directly on all life forms. There is therefore urgent need for a concerted effort to integrate cleaner production technologies coupled with the formulation and implementation of appropriate policies geared towards solving the global threatening environmental issues (Dahunsi et al., 2017a,b). Paramount of these issues is that of energy production and usage (Klemes et al., 2012; Kalbar et al., 2016) and anaerobic digestion is one of the sustainable approach to generate such environmental friendly energies (Klemes and Varbanov, 2013; Liew et al., 2017).

The anaerobic treatment of organic wastes and biomass for the purpose of generating a mixture called biogas is an advanced technology (Patinvoh et al., 2017; Pavi et al., 2017 Nemestothy et al., 2018). Biogas in turn consists largely of methane followed by carbon dioxide, hydrogen sulfide, water vapor and other impurities (Koido et al., 2018; Brunklaus et al., 2018). Biogas is usually made up of between 50 and 70% methane content which makes the gas a potential replacement to replace fossil fuels and has also found wide application in several in cooking, heat and electricity generation via the Combined Heat and Power (CHP) systems and in internal combustion engines (Ferella et al., 2017; Miltner et al., 2017;

* Corresponding author.
E-mail address: dahunsi.olatunde.samuel@tdtu.edu.vn (S.O. Dahunsi).

https://doi.org/10.1016/j.jclepro.2019.04.112
0959-6526/

Morero et al., 2017). Often, biogas is upgraded to biomethane employing different processes in order to use the purified fuel in the transportation industry as a vehicular fuel and can also be fed into the energy grid following laid down requirements (Chen et al., 2015).

One of the efficient approaches to solving the world's growing energy demand, reduce over-dependence on fossil fuels and drastically reduce the emission of greenhouse gasses is the conversion of lignocelluloses into biogas and other biochemicals (Dollhofera et al., 2018). Materials that fits into the lignocellulosic class includes agricultural residues, green biomass, forest residues, mill wastes, fractions of municipal solid wastes, horticultural wastes, food wastes etc. which are abundantly available in most locations of the world (Gerbrandt et al., 2016; Wei, 2016; Williams et al., 2016). The advantage of using these materials for energy generation is in that they are abundant, easily and cheaply accessible as against the huge cost involved in the cultivation of energy crops for the same purpose. Besides, use of lignocellulose materials eliminates the need for land for cultivation and is devoid of the famous "food versus fuel" debate.

However, the full exploitation of lignocellulosic biomass has not been achieved and their usage is regarded as not economically feasible in most environments due to their complex chemical constituent which makes them recalcitrant to microbial attack during digestion (Dahunsi et al., 2017c,d, 2018a,b,c). Their structure usually contains a fibrous and interwoven fabric of L, C and H making it very difficult or almost undegradable. A major disadvantage in this is that anaerobic organisms (Bacteria and archaea) are usually not able to degrade these components especially the L coat thereby failing to utilize the abundant hydrolysable sugars these materials contain for biogas production (Dahunsi et al., 2017e,f).

In this regard, the application of appropriate pretreatment technologies is necessary in order to break the coherent recalcitrance of the biomass thereby providing accessibility for cellulolytic microorganism with the sole aim of producing more biogas (Patinvoh et al., 2017). There are many methods which includes mechanical, chemical, biological and sometimes combination of some of these approaches. However, the cost effectiveness of any chosen must be well considered before adoption in order to justify the investment (Patinvoh et al., 2017).

Theobroma cacao is known to have originated from Latin America but is now found being largely cultivated globally especially in the Americas, Asia and Africa (Kaufman and Justeson, 2006). West African countries: Ivory Coast, Ghana, Nigeria, and Cameroon are responsible for about 70 percent of total production of Cocoa globally with majority coming from Cote d'Ivoire and Ghana. Other leading producers include Indonesia, Nigeria, Cameroon, Brazil, Ecuador, Mexico, Peru and Dominican Republic (Food and Agriculture Organization of the United Nations Statistics Division, 2017). In 2017 global production of Cocoa was approximately 5, 000, 000 MT. Nigeria occupies the 4th position in Cocoa production globally with an average of 367,000 metric tons of Cocoa beans annually (Food and Agriculture Organization of the United Nations Statistics Division, 2017). During harvesting and processing of Cocoa for several purposes, the pod husk is usually separated and thrown away as solid wastes thereby constituting environmental nuisance as they serve as medium for transporting pathogenic microorganisms to humans and animals alike. Even though few usages had been sought for the husk in some localities, there are no documented sustainable methods of treatment till date. With the huge biomass accrued from this economic crop and need for cleaner production strategies geared towards environmental protection and generation of sustainable biofuels, there is need to explore means of converting Cocoa pod husk into value-added products.

The aim of this study therefore is to evaluate the energy producing potential of Cocoa pod husk in anaerobic monofermentation and to achieve the optimal treatment for the biomass. If successful, this will help situate Cocoa pod husk as a profound biofuel feedstock and will boost the economy of Cocoa producing nations by gaining additional energy from what is being regarded as wastes.

2. Materials and methods

2.1. Sample collection

Cocoa pods were collected from Ile-Ife, Osun State, Southwestern Nigeria which is renowned for Cocoa production among many other locations. After separation of the bean, the remaining pod husks were further cut into pieces and sun dried to achieve constant dried weight. Afterwards, a knife mill (SOLAB, SL-31, Brazil) was used to grind the dried husks and was then sieved using sieves of mesh sizes ranging between 0.075 and 4.750 mm following standard procedures (Dahunsi et al., 2016a,b). However, a portion of the ground biomass was not sieved so it could be compared with the sifted during analysis and biogas generation. All samples were kept in the refrigerator at 4 °C before next usage.

2.2. Reagents

All the reagents used in this study were analytical grades. Sulfuric acid (98% W/W minimum) was purchased from Panoli Intermediates, India and used for the acid pretreatment while the Standard Grade 70% hydrogen peroxide (PeroxyChem LLC, US) was used for the alkaline treatment after adjusting the pH to 11.5 by the addition of 5 M NaOH solution (Li et al., 2012).

2.3. Experimental design of pretreatments

The Response Surface Methodology (RSM), a component of the Design-Expert software (Version 9.0.3.1) which is a common and reliable experimental design tool was employed in this study. Two designs were carried in order to reflect the types of pretreatments i.e. (i) the acidic pretreatment with sulfuric acid (H_2SO_4) in humid steam using an autoclave and (ii) alkaline hydrogen peroxide (H_2O_2) pretreatment in an orbital shaker. In the design for the acidic pretreatment, the input variables considered include 'Exposure time' in minutes, 'Temperature' in degree Celsius, 'H_2SO_4 concentration' in percentage and the 'Dry mass' in grams. Same variables were employed in the alkaline pretreatment except with the addition of 'Agitation' in rotation per minute (rpm) since the experiment was done in an orbital shaker. The percentage composition of lignin (L), cellulose (C) and hemicellulose (H) in $m.m^{-1}$ was the evaluated response in both designs (Venturin et al., 2018).

2.3.1. Pretreatments

A range of values were chosen according to the experimental design to maximize the acidic treatment of the biomass in this study. These values are exposure time of 5, 15, 25, 35 and 45 min, temperature of 80, 90, 100, 110 and 120 °C, sulfuric acid 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 previous designs (Baadhe et al., 2014; Venturin et al., 2018). For the alkaline pretreatment, the values considered are exposure time of 50, 60, 70, 80 and 90 min, 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_2O_2 concentration of 3, 6, 9, 12 and 15% $v.v^{-1}$. An antifoam reagent (0.5 mL) (Biocane FC

500, Brazil) was added to the alkaline pretreatment setup so as to reduce foaming. All values were chosen according to standard methods (Rabelo et al., 2011; Sun et al., 2013; Venturin et al., 2018) with some modifications.

2.4. Physicochemical analyses

The physicochemical composition of the biomass in terms of the values of all important parameters were all carried out using the inductively coupled plasma mass spectrometry while the American Public Health Association's method (APHA, 2012) was used in the determination of Chemical Oxygen Demand (COD). A Clarus 580 GC gas chromatography (PerkinElmer, USA) was used to determine the concentration of Volatile fatty acids (VFAs) while those of total solids (TS) and volatile solids (VS) were done using the method of the Finnish Standard Association (SFS 3008 protocol) (1990). For the determination of total phenolics, a Spectroquant microtube (Merck) test was employed followed by a measurement of 4-amino antipyrine by colorimetry according to the method of Monlau et al. (2012).

2.5. Biomass structural composition

Concentrations of L, C and H, fixed and extractive solids in the pretreated and untreated samples were all determined using standard methodology (Sluiter et al., 2012). A portion of each treated and untreated biomass was divided into two with one portion heated for 6 h in a Soxhlet apparatus in order to obtain the extractable components while the muffle furnace was used in burning the other portion for the determination of fixed solids (Sluiter et al., 2008a,b). For total L, C and H determination, 0.3 g of each sample was heated in a thermostatic bath with the addition of 72% sulfuric acid (3 mL) ($v.v^{-1}$) at 30 °C for 1 h after which the concentration of each structural material was determine. The resulting filtrate was afterwards used for carbohydrate concentration determination (Sluiter et al., 2012). For the determination of sugars and acetic acid in the samples, the liquid chromatographic method i.e. LC-MS mass spectrometer (SHIMADZU, Japan) in a refractive index detector (DIR-10A) was used and was operated with an AMINEX® BIORAD HPX87H column. A total of 0.005 $mol.L^{-1}$ sulfuric acids were used as the mobile phase in an isocratic mode, at a temperature of 45 °C, injection volume of 20 μL and a flow of 0.6 mL min^{-1}. After these, the concentration of each of sugars and acetic acid was then determined using calibration curves with LC-MS standard from Sigma-Aldrich for each sugar and acid (Bazoti et al., 2017). The same procedure was followed in determining the concentration of furfural and hydroxymethylfurfural (HMF) with some modifications e.g. a diode array detector (DAD) was attached to the LC-MS, a C18 column was used, mobile phase was 1:8 acetonitrile/water with the addition of 1% acetic acid, an isocratic mode, temperature of 30 °C, injection volume of 20 μL and flow of 0.8 mL min^{-1}.

2.6. Determination of functional groups

In order to determine the extent of chemical disruptions in the pretreated biomass, their functional groups were determined by FTIR (IR tracer-100, SHIMADZU, Japan) spectroscopy following the method of Zhao et al. (2016).

2.7. Biogas potential (BP) test

The potential of Cocoa pod husk for biogas generation under constant condition was evaluated in this study. In doing this, a temperature of 37 °C with constant pressure over a retention period of 30 days was chosen. Due to the small quantities of substrate used for the test, 250 mL mini batch reactors in close connection to 500 mL eudiometer tubes containing 10% ($m.v^{-1}$) volatile solid contents were employed (Steinmetz et al., 2016; Dahunsi et al., 2019). Also, compliance with the VDI 4630 (2006) standard for BMP testing was ensured.

2.8. Digestion

Anaerobic mono-fermentation was carried out for all the samples of Cocoa pod husk based on the values from pretreatment optimization experiments and this was done using the Batch digester (EDIBON, United Kingdom) as shown in Fig. 1. The digesters were seeded with inoculum from a digester treating animal manure as the sole substrate at mesophilic temperature (Steinmetz et al., 2016). The digester has an in-built downward displacement of water trough and this was used for the collection of produced biogas. At the end of the experiments, the data obtained from all digestion set ups were computed and this was followed by analyses of the biogas major components (CH_4, CO_2, and H_2S) by infrared and electrochemical sensors (BIOGASS5000, USA).

2.9. Analysis of microbial community

On days 6, 12, 18, 24 and 30 of the anaerobic digestion in each experiment, samples (45 mL each) were taken for the analyses of the microbial community of the fermenting materials and effluents obtained after digestion. Samples were stored at −20 °C prior to the total DNA extraction which was done following standard method (Vilchez-Vargas et al., 2013) after which a conventional PCR targeting the total bacterial and archaeal population with the aid of the P338f and P518r primers was carried out (Muyzer et al., 1993; Boon et al., 2002). After extraction of DNA, its purity and those of the PCR products were checked using Agarose gel electrophoresis after which a Real-time PCR analysis was carried out using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). In order to check the integrity of the products of the Real-time PCR, different parameters obtained with the StepOnePlus software V2.3 were analyzed in triplicate and results recorded.

2.10. Optimization and statistical analysis of data

After the anaerobic digestion and all analyses, the RSM was employed for the statistical interpretation of all the data based on the initial design of the pretreatment procedures so as to fit the polynomial equations already generated by the Design-Expert software. Multiple regressions were used to fit the coefficient of the polynomial model in order to correlate the responses and the independent factors. The model's quality was evaluated using the test of significance and analysis of variance (ANOVA). The equation below shows the quadratic model equation:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \quad (1)$$

where: Y is the variable of the response; b_o is the value of the intercept; b_i (i = 1, 2, k) is the first order model coefficient; b_{ij} is the interaction effect; b_{ii} is the quadratic coefficients of X_i while e represents the random error.

The 3-D graphs of the responses in terms of percentage levels of L, C and H were then constructed. Afterwards, the STATISTICA V. 12 software (StatSoft, Tulsa, USA) was used to analyze the different responses considering a 95% ($p < 0.05$) confidence interval after which the Tukey's test was used for comparison of means.

Fig. 1. The Computer Controlled Anaerobic Digester used in the study (Before loading).

3. Results and discussion

3.1. Structural and elemental characteristics of raw cocoa pod husk

After the grinding and sieving of the dried cocoa pod husk, sizes of 0.622, 0.401, 0.261, 0.109 and 0.073 mm were obtained out of which 0.401 mm was the most abundant and this was subsequently used in the acidic and alkaline pretreatments. The structural composition of the husk (Treated and untreated) samples (Table 1). The composition of L, C and H in the untreated not sifted biomass was 21.7 ± 0.01, 31.7 ± 0.10 and 27.0 ± 0.10 respectively while 19.2 ± 0.11, 29.2 ± 0.10 and 25.2 ± 0.01 were the composition of the three parameters in the untreated sifted sample respectively. These are in conformity with the earlier result from the different experiments to characterize the structural components of corn stalk (Cai et al., 2016; Venturin et al., 2018).

According to Table 2, Cocoa pod husk is very rich in nutrients making it a suitable biomass for anaerobic digestion. Also, the concentration of soluble sugar in the cocoa pod husk is moderate and these are fermentable during hydrolysis where they are converted to alcohols by microorganisms as a precursor to biogas formation. Different biomass used for biogas production have been characterized and also showed the presence of such nutrient elements (Dahunsi et al., 2016a; 2017c,d,e).

3.2. Characteristics of pretreated sifted cocoa pod husk

3.2.1. Acidic pretreatment

As shown in Table 3a, sulfuric acid treatment of Cocoa pod husk caused the H component of the biomass to be solubilized as evident in the breakdown of major chemical bonds in the biomass. Another observation was the depolymerization of the H through xylose hydrolysis in order to form monosaccharide. However, the other two structural components i.e. L and C remained largely unaffected by the actions of the acid but were rather strengthened as evident in their increased concentration.

From the experimental design of the acidic pretreatment, the most efficient condition that achieved the best results in terms of H solubilization was 2% (w/v) H_2SO_4, temperature of 121 °C and 4.01 g dry mass for an experimental duration of 60 min in the autoclave. By adopting this set condition, the H content of the biomass reduced from the initial 27.0 ± 0.01 to $8.5 \pm 0.01\%$ $m.m^{-1}$ after pretreatment which amount to a 69% reduction. At the same time, total L content increased from the initial 21.7 ± 0.01 to $28.6 \pm 0.20\%$ $m.m^{-1}$ which equals 24% increment while C also increased from 31.7 ± 0.10 to $49.0 \pm 0.01\%$ $m.m^{-1}$ which amount to 35% increment.

Table 1
Characterization of Cocoa pod husk and inoculum.

Parameter	Inoculum	Cellulose Standard	Pretreated Cocoa Pod husk		Untreated Cocoa pod husk	
			H_2SO_4	H_2O_2	Sifted	Not Sifted
pH (Sample + Inoculum)	7.79 ± 0.02	7.85 ± 0.10	7.88 ± 0.11	7.90 ± 0.10	7.82 ± 0.10^{a}	7.83 ± 0.10^{a}
Total solids (% $m.m^{-1}$)	4.2 ± 0.02	95.1 ± 1.00	88.9 ± 0.11	90.4 ± 0.01	96.5 ± 1.02^{b}	94.1 ± 0.02^{b}
Volatile solids (% $m.m^{-1}$)	3.0 ± 0.01	95.3 ± 2.00	85.9 ± 2.01	70.5 ± 1.02	92.6 ± 2.01^{c}	91.4 ± 0.01^{c}
Total Lignin (% $m.m^{-1}$)	29.5 ± 2.01	ND	28.6 ± 0.20	4.2 ± 0.02	19.2 ± 0.11^{d}	21.7 ± 0.01^{d}
Cellulose (% $m.m^{-1}$)	3.3 ± 0.01	99 ± 1.01	49.0 ± 0.01	39.8 ± 1.01	29.2 ± 0.10	31.7 ± 0.10
Hemicellulose (% $m.m^{-1}$)	20.9 ± 0.01	ND	8.5 ± 0.01	8.7 ± 0.11	25.2 ± 0.01	27.0 ± 0.10
Fixed solids (% $m.m^{-1}$)	1.4 ± 0.01	0 ± 0.00	1.9 ± 0.01	1.1 ± 0.01	3.8 ± 0.02	3.7 ± 0.10
Extractives (% $m.m^{-1}$)	ND	ND	ND	ND	17.9 ± 0.01	16.8 ± 0.02
Solids after pretreatment (% $m.m^{-1}$)	–	–	3.8 ± 0.00	2.4 ± 0.02	–	–
Added sample (g)	0 ± 0.00	1 ± 0.10	2.4 ± 0.12	2.3 ± 0.10	2.5 ± 0.00	2.4 ± 0.10
COD (g COD/g VS)	148.08 ± 1.10	ND	212.32 ± 1.20	204.62 ± 0.10	206.72 ± 5.00	211.05 ± 0.10
BP ($L_{Nbiogas.}$ Kg VS_{ad}^{-1})	25.6	617.4 ± 2.01	203.6 ± 2.10	633.2 ± 3.10	321.1 ± 3.10^{f}	382.4 ± 3.00
μmax ($L_{Nbiogas.}$ Kg VS_{ad}^{-1}).d^{-1}	ND	154.0 ± 2.02	222.6 ± 0.01	275.5 ± 2.10	44.5 ± 0.10^{g}	52.2 ± 0.10
Day of μmax	ND	3–4	4–6	1–2	4–6	3–4

Table 2
Elemental composition of Pretreated and Untreated Cocoa Pod husk, inoculum and Digestates.

Parameter	Inoculum	H_2SO_4 Pretreated Cocoa Pod husk		H_2O_2 Pretreated Cocoa Pod husk		Untreated Sifted Cocoa Pod husk		Untreated not Sifted Cocoa Pod husk	
		Pretreated biomass	Digestate	Pretreated biomass	Digestate	Untreated biomass	Digestate	Untreated biomass	Digestate
Ash Content (%)	5.56 ± 1.02	4.60 ± 0.01	5.51 ± 0.02	3.51 ± 1.00	4.16 ± 1.00	4.20 ± 0.01	4.31 ± 0.01	4.71 ± 0.00	4.77 ± 1.01
Moisture Content (%)	90.48 ± 3.02	81.19 ± 3.01	84.91 ± 2.01	93.21 ± 1.05	96.01 ± 3.02	80.51 ± 1.01	82.41 ± 3.05	87.2 ± 0.02	89.6 ± 4.01
Total Carbon (g/kg TS)	265.21 ± 0.10	635.09 ± 4.02	345.03 ± 3.01	722.55 ± 5.21	482.61 ± 4.11	436.11 ± 2.05	321.20 ± 3.05	435.04 ± 1.23	305.13 ± 5.21
Total Nitrogen (g/kg TS)	48.00 ± 2.02	27.56 ± 0.22	32.60 ± 1.02	30.04 ± 0.25	36.43 ± 1.51	21.04 ± 0.02	24.06 ± 2.01	22.43 ± 1.20	26.33 ± 3.20
C/N	6/1	23/1	11/1	24/1	13/1	21/1	13/1	19/1	12/1
Acetate (g COD/g VS)	1.04 ± 0.10	0.09 ± 0.01	0.03 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.04 ± 0.10	0.03 ± 0.10	0.04 ± 0.10	0.02 ± 0.01
Propionate (g COD/g VS)	1.07 ± 0.02	0.11 ± 0.01	0.6 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
TVFAs (g COD/g VS)	2.44 ± 0.10	1.19 ± 0.10	1.11 ± 0.00	1.12 ± 0.10	1.02 ± 0.10	0.11 ± 0.01	0.07 ± 0.01	1.02 ± 0.01	0.09 ± 0.01
Ammonia (mg/g VS)	4.97 ± 1.01	2.03 ± 0.10	1.01 ± 0.01	2.09 ± 0.03	2.00 ± 0.01	1.20 ± 0.01	1.03 ± 0.01	1.07 ± 0.10	1.03 ± 0.01
Uronic acids (% VS)	1.67 ± 1.11	2.56 ± 0.10	2.88 ± 0.10	2.08 ± 0.10	2.18 ± 0.10	1.61 ± 1.00	1.64 ± 0.00	1.01 ± 0.10	1.03 ± 0.10
[@]Soluble sugars (% VS)	4.02 ± 2.10	6.01 ± 0.11	7.03 ± 0.01	7.35 ± 0.10	7.83 ± 1.10	3.04 ± 1.00	3.11 ± 1.00	3.17 ± 0.10	3.21 ± 0.10
Phenols (mg L^{-1})	4.71 ± 2.10	0.005 ± 0.01	0.002 ± 0.01	0.003 ± 0.01	0.004 ± 0.00	0.001 ± 0.01	0.001 ± 0.01	0.001 ± 0.10	0.001 ± 0.00
Total Phosphorus (g/kg TS)	6.30 ± 0.02	4.64 ± 0.02	5.44 ± 0.05	5.58 ± 0.01	6.28 ± 0.02	3.00 ± 0.01	3.66 ± 0.02	3.37 ± 0.01	3.71 ± 0.03
Potassium (g/kg TS)	7.20 ± 0.11	7.2 ± 0.11	7.8 ± 1.01	9.23 ± 0.01	10.13 ± 0.02	3.03 ± 0.01	4.00 ± 0.02	3.25 ± 0.01	3.85 ± 0.02
Phosphate (g/g TS)	3.00 ± 0.02	3.30 ± 0.11	3.35 ± 0.01	3.40 ± 0.10	3.41 ± 0.01	1.04 ± 0.01	1.06 ± 0.01	1.20 ± 0.20	1.23 ± 0.10
Sulphate (g/kg TS)	134 ± 2.00	100.00 ± 3.00	104.30 ± 2.10	111.10 ± 3.01	112.11 ± 4.01	54.00 ± 2.00	54.30 ± 3.00	61.04 ± 1.02	62.41 ± 3.02
Calcium (g/kg TS)	80.00 ± 0.10	523.50 ± 1.42	366.11 ± 5.02	584.03 ± 5.01	334.13 ± 3.01	333.7 ± 0.22	200.5 ± 3.10	361.30 ± 2.03	212.02 ± 4.03
Magnesium (g/kg TS)	96.00 ± 0.10	47.50 ± 1.02	51.12 ± 3.02	70.10 ± 1.40	74.05 ± 2.20	35.22 ± 0.02	36.01 ± 2.02	39.40 ± 1.10	43.30 ± 3.10
Manganese (g/kg TS)	1.18 ± 0.22	0.014 ± 0.04	0.016 ± 0.02	0.020 ± 0.00	0.022 ± 1.00	0.009 ± 0.01	0.010 ± 0.01	0.010 ± 0.10	0.013 ± 0.10
Iron (g/kg TS)	1.18 ± 0.11	1.72 ± 0.01	1.93 ± 0.02	1.43 ± 0.01	1.46 ± 0.03	0.40 ± 0.01	0.41 ± 0.01	0.46 ± 0.01	0.55 ± 0.01
Zinc (g/kg TS)	38.00 ± 0.02	34.30 ± 0.02	36.70 ± 0.02	42.04 ± 0.01	44.03 ± 1.01	14.40 ± 0.02	14.42 ± 0.03	18.20 ± 0.01	18.41 ± 0.03
Aluminium (g/kg TS)	0.80 ± 0.11	1.27 ± 0.01	1.29 ± 0.01	1.43 ± 0.02	1.44 ± 0.01	0.15 ± 0.02	0.16 ± 0.02	0.20 ± 0.10	0.23 ± 0.10
Copper (g/kg TS)	4.80 ± 0.10	3.33 ± 0.11	3.76 ± 0.01	4.02 ± 0.10	4.03 ± 0.01	2.02 ± 0.10	2.03 ± 0.11	2.31 ± 0.10	2.34 ± 0.11

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

Table 3a
Structural composition of crushed and sifted Cocoa Pod husk 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 (%)	Desirability (%)
1	60.00	121.00	2.00	4.01	50.46	32.48	15.82	99.1
2	60.56	119.99	2.00	4.09	50.65	32.56	15.72	99.1
3	60.39	119.99	2.00	4.07	50.66	32.57	15.71	99.0
4	61.25	120.00	2.00	4.19	50.54	32.48	15.79	99.0
5	61.28	120.00	2.00	4.46	50.60	32.49	15.76	99.0
6	50.58	120.00	2.00	4.10	50.93	32.64	15.58	99.0
7	51.56	120.00	2.00	4.14	50.33	32.39	15.89	99.0
8	69.86	120.00	2.00	4.11	50.48	32.54	15.77	99.0
9	50.76	120.00	1.99	4.19	50.48	32.50	15.77	99.0
10	40.80	120.00	2.00	3.73	49.92	32.30	16.05	99.0
11	42.45	120.00	2.00	4.21	50.11	32.26	15.99	98.9
12	40.64	120.00	2.00	3.43	49.61	32.19	16.16	98.9
13	41.88	120.00	2.00	3.48	49.44	32.08	16.27	98.8
14	50.77	120.00	2.00	5.36	51.40	32.74	15.29	98.7
15	50.78	120.00	1.98	4.56	50.62	32.56	15.62	98.7
16	51.88	120.00	2.00	5.22	51.09	32.55	15.45	98.6
17	41.83	120.00	2.00	5.30	51.16	32.57	15.41	98.4
18	39.08	120.00	2.00	5.90	51.95	32.9	14.86	98.3
19	60.82	120.00	2.00	2.87	48.83	31.89	16.43	98.3
20	53.41	119.98	2.00	2.93	48.37	31.62	16.68	98.2
21	44.89	120.00	2.00	4.08	49.27	31.77	16.25	98.2
22	38.75	120.00	2.00	6.38	52.20	33.05	14.60	97.9
23	44.35	120.00	2.00	2.87	48.03	31.45	16.79	97.6
24	39.23	119.30	1.97	5.12	50.97	32.58	15.10	97.6
25	41.58	120.00	2.00	2.02	47.43	31.32	16.89	97.3
26	36.81	119.34	2.00	3.44	49.65	32.11	15.59	96.5
27	35.15	120.00	2.00	3.40	49.79	32.26	15.46	96.4
28	45.00	120.00	1.91	4.51	49.08	31.75	15.88	96.1
29	42.88	118.98	2.00	2.00	46.68	30.81	16.88	93.7
30	40.69	120.00	2.00	7.80	52.19	32.56	14.09	92.7

These results agrees with earlier reports (Baadhe et al., 2014; Cai et al., 2016; Venturin et al., 2018) in which H was almost completely removed with the use of acids for corn stalk pretreatment. The improvement observed in this study was the use of lower acid volume to achieve higher solubilization of H. Another effect of the acid on Cocoa pod husk was the increase in the concentration of L and C which is very similar to the submission of Cai et al. (2016) in which corn stalk lost 87% of its H content while both L and C increased by 25% each when acidic pretreatment was carried out on the stalk. Similar results were obtained by other authors after the

application of acidic pretreatment to different biomass (Guo et al., 2011). The effect of the acid on L was further shown as the important chemical groups associated with L i.e. the 1734, 1716, 1633, and 1604 cm^{-1} bands were all reduced after the pretreatment. C was also modified after the acidic pretreatment as a result of exposure to environmental factors. This agrees with the results of Cai et al., (2016) and Zhao et al. (2018).

3.2.2. Alkaline pretreatment

The use of alkaline H_2O_2 in pretreating the biomass also achieved a commendable structural deconstruction as seen in the results of the acidic pretreatment. The only difference is that L was the target of solubilization by hydrogen peroxide while the effect on H was partial. From the different runs that were experimented, the most efficient in Cocoa pod husk deconstruction for the purpose of L reduction was 7.5% (w/v) H_2O_2, temperature of 30 °C, agitation at 130 rpm for 75 min using 3 g of biomass. By using these set of values, the L composition was reduced from the initial 17.8 to 5.1% $m.m^{-1}$ amounting to 71.34% reduction, C was increased from 26.6 to 43.3% $m.m^{-1}$ which equals 39% increment in value while reduction was observed for H from 22.8 to 8.8% $m.m^{-1}$ i.e. 39% reduction as shown in Table 3b.

The effect of H_2O_2 on the Cocoa pod husk was enormous as pronounced breakdown of L bonds was seen to have taken place. This was most evident in the flattening/rupturing and complete disappearance in some cases of all the bands commonly associated with L which include the 1734, 1716, 1633, 1604 and 1516 cm^{-1} thereby resulting in the high solubilization of L. It has been widely reported that alkalis (H_2O_2, NaOH, and KOH) causes L reduction (Dahunsi et al., 2016a; 2017c). Specifically, only few studies have reported the use H_2O_2 for pretreatment of biomass. In Cai et al. (2016), corn straw was pretreated with hydrogen peroxide and this caused reduction of 19.6, 32.8 and 6.2% respectively in the composition of L, C and H. In another study, Sun et al. (2013) also pretreated corn straw with H_2O_2 and reported 38.9% L reduction with increase of 31.4 and 33.3% respectively in the composition of C and H.

3.3. Structural changes in cocoa pod husk

As shown in Table 4, the application of both acidic and alkaline pretreatments has profound effect on the molecular structure of the pretreated biomass which also created a wide variation between the pretreated and not treated samples of Cocoa pod husk. All the bands revealed by the FTIR spectroscopy were between 3348 and 2900 cm^{-1} signifying the presence of bonds of C. Due to the application of alkaline treatment, there was increase in the cellulosic content of the biomass by 20.8% as well as an increase in absorbance to by 52.5%. Also, there was an increase in the 1373 cm^{-1}. By using acidic pretreatment, the cellulosic O–H bonds in the 3448 cm^{-1} band were reduced. In all, over 75% reduction in the L and C ratio was obtained via the use of the hydrogen peroxide for pretreatment of Cocoa pod husk in this study but an increase in this ratio was observed with the use of acidic pretreatment. The increase in L content observed in the AcP biomass was caused by the formation of pseudo-L. This trend has been reported in previous studies (Cai et al., 2016) and the effect is inhibition of anaerobic digestion which ultimately affects both the rate and final volume of biogas.

3.4. Composition of microbes and dynamics of volatile fatty acids (VFAs)

Several groups of microorganisms were identified throughout the stages of digestion in this study. They include aerobes such as *Bacillus pantothenticus, Bacillus stearothermophilus, Serratia plymuthica* and *Proteus vulgaris* while *Clostridium clostridioforme, Fusobacterium mortiferum* and *Porphyromonas assacharolyticum*

Table 3b
Structural composition of crushed and sifted Cocoa Pod husk 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 (%)	Hemi cellulose (%)	Desirability (%)
1	75.00	30.00	130.00	7.50	3.00	14.84	36.29	15.58	100
2	76.66	30.00	130.03	7.50	3.01	14.47	36.63	15.34	100
3	70.67	30.00	130.44	7.43	3.00	14.85	35.98	15.58	100
4	71.05	31.52	130.00	7.50	3.00	14.57	35.79	15.61	100
5	71.66	30.06	131.13	7.50	3.00	14.81	35.63	15.54	100
6	79.39	30.00	130.00	7.24	3.73	15.02	33.97	15.78	100
7	71.27	30.00	133.33	7.50	3.00	14.25	34.89	15.17	100
8	67.65	30.02	130.01	5.55	3.00	14.08	34.93	15.21	100
9	61.83	62.00	170.00	1.50	15.00	13.46	35.89	15.07	100
10	63.46	62.00	170.00	1.51	15.00	13.33	36.07	15.03	100
11	57.60	61.74	170.00	1.50	15.00	13.67	35.27	15.14	98.5
12	65.82	61.44	169.98	1.50	15.00	13.06	36.23	14.97	97.0
13	73.50	30.00	130.00	6.84	4.07	13.81	34.38	15.30	96.2
14	84.94	30.00	130.00	7.50	3.74	15.78	33.08	15.99	94.8
15	80.27	32.14	131.08	7.50	3.76	14.30	33.31	15.54	94.4
16	60.21	62.00	169.21	1.50	14.83	13.22	35.26	14.93	93.6
17	57.67	30.00	130.00	5.73	3.00	13.18	36.25	14.38	90.5
18	62.04	60.54	170.00	1.50	14.74	12.94	35.43	15.01	89.3
19	51.66	62.00	169.93	1.60	14.84	13.72	33.92	14.99	88.7
20	65.50	61.97	169.50	1.50	14.28	12.52	35.39	14.71	87.0
21	53.05	30.14	130.00	1.52	3.00	15.11	33.61	14.09	86.7
22	54.38	30.00	131.51	2.07	3.00	14.30	33.38	14.11	85.5
23	57.28	30.00	130.00	1.53	3.35	14.76	32.79	14.37	83.1
24	55.01	30.00	131.49	1.50	3.11	14.68	32.77	14.21	82.6
25	61.20	53.55	170.00	1.50	14.49	11.60	34.19	15.00	81.7
26	57.01	44.53	170.00	1.50	15.00	10.99	33.58	15.28	80.2
27	61.86	42.96	170.00	1.50	14.85	10.60	33.48	15.24	79.9
28	52.33	33.21	169.99	1.50	15.00	10.38	32.95	15.39	78.3
29	53.75	34.47	170.00	1.51	14.91	10.35	32.91	15.37	77.3
30	55.80	33.69	170.00	1.50	14.87	10.29	32.87	15.41	72.6

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 Cocoa Pod husk (Dahunsi et al., 2019).

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)	0.3261	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 (Iα)	0.0188	0.0115	38.8	0.0280	−48.9
719	Crystalline cellulose (Iβ)	0.0344	0.0271	21.2	0.0372	−8.1
771/719	Ratio of crystalline cellulose polymorphs (Iα/Iβ)	0.3	0.2	33.3	0.4	−33.3

ND = Not determined; All positive values indicates decrease.

were the anaerobes. The identified methane producers include members of the genera *Methanobacteriales, Methanosaetaceae* and *Methanosarcinaceae.* This diversity and microbial population caused enormous microbial activities i.e. production of intermediate acids which resulted in the production and accumulation of VFAs. Such acid causes the inhibition of anaerobic digestion processes especially when they are in high quantities or are not properly consumed by the microbial community. In this study, the profound VFAs which were slightly accumulated in the digesters are acetate and propionate. They however reached peak of accumulation between the 12th 15th days of experiment. This indicates an imbalance between the stages involved in the digestion process. The types and concentration of volatile fatty acids (VFA)'s reported in this study have been previously reported (Riggio et al., 2017). As shown in the diversity of microorganisms implicated in this study which were dominated by members of the genera *Clostridia*, the last two stages of digestion were pronounced. *Clostridium* species can efficiently break down amino-acids so as to produce acetic and propionic acids coupled with ammonia as end-product (Degueurce et al., 2016). The microbes also consumed the VS maximally due to their high population.

3.5. Stoichiometry and mass balance

In computing the mass balance in all digestions carried out in this study, the mixture of the main substrate i.e. Cocoa pod husk and inoculum was used as the input variable while all the products of the anaerobic digestion process i.e. CH_4, CO_2 and the anaerobic digestate were used as the output variables. Based on computation, the mass balance for each of the AcP, AlP and the NsU Cocoa pod husk is 0.25, 0.39 and 0.33 respectively while the VS consumption/removal in all three experiments was 35, 52 and 41% respectively.

3.6. Results of biogas potential test

The result of the biogas potential showed that the inoculum only produced less than 10% of total biogas production from all experiment. This shows that the inoculum used in this study has low biogas-producing potential under constant operational condition. The average total biogas volume from all the four experiments i.e. the AcP, AlP, NsU and SU is 162.8 ± 5.0, 564.8 ± 5.1, 243.3 ± 4.1 and 220.8 ± 3.3 respectively. This show that the hydrogen peroxide treated Cocoa pod husk yielded the highest biogas volume followed by the NsU Cocoa pod husk and the SU husk while the lowest was obtained from the sulfuric acid treated biomass. Overall, the AlP biomass produced 71% more total biogas than the AcP biomass and also produced 57% more biogas than the NsU Cocoa pod husk.

The AlP Cocoa pod husk did not only produce the highest biogas volume but also achieved peak of production faster than all the other experimental setups. Total biogas generation was achieved in just 12 days out of the 30-day retention period used in the study in which biogas production started on the 3rd day and climaxed on the 15th experimental day whereas, biogas generation did not commence until after the 5th and 6th days in other experiments and climax was not reached until between the 18th and 21st days (Fig. 2). The higher volume of biogas produced by the AlP biomass over other treatments indicate that the highest treatment efficiency was achieved with the use of alkali over acid. A similar finding has been earlier reported (Venturin et al., 2018). This calls for the need to pretreat biomass before anaerobic digestion as this will affect the rate of biogas generation as well as the time taken to reach production peak (Mancini et al., 2018). From the analysis of all produced biogas, the average methane content between 58.2 ± 4.1 and 65.2 ± 5.1 while the carbon dioxide content ranged between 21.4 ± 1.5 and 23.5 ± 0.5.

3.7. RSM optimization of pretreatment

The coefficients of the model equation and their statistical significance were evaluated in this study. The significance test and the ANOVA for all regression coefficients are as shown in Tables 5 and 6. Most of the model terms are significant based on the F-values and p-values. For the acidic pretreatment, the Model F-values of 0.65, 0.54 and 0.85 for L, C and H respectively implies significance of the model. For the optimization of L reduction, the linear terms of Q, *R, OQ, OR, PQ, PR,* O^2 and P^2, were the most significant model terms ($p < 0.05$). For the optimization of C reduction, Q, *R, OQ, OR, PR, QR,* Q^2 and R^2 were the most significant terms while for the optimization of H reduction, the most significant term were *O, OP, OQ, OR, PQ, PR, QR,* O^2, P^2, and Q^2 (Table 5a). In the pretreatment by alkaline

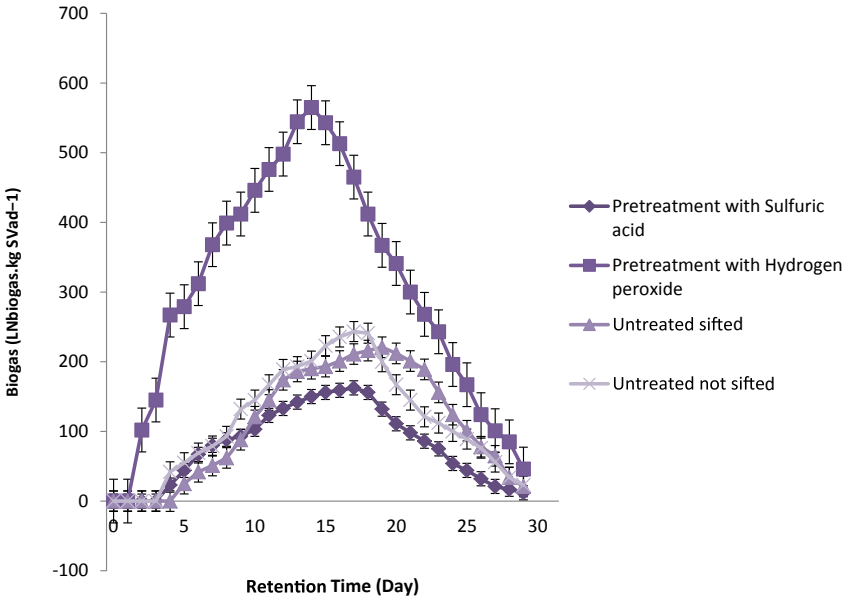

Fig. 2. Cumulative biogas production per kilogram of added volatile solids for H_2S_2 pretreated, H_2SO_4 pretreated, untreated sifted and untreated not sifted Cocoa pod husk (Error bars are showing Standard errors).

Table 5
Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for Sulfuric pretreatment of Cocoa Pod husk.

Source	df	Lignin				Cellulose				Hemicellulose			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value
O	1	721.50	721.50	2.54	0.2123	303.95	303.95	2.13	0.8750	46.00	46.00	2.03	**0.0144**
P	1	114.35	114.35	0.40	0.3017	27.11	27.11	0.19	0.1649	5.17	5.17	0.23	0.0628
Q	1	38.61	38.61	0.14	**0.0623**	16.71	16.71	0.12	**0.0090**	7.24	7.24	0.32	0.1086
R	1	17.59	17.59	0.06	**0.0222**	3.77	3.77	0.03	**0.0152**	32.92	32.92	1.45	0.1252
OP	1	67.19	67.19	0.24	0.3112	17.93	17.93	0.13	0.3312	15.35	15.35	0.68	**0.0322**
OQ	1	18.85	18.85	0.07	**0.1165**	15.87	15.87	0.11	**0.0211**	1.56	1.56	0.07	**0.0214**
OR	1	1.84	1.84	6.45	**0.0301**	41.14	41.14	0.29	**0.0461**	5.25	5.25	0.23	**0.0162**
PQ	1	53.70	53.70	0.19	**0.0445**	83.55	83.55	0.59	0.1100	3.18	3.18	0.14	**0.0102**
PR	1	3.43	3.43	0.02	**0.0400**	2.15	2.15	0.02	**0.0112**	13.58	13.58	0.60	**0.0312**
QR	1	11.84	11.84	0.04	0.1163	1.34	1.34	9.38	**0.0276**	12.04	12.04	0.53	**0.0276**
O^2	1	9389.38	9389.38	3.30	**0.0002**	394.20	394.20	2.77	0.2842	25.08	25.08	1.11	**0.0311**
P^2	1	5.10	5.10	0.12	**0.0011**	29.22	29.22	0.20	0.2331	0.10	0.10	4.53	**0.0331**
QC^2	1	28.59	28.59	0.10	0.2249	11.92	11.92	0.08	**0.0120**	0.06	0.06	2.83	**0.0212**
R^2	1	75.27	75.27	0.26	0.1220	25.98	25.98	0.18	**0.0102**	0.14	0.14	6.00	0.5107
Model	14	2575.81	183.99	0.65	**0.0004**	1068.03	76.29	0.54	**0.0261**	269.33	19.24	0.85	**0.0411**
Residual	15	4264.43	284.28			2138.50	142.57			340.10	22.67		
Lack of Fit	7	2393.43	341.92	1.46	0.0788	1436.57	205.22	2.34	0.1012	245.64	35.09	2.97	0.0733
Pure Error	8	11.22	4.46			9.83	30.23			14.46	11.81		
R-Squared		0.9902				0.9859				0.9777			
Adequate Precision		13.67				16.27				15.59			

df = degree of freedom; SS = Sum of square; MS = Mean square.

hydrogen peroxide as shown in Table 5b, the Model F-values of 0.57, 0.38 and 0.53 for L, C and H respectively signifies that the model is significant. For the optimization of L reduction, the model terms *O, P, S, OP, OQ, OR, OS, PQ, QR, QS, RS,* R^2 and S^2 were the most significant. For the optimization of C reduction, the terms *O, Q, R, S, OP, OQ, OS, PQ, PR, QS, RS,* O^2, P^2 and Q^2 were the significant ones while for H reduction optimization, the significant terms were *P, Q, R, S, OR, OS, PS, QR, QS, RS,* O^2, R^2 and S^2. The values obtained for the 'Adequate Precision' i.e. 13.67, 16.27 and 15.59 for L, C and H in the acidic pretreatment gives an indication that the signal is adequatel and can be used to navigate the design space. Similarly, the values of 16.63, 14.46 and 18.03 obtained for the three structural components after alkaline pretreatment indicates adequate signal.

The R^2 (coefficient of determination) value obtained was used to check the goodness of fit of the model. In doing this, the Lack of Fit F-values of 1.46, 2.34 and 2.97 for the optimization of L, C and H reductions respectively in the acidic pretreatment implies non significance. In these cases, non-significant lack of fits is good thus making the model fit. In the alkaline pretreatment, the values of 3.38, 3.22 and 3.16 obtained from the optimization of the reduction of the three structural components also show non-significance. The developed regression model equations describing the relationship between the response i.e. percentage reduction in each structural material and the coded values of independent factors [Exposure time (*O*), Temperature (*P*), Acid concentration (*Q*) and Dry mass (*R*)] and their respective interactions for the acidic pretreatment are described in the following equation:

Final equation in terms of coded factors:

Table 6
Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for Hydrogen peroxide pretreatment of Cocoa Pod husk.

Source	df	Lignin				Cellulose				Hemicellulose			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value
O	1	5.04	5.04	0.19	**0.0143**	7.15	7.15	0.11	**0.1044**	1.31	1.31	0.14	0.3211
P	1	22.04	22.04	0.85	**0.0597**	4.08	4.08	0.07	0.3403	5.80	5.80	0.63	**0.0224**
Q	1	0.03	0.03	1.03	0.8202	4.77	4.77	0.08	**0.0044**	0.60	0.60	0.07	**0.0123**
R	1	23.64	23.64	0.91	0.0742	2.94	2.94	0.05	**0.0423**	9.60	9.60	1.05	**0.0422**
S	1	25.22	25.22	0.97	**0.0302**	8.02	8.02	0.13	**0.0012**	4.29	4.29	0.47	**0.0103**
OP	1	3.61	3.61	0.14	**0.0022**	56.63	56.63	0.91	**0.0312**	0.49	0.49	0.05	0.1043
OQ	1	2.72	2.72	0.10	**0.0303**	32.21	32.21	0.52	**0.0003**	4.41	4.41	0.48	0.1611
OR	1	39.69	39.69	1.53	**0.0148**	37.52	37.52	0.60	0.3024	2.10	2.10	0.23	**0.0102**
OS	1	2.25	2.25	0.09	**0.0212**	0.23	0.23	3.62	**0.0210**	0.36	0.36	0.04	**0.0212**
PQ	1	11.56	11.56	0.44	**0.0214**	31.08	31.08	0.50	**0.0021**	11.90	11.90	1.30	0.2018
PR	1	7.02	7.02	0.27	**0.1157**	18.28	18.28	0.29	**0.0012**	4.84	4.84	0.53	0.1571
PS	1	11.90	11.90	0.46	0.1061	1.89	1.89	0.03	0.2001	0.30	0.30	0.03	**0.0162**
QR	1	0.36	0.36	0.02	**0.2101**	17.85	17.85	0.29	0.4620	18.49	18.49	2.01	**0.0111**
QS	1	10.89	10.89	0.42	**0.0107**	74.39	74.39	1.19	**0.0103**	0.90	0.90	0.10	**0.0075**
RS	1	6.00	6.00	0.23	**0.0300**	32.78	32.78	0.53	**0.0110**	2.56	2.56	0.28	**0.0300**
O^2	1	3.12	3.12	0.12	0.3786	42.91	42.91	0.69	**0.0276**	9.63	9.63	1.05	**0.0306**
P^2	1	17.12	17.12	0.66	0.1027	2.67	2.67	0.04	**0.0122**	0.16	0.16	0.02	0.1027
Q	1	73.61	73.61	2.83	0.3112	37.63	37.63	0.60	**0.0113**	4.16	4.16	0.45	0.1012
R^2	1	7.19	7.19	0.28	**0.0149**	1.82	1.82	0.03	0.0972	0.20	0.20	0.02	**0.0131**
S^2	1	48.82	48.82	1.88	**0.0202**	11.74	11.74	0.19	0.0907	9.50	9.50	1.04	**0.0220**
Model	14	297.71	14.89	0.57	**0.0209**	478.90	23.95	0.38	**0.0222**	97.21	4.85	0.53	**0.0043**
Residual	9	156.04	26.01			374.25	21.91			55.08	9.18		
Lack of Fit	6	341.31	301.23	3.38	0.1203	235.12	201.21	3.22	0.0834	223.02	115.11	3.16	0.1120
Pure Error	3	21.34	22.02			34.01	16.91			18.09	12.64		
R-Squared		0.9912				0.9899				0.9939			
Adequate Precision		16.63				14.46				18.03			

df = degree of freedom; SS = Sum of square; MS = Mean square.

$$L = 39.19 + 9.16O + 2.66P + 2.61Q + 1.33R + 2.26OP - 1.85OQ - 0.39O + 2.66PQ - 0.53PR + 1.44QR - 8.25O2 + 0.42P^2 + 1.81Q2 - 2.29R^2 \quad (2)$$

$$C = 25.66 + 5.95O + 1.29P + 1.71Q + 0.62R + 1.17OP - 1.70OQ - 1.83OR + 3.32PQ + 0.42PR + 0.48QR - 5.34O^2 + 1.01P^2 - 1.17Q2 - 1.35R^2 \quad (3)$$

$$H = 10.23 + 2.31O + 0.56P + 1.13Q - 1.82R + 1.08OP + 0.53OQ - 5OR + 0.65PQ - 1.05PR + 1.45QR - 1.35O^2 + 0.060P^2 + 0.086Q^2 - 0.098R^2 \quad (4)$$

The model equations describing the relationship between the response i.e. percentage reduction in each of L, C and H and the coded values of independent factors [Exposure time (*O*), Temperature (*P*), Agitation (*Q*), Dry mass (*R*) and Alkaline concentration (*S*)] and their respective interactions for the acidic pretreatment are described in the following equation:

$$L = 4.89 + 0.46O - 0.96P - 0.033Q - 1.13R - 1.16S - 0.48OP - 0.41OQ + 1.57OR + 0.38OS + 0.85PQ - 0.66PR + 0.86PS + 0.15QR + 0.83QS - 0.61RS - 0.38O^2 + 0.88P^2 + 1.83Q^2 + 0.73R^2 + 1.90S \quad (5)$$

$$C = 26.81 - 0.55O - 0.41P - 0.45Q - 0.40R + 0.66S + 1.88OP + 1.42OQ + 1.53OR + 0.12OS + 1.39PQ - 1.07PR + 0.34PS - 1.06QR + 2.16QS - 1.43RS - 1.40O^2 + 0.35P^2 + 1.31Q^2 - 0.37R^2 + 0.93S^2 \quad (6)$$

$$H = 11.83 + 0.23O - 0.49P - 0.16Q - 0.72R - 0.48S - 0.17OP - 53OQ + 0.36OR - 0.15OS + 0.86PQ + 0.55PR - 0.14PS - 1.07QR + 0.24QS - 0.40RS - 0.66O^2 + 0.086P^2 + 0.44Q^2 + 0.12R^2 + 0.84S^2 \quad (7)$$

The three-dimensional (3D) response surface plots which are graphical representations of the above regression equations showing the interactions between all the variables in the optimization of the acidic and alkaline pretreatment procedures are represented in figures S1 and S2 (Supplementary materials).

In the optimization of acidic and alkaline pretreatments of Cocoa pod husk in this study, the significance of the regression models were validated using the F-values with their respective p-values. These values coupled with the coefficient of determination (R^2) showed significance for all the models. To further determine suitability of the models for the experimental design, the 'adequate precision' was employed. Usually, a value of 4 or more is required for a model to be fit for usage in an experiment. In this study, the values of 13.67, 16.27 and 15.59 for L, C and H in the acidic pretreatment and 16.63, 14.46 and 18.03 obtained for the three structural components after alkaline pretreatment indicates good fit, suitability, adequate signal and high significance of the models by all the significant model terms with $p < 0.05$. The lack-of-fit terms of 1.46, 2.34 and 2.97 for the optimization of L, C and H

reductions respectively in the acidic pretreatment and 3.38, 3.22 and 3.16 for the alkaline pretreatment all implies non significance. Non significant lack of fit terms signifies the goodness of a model. Considering the curvature nature of all the 3-Dimensional plots for the optimization of L, C and H reductions respectively in the acidic pretreatment, there was moderate to high interactions among the process parameters i.e. exposure time, temperature, acid concentration and dry mass while lesser interactions were shown among the process parameters used in the alkaline pretreatment i.e. exposure time, temperature, agitation, dry mass and alkali concentration. This kind of interaction have been previously documented (Dahunsi et al., 2017c,d). In order to estimate the accuracy of the models in this study, the mean squared error (RSME) and the R^2 values were used. All the R^2 values i.e. 0.9902, 0.9859 and 0.9777 from the acidic pretreatment and 0.9912, 0.9899 and 0.9939 from the alkaline pretreatment showed high accuracy and suitability for the respective experiments.

In order to validate the results of this study, new experiments were carried out entirely. In these, fresh samples of Cocoa pod husk were pretreated using the optimal values obtained earlier in the study and deconstruction of L in the alkaline pretreatment i.e. 2% (w/v) H_2SO_4, temperature of 121 °C and 4.01 g dry mass for 60 min in the autoclave for the most efficient solubilization of H in the acidic pretreatment and 7.5% (w/v) H_2O_2, temperature of 30 °C, agitation at 130 rpm for 75 min using 3 g of biomass for the deconstruction of L. In the acidic pretreatment, the validated results for the optimal solubilization of H was 2.2% (w/v) H_2SO_4, temperature of 120 °C and 4.25 g biomass for an experimental duration of 62 min in the autoclave. Whereas for the alkaline pretreatment, the validated result for the optimal deconstruction/reduction of L was 7.4% (w/v) H_2O_2, temperature of 32 °C, agitation at 133 rpm for 71 min using 3.1 g biomass. All these values are very close to the earlier reported values in the main experiments. This further confirmed the accuracy and suitability of the models for use in the optimization of acidic and alkaline pretreatments of Cocoa pod husk.

3.8. Digestate composition

All the digestates in this study were nutrient and microbiologically rich. Virtually all the major and minor element present in the digestates had their values higher than it was in the initial biomass before anaerobic digestion (Table 2). There was also increase in the L to C–H complex ratio. Considering the quality of digestates obtained after the anaerobic digestions in this study, they could be useful as biofertilizers or soil conditioners when applied and this is due to the fact that they are enormously rich in nutrients and microbial inoculants needed by the soil for crop plant' wellbeing. This will have more practicability in Sub-Saharan African nations that are currently bedevilled with multiple menace of depletion/loss of soil nutrient, soil pollution, toxicity to soil microorganisms due to overuse of chemical fertilizers. Researches proposing the use of digestates as biofertilizer abound in the literature (Westphal et al., 2016; Dahunsi et al., 2017a,e).

4. Conclusions

As seen in this study, the efficacy of hydrogen peroxide alkaline pretreatment of Cocoa pod husk was established over the use of sulfuric acid for the same purpose. All the structural components of the pod experienced solubilization with the use of the alkali and this led to an upsurge in biogas volume and shortening of retention time. Overall, the alkaline treated biomass produced more total biogas than the AcP biomass and also more than the NsU Cocoa pod husk. The Response Surface Methodology has also been shown to be a suitable model for the optimization of biogas generation from the biomass used in this study. Therefore, further use of alkaline pretreatment for Cocoa pod husk under different condition as well as for other biomass for the purpose of biogas generation is hereby advocated.

Funding Source

This research received funding from the Ton Duc Thang University, Ho Chi Minh City, Vietnam.

Conflicts of interest

The author declares no conflict of interest whatsoever.

Acknowledgments

The author appreciates the efforts of the technical staffs in the Environmental Engineering Laboratory of Landmark University, Omu-Aran, Nigeria.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2019.04.112.

List of abbreviations

L =	lignin
C =	cellulose
H =	hemicellulose
AcP =	acidic pretreated
AlP =	alkaline pretreated
NsU =	not sifted untreated
SU =	sifted untreated
TS =	total solids
VS =	volatile solids

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.

References

APHA, 2012. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC, USA.

Baadhe, R.R., Potumarthi, R., Mekala, N.K., 2014. Influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by crude enzymatic method: a comparative study. Bioresour. Technol. 162, 213–217.

Bazoti, S.F., Golunski, S., Pereira, S.D., Scapini, T., Barrilli, É.T., Alex, M.D., Barros, K.O., Rosa, C.A., Stambuk, B.U., Alves, S.L., Valério, A., de Oliveira, D., Treichel, H., 2017. Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain. Bioresour. Technol. 244, 582–587.

Boon, N., De Windt, W., Verstraete, W., Top, E.M., 2002. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. FEMS Microbiol. Ecol. 39, 101–112.

Brunklaus, B., Rex, E., Carlsson, E., Berlin, J., 2018. The future of Swedish food waste: an environmental assessment of existing and prospective valorization techniques. J. Clean. Prod. 202, 1–10.

Cai, D., Li, P., Chen, C., Wang, Y., Hu, S., Cui, C., Qin, P., Tan, T., 2016. Effect of chemical pretreatments on corn stalk bagasse as immobilizing carrier of *Clostridium acetobutylicum* in the performance of a fermentation-pervaporation coupled system. Bioresour. Technol. 220, 68–75.

Chen, X.Y., Vinh-Thang, H., Ramirez, A.A., Rodrigue, D., Kaliaquine, S., 2015. Membrane gas separation technologies for biogas upgrading. RSC Adv. 5, 24399–24448.

Dahunsi, S.O., Olayanju, A., Izebere, J.O., Oluyori, A.P., 2018a. Data on Energy and Economic evaluation and microbial assessment of anaerobic co-digestion of fruit rind of *Telfairia occidentalis* (Fluted Pumpkin) and Poultry Manure. Data Brief 21, 97–104.

Dahunsi, S.O., Oranusi, O., Efeovbokhan, V.E., 2017a. Anaerobic mono-digestion of *Tithoniadiversifolia* (wild Mexican sunflower). Energy Convers. Manag. 148, 128–145.
Dahunsi, S.O., Oranusi, O., Efeovbokhan, V.E., 2017b. Pretreatment optimization, Process control, Mass and Energy balances and Economics of anaerobic co-digestion of *Arachis hypogaea* (Peanut) hull and poultry manure. Bioresour. Technol. 241, 454–464.
Dahunsi, S.O., Oranusi, S., Efeovbokhan, V.E., 2017c. Bioconversion of *Tithonia diversifolia* (Mexican Sunflower) and poultry droppings for energy generation: optimization, mass and energy balances, and economic benefits. Energy Fuels 31, 5145–5157.
Dahunsi, S.O., Oranusi, S., Efeovbokhan, V.E., 2017d. Cleaner energy for cleaner production: modeling and optimization of biogas generation from *Carica papayas* (Pawpaw) fruit peels. J. Clean. Prod. 156, 19–29.
Dahunsi, S.O., Oranusi, S., Efeovbokhan, V.E., 2017e. Optimization of pretreatment, process performance, Mass and Energy balance in the anaerobic digestion of *Arachis hypogaea* (Peanut) hull. Energy Convers. Manag. 139, 260–275.
Dahunsi, S.O., Oranusi, S., Efeovbokhan, V.E., Olayanju, A., Zahedi, S., Ojediran, J.O., Izebere, J.O., Aladegboye, O.J., 2018b. Anaerobic conversion of *Chromolaena odorata* (Siam weed) to biogas. Energy Rep. 4, 691–700.
Dahunsi, S.O., Oranusi, S., Efeovbokhan, V.E., Zahedi, S., Ojediran, J.O., Olayanju, A., Oluyori, A.P., Adekanye, T.A., Izebere, J.O., Enyinnaya, M., 2018c. Biochemical conversion of fruit rind of *Telfairia occidentalis* (fluted pumpkin) and poultry manure. Energy Sources (Part A) Utilization Environ. Effects 40 (23), 2799–2811.
Dahunsi, S.O., Oranusi, S., Owolabi, J.B., Efeovbokhan, V.E., 2017f. Synergy of Siam weed (*Chromolaena odorata*) and poultry manure for energy generation: effects of pretreatment methods, modeling and process optimization. Bioresour. Technol. 225, 409–417.
Dahunsi, S.O., Oranusi, S., Owolabi, J.B., Efeovbokhan, V.E., 2016a. Mesophilic anaerobic co-digestion of poultry droppings and *Carica papaya* peels: modelling and process parameter optimization study. Bioresour. Technol. 216, 587–600.
Dahunsi, S.O., Oranusi, S., Owolabi, J.B., Efeovbokhan, V.E., 2016b. Comparative biogas generation from fruit peels of Fluted Pumpkin (*Telfairia occidentalis*) and its optimization. Bioresour. Technol. 221, 517–525.
Dahunsi, S.O., Osueke, C.O., Olayanju, T.M.A., Lawal, A.I., 2019. Co-digestion of *Theobroma cacao* (Cocoa) pod husk and poultry manure for energy generation: effects of pretreatment methods. Bioresour. Technol. 283, 229–241.
Degueurce, A., Tomas, N., Le Roux, S., Martinez, J., Peu, P., 2016. Biotic and abiotic roles of leachate recirculation in batch mode solid-state anaerobic digestion of cattle manure. Bioresour. Technol. 200, 388–395.
Dollhofera, V., Dandikas, V., Dorn-Inc, S., Bauera, C., Lebuhna, M., Bauer, J., 2018. Accelerated biogas production from lignocellulosic biomass after pretreatment with *Neocallimastix frontalis*. Bioresour. Technol. 264, 219–227.
Ferella, F., Puca, A., Taglieri, G., Rossi, L., Gallucci, K., 2017. Separation of carbon dioxide for biogas upgrading to biomethane. J. Clean. Prod. 164, 1205–1218.
Finnish Standard Association SFS 3008, 1990. Determination of Total Residue and Total Fixed Residue in Water, Sludge, and sediment.
Food and Agriculture Organization of the United Nations Statistics Division, 2017.
Gerbrandt, K., Chu, P.L., Simmonds, A., Mullins, K.A., MacLean, H.L., Griffin, W.M., Saville, B.A., 2016. Life cycle assessment of lignocellulosic ethanol: a review of key factors and methods affecting calculated GHG emissions and energy use. Curr. Opin. Biotechnol. 38, 63–70.
Guo, P., Mochidzuki, K., Cheng, W., Zhou, M., Gao, H., Zheng, D., Wang, X., Cui, Z., 2011. Effects of different pretreatment strategies on corn stalk acidogenic fermentation using a microbial consortium. Bioresour. Technol. 102, 7526–7531.
Kalbar, P.P., Karmakar, S., Asolekar, S.R., 2016. Life cycle-based decision support tool for selection of wastewater treatment alternatives. J. Clean. Prod. 117, 64–72.
Kaufman, T., Justeson, J., 2006. History of the word for 'cacao' and related terms in ancient meso-America. Cameron L. McNeil. Chocolate in Mesoamerica: A Cultural History of Cacao. University Press of Florida, p. 121.
Klemes, J.J., Varbanov, P.S., 2013. Process intensification and integration: an assessment. Clean Technol. Environ. Policy 15 (3), 417–422.
Klemes, J.J., Varbanov, P.S., Huisingh, D., 2012. Recent cleaner production advances in process monitoring and optimization. J. Clean. Prod. 34, 1–8.
Koido, K., Takeuchi, H., Hasegawa, T., 2018. Life cycle environmental and economic analysis of regional-scale food-waste biogas production with digestate nutrient management for fig fertilization. J. Clean. Prod. 190, 552–562.
Li, M., Foster, C., Kelkar, S., Pu, Y., Holmes, D., Ragauskas, A., Saffron, C.M., Hodge, D.B., 2012. Structural characterization of alkaline hydrogen peroxide pretreated grasses exhibiting diverse lignin phenotypes. Biotechnol. Biofuels 5, 38.
Liew, P.Y., Theo, W.L., Alwi, S.R.W., Lim, J.S., Manan, Z.A., Klemes, J.J., Varbanov, P.S., 2017. Total site heat integration planning and design for industrial, urban and renewable systems. Renew. Sustain. Energy Rev. 68, 964–985.
Mancini, G., Papirio, S., Riccardelli, G., Lens, P.N.L., Esposito, G., 2018. Trace elements dosing and alkaline pretreatment in the anaerobic digestion of rice straw. Bioresour. Technol. 247, 897–903.
Miltner, M., Makaruk, A., Harasek, M., 2017. Review on available biogas upgrading technologies and innovations towards advanced solutions. J. Clean. Prod. 161, 1329e1337.
Monlau, F., Barakat, A., Steyer, J.P., Carrere, H., 2012. Comparison of seven types of thermo-chemical pretreatment on the structural features and anaerobic digestion of sunflower stalks. Bioresour. Technol. 120, 241–247.
Morero, B., Groppelli, E.S., Campanella, E.A., 2017. Evaluation of biogas upgrading technologies using a response surface methodology for process simulation. J. Clean. Prod. 141, 978–988.
Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-rna. Appl. Environ. Microbiol. 59, 695–700.
Nemestothy, N., Bakonyi, P., Szentgy€orgyi, E., Kumar, G., Nguyen, D.D., Chang, S.W., Kim, S., Belafi-Bako, K., 2018. Evaluation of a membrane permeation system for biogas upgrading using model and real gaseous mixtures: the effect of operating conditions on separation behaviour, methane recovery and process stability. J. Clean. Prod. 185, 44–51.
Patinvoh, R.J., Osadolor, O.A., Chandolias, K., Horvath, S.I., Taherzadeh, M.J., 2017. Innovative pretreatment strategies for biogas production. Bioresour. Technol. 224, 13–24.
Pavi, S., Kramer, L.E., Gomes, L.P., Miranda, L.A.S., 2017. Biogas production from codigestion of organic fraction of municipal solid waste and fruit and vegetable waste. Bioresour. Technol. 228, 362–367.
Rabelo, S.C., Amezquita, F.N.A., Andrade, R.R., Maciel, F.R., Costa, A.C., 2011. Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. Biomass Bioenergy 35, 2600–2607.
Riggio, S., Torrijos, M., Debord, R., Esposito, G., van Hullebusch, E.D., Steyer, J.P., 2017. Mesophilic anaerobic digestion of several types of spent livestock bedding in a batch leach-bed reactor: substrate characterization and process performance. Waste Manag. 9, 129–139.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008a. Determination of ash in Biomass. Laboratory Analytical Procedures (LAP). Natl. Renew Energy Lab. 1–8 Report No. TP-510-42622.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of Structural Carbohydrates and Lignin in BIOMASS. Laboratory Analytical Procedures (LAP). Natl. Renew Energy Lab. 1–18 Report No. TP-510-42618.
Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008b. Determination of Extractives in Biomass. Laboratory Analytical Procedures (LAP). Natl. Renew. Energy Lab. 1–12 Report No. TP-510-42619.
Steinmetz, R.L.R., Mezzari, M.P., da Silva, M.L.B., Kunz, A., do Amaral, A.C., Tápparo, D.C., Soares, H.M., 2016. Enrichment and acclimation of an anaerobic mesophilic microorganism's inoculum for standardization of BMP assays. Bioresour. Technol. 219, 21–28.
Sun, S.N., Li, M.F., Yuan, T.Q., Xu, F., Sun, R.C., 2013. Effect of ionic liquid/organic solvent pretreatment on the enzymatic hydrolysis of corncob for bioethanol production. Part 1: structural characterization of the lignins. Ind. Crops Prod. 43, 570–577.
UN, 2017. United Nations Sustainable Development Agenda. www.un.org/sustainabledevelopment/development-agenda/. (Accessed 20 September 2017).
Venturin, B., Camargo, A.F., Scapini, T., Mulinari, J., Bonatto, C., Bazoti, S., Siqueira, D.P., Colla, L.M., Alves Jr., S.L., Bender, J.P., Steinmetz, R.L.R., Kunze, A., Fongaro, G., Treichel, H., 2018. Effect of pretreatments on corn stalk chemical properties for biogas production purposes. Bioresour. Technol. 266, 116–124.
Vilchez-Vargas, R., Geffers, R., Suarez-Diez, M., Conte, I., Waliczek, A., Kaser, V.S., Kralova, M., Junca, H., Pieper, D.H., 2013. Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system. Environ. Microbiol. 15, 1016–1039.
Wei, S., 2016. The application of biotechnology on the enhancing of biogas production from lignocellulosic waste. Appl. Microbiol. Biotechnol. 100 (23), 9821–9836.
Westphal, A., Kücke, M., Heuer, H., 2016. Soil amendment with digestate from bioenergy fermenters for mitigating damage to *Beta vulgaris subspp.* by *Heterodera schachtii*. Appl. Soil Ecol. 99, 129–136.
Williams, L., Gallagher, J., Bryant, D., Ravella, S.R., 2016. Anaerobic digestion and the use of pre-treatments on lignocellulosic feedstocks to improve biogas production and process economics. Adv. Biofeeds. Biofuels. 1, 121.
Zhao, C., Shao, Q., Ma, Z., Li, B., Zhao, X., 2016. Physical and chemical characterizations of corn stalk resulting from hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment. Ind. Crops Prod. 83, 86–93.
Zhao, X., Luo, K., Zhang, Y., Zheng, Z., Cai, Y., Wen, B., Cui, Z., Wang, X., 2018. Improving the methane yield of maize straw: focus on the effects of pretreatment with fungi and their secreted enzymes combined with sodium hydroxide. Bioresour. Technol. 250, 204–213.