



Liquefaction of pineapple peel: Pretreatment and process optimization

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ABSTRACT

This study explored the optimization of pretreatment of pineapple peel for biogas generation. Pretreatments were carried out sulfuric acid and alkaline hydrogen peroxide prior to anaerobic digestion while the response surface methodology (RSM) was used for optimization of the pretreatment procedures. The physical, chemical, proximate and structural compositions of the peels were determined prior to and at the end of the pretreatment procedures. The dynamics of microorganisms in the reactors were also evaluated by rapid molecular methods while the Fourier Transform Infra-red (FTIR) spectroscopy was employed in the identification of the chemical changes as a result of pretreatments. The use of H_2O_2 pretreatment caused enormous lignin solubilization in the pineapple peel. In comparison, biogas production was 67% more in the alkaline pretreated pineapple peel than the biomass treated with acid and also 51% over the untreated samples. The total biogas volume produced from the acidic pretreated, alkaline pretreated, not sifted untreated and sifted untreated samples are 194.2 ± 3.0 , 587.5 ± 5.2 , 287.8 ± 2.1 and 245.4 ± 3.1 respectively. Thus, the alkaline pretreated experiment used lower retention time to achieve maximum gas production in this study. The use of alkaline H_2O_2 on lignocelluloses has remained unpopular prior to this study. However, its usage in this study yielded better result than all the conventional treatments in terms of lignin solubilization and improvement in methane yield. Economically, the use of H_2O_2 for pretreatment is adjudged feasible because the $1504 \text{ kWh t}^{-1} \text{ TS}$ thermal energy gain obtained from the biogas produced by the alkaline treated peel exceeded the $921 \text{ kWh t}^{-1} \text{ TS}$ used in the pretreatment. This gives a net thermal energy of $583 \text{ kWh t}^{-1} \text{ TS}$. Whereas, the investment into acidic pretreatment of pineapple peel may not be economically justified because the total thermal energy gain of $-200 \text{ kWh t}^{-1} \text{ TS}$ was far lower than the $1236 \text{ kWh t}^{-1} \text{ TS}$ thermal energy that was consumed during the pretreatment giving a net thermal energy of $-1436 \text{ kWh t}^{-1} \text{ TS}$. Therefore, the use of mild alkaline pretreatment is advocated in biogas generation from pineapple peel and also for biofertilizer production mostly in localities of mass production.

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1. Introduction

Pineapple (*Ananas comosus*) is a very important fruit among the very common the world has ever known with over 20% contributions to the global production of tropical fruits [1]. Its origin is traced to the Amazon basin of South America and specifically to Brazil and Paraguay. It is now found as an abundant fruit with several domestic and industrial applications worldwide. Pineapple is very rich in sugar, enzymes e.g. bromelain, digestive acids,

vitamins, minerals and fiber [2]. Approximately 24.8 million tons of pineapple is produced globally in recent years most of which comes from Costa Rica, Brazil, Philippines, Thailand, Indonesia, India, Nigeria, China, Mexico and Columbia [3]. Being the world number 7 and African number 1 producer of pineapple, Nigeria produces about 1.42 million tons yearly. Judging by this enormous production, it is expected that huge biomass accruing from the peel of this fruit is generated most of which ends up as waste in most localities of production and marketing. These peels end up as environmental nuisance conveying pathogens of global health importance due to their perishability. Though a few usage has been sought for the peels, most of them are not sustainable and the problem of dealing with this huge resources remained unsolved, hence the need to

Abbreviations: AD, anaerobic digestion; RSM, response surface methodology; TS, total solids; VS, volatile solids.

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explore the bioconversion of these peels to sustainable energy sources using adequate pretreatment methods considering the fibrous nature of the peel as a result of its high lignin content.

Fossil fuels constitute well over 80% of global energy resources. However, these fuels are not renewable and cause the bulk of environmental degradation via the enormous production of CO₂ and other greenhouse gases [4]. This phenomenon has therefore given rise to alternative and renewable energy generation from wastes and biomass [5–7]. Among such sustainable materials for renewable fuels generation are lignocelluloses which are very abundant globally as huge energy carriers due to their ability to be converted into various forms of energy [8–11]. Among the numerous energy form derived from lignocellulose is methane which has found lots of useful applications domestically and industrially especially because it is a source of clean energy with high hydrogen to carbon ratio [12]. The process of methane formation from wastes, biomass or any other resource is called anaerobic digestion (AD) which is a microbial mediated/controlled process usually progressing in four different stages with each having diverse array of microorganisms in succession. This makes anaerobic digestion a very energy-efficient and environmentally beneficial technology [13]. However, one major factor that limits the efficiency of the AD process is the recalcitrance posed by lignocelluloses due to the abundant lignin and cellulose composed in them [14–17]. This phenomenon is usually encountered during the first stage of AD i.e. hydrolysis in which microbial enzymes fails to degrade these materials because of the lignin-cellulose matrix [18,19]. This has made the hydrolysis process rate-limiting thereby requiring prior biomass pretreatment for easy digestion in the digester [20,21].

Pretreatment helps in changing the complex chemical structure of the lignocellulose causing quick hydrolysis and higher rate of digestion [22]. There are many pretreatment methods that have been applied to diverse lignocellulosic biomass over time. Some of the very common ones are physical/mechanical comminution, sonication, alkaline and acidic (Chemical), use of microorganisms (Bacteria and fungi) and the use of combined pretreatments [23,24]. The advantages derive from such treatments though at different rates depending on the methods includes easing the AD process, preservation of sugars, solubilization of lignin, cellulose and hemicellulose and prevention of process inhibition [25].

Acids have been used in biomass pretreatment with some improvements in the digestion performance. The most common acid in this process has been sulfuric acid which have been documented to be very potent in the total removal of the hemicellulose component of corn stalk, whole corn stalk, Sorghum stalk, grasses and other lignocelluloses after pretreatment [12,18,19].

Alkaline pretreatment of biomass is not new in the energy literature. Alkalis such as NaOH, KOH and others have been extensively used in treating different biomass [16,17]. Few of the previous researches with pretreatment of biomass are hydrolysis of cellulose into methane [21], kitchen wastes [26], food waste [27], *Tithonia diversifolia* shoot [9], *Chromolaena odorata* shoot [5], *Arachis hypogaea* hull [10], *Telfairia occidentalis* fruit peel [6], *Carica papaya* fruit peel [8]. There is high preference for alkali pretreatment over the acidic one simply because a pH buffering by increased alkalinity is usually required for successful digestion process [28]. Enhancement of methane production has been severally reported in some recent studies with the application of alkaline treatments e.g. the digestion of sunflower stalks [29] and ensiled Sorghum forage [30]. Generally, alkaline methods are inappropriate for highly perishable materials due to the ease of degradation and the tendencies to produce high amount of volatile fatty acids (VFA's) with the potential to inhibit the methanogenesis stage of digestion. However, the alkalis are very efficient in

pretreating lignocellulosic biomass because of their complex nature composing of enormous lignin, cellulose and hemicelluloses [31,32].

However, the use of H₂O₂ as an alkaline pretreatment agent is not well documented. Only recently, few studies have documented the application of H₂O₂ to some lignocellulosic biomass including grasses [33] *Theobroma cacao* [34,35], *Sorghum bicolor* [36]. In all these studies, there was enormous solubilization of structural materials (Lignin, cellulose and hemicellulose) with a corresponding increase in biogas production over the untreated and acidic treated biomass. There is therefore need to evaluate the use of this alkaline pretreatment on other lignocelluloses as well to assess the optimal condition for the process.

Even though, few studies have investigated the biogas potentials of pineapple wastes and pulp [37–39], no attempt has been made prior to now to evaluate the appropriate pretreatment method or to unravel the optimal conditions of pretreatment and biogas generation from pineapple peels. According to information in literature, this is the first report on the liquefaction of pineapple peel with the elucidation of the structural components of the peel. This study has also documented the numerous changes caused by the application of pretreatments. Another major gap this research has covered is in the establishment of the appropriate low-cost pretreatment method which caused maximum solubilization of lignin with attendant enhanced biogas yield in the long run. Besides, this study also assessed the feasibility of the pretreatment application and the combustibility of the produced gas in terms of economics of the process. Therefore, the objective of this study was to establish the optimal operating conditions for the maximum pretreatment and biogas yield from the liquefaction of pineapple peel based on experimental design. If this is successfully implemented, it will result in the optimal and efficient use of this abundant bioresource for energy generation. It will also help in the analysis, optimization and establishment of the anaerobic digestion technology as an effective treatment process for pineapple peel at industrial scale.

2. Materials and methods

2.1. Collection of sample

The main bioresource used in this research was Pineapple peels which was obtained from the residue of pineapple consumption at the staff quarters of Landmark University, Omu-Aran, Kwara State, Nigeria. They peels were sun-dried to achieve constant weight after which it was ground with a SOLAB knife mill (SL-31, Brazil) and immediately sieved using sizes between 0.075 and 4.750 mm of the mesh [12]. A portion of the ground peel was however not sifted in order to compare the biogas yield with that of the sifted peel. Both samples (Sifted and not sifted) ground pineapple peel were then stored at 4 °C prior to analyses.

2.2. Pretreatment's experimental design

The design of both acidic and alkaline pretreatment procedures was done employing Response Surface Methodology (RSM). The entire acidic pretreatment was done in an autoclave using sulfuric acid (H₂SO₄) as the pretreating agent. The following parameters: Exposure time (min), Temperature (° C), H₂SO₄ concentration (%) and Dry mass (g) were used as the input variables. However, the alkaline pretreatment was carried out in an orbital shaker using hydrogen peroxide (H₂O₂) as the pretreatment agent. Same input variables were used except that Rotation (rpm) was added as an extra parameter during the evaluation. The residual composition of lignin, cellulose and hemicellulose (%) after pretreatments was used as the sole response in both designs.

2.2.1. Acidic pretreatment

According to the experimental design used, the value of each variable studied was: (i) exposure time (6, 16, 26, 36 and 46 min), (ii) autoclave working temperature of (75, 85, 95, 105 and 115 °C), (iii) H₂SO₄ concentration (0, 0.5, 1, 1.5 and 2% (v.v⁻¹)), and (iv) dry mass (1, 2, 3, 4 and 5 g), chosen according to standard methods with as modified [12,19].

2.2.2. Alkaline pretreatment

A modification of earlier methods [12,15,40] was used in this design, the value employed for the various variables were: (i) Exposure time (55, 65, 75, 85 and 95 min) (ii) shaker temperature (20, 25, 30, 35 and 40 °C), (iii) Agitation (115, 125, 135, 145 and 155 rpm), (iv) Dry mass (3, 4.5, 6, 7.5 and 9 g) and (v) H₂O₂ concentration (2, 4, 6, 8 and 10% (v.v⁻¹)). In order to reduce the incidence of foaming due to the presence of hydrogen peroxide, 0.5 mL of antifoam agent (Biocane FC 500, Brazil) was added to the setup.

2.3. Physicochemical analyses

Determination of the concentration of all important elements as shown in Table 1a was done using an inductively coupled plasma mass spectrometry. Chemical Oxygen Demand (COD) concentrations were determined using standard method [41] was employed. A Clarus 580 gas chromatography (PerkinElmer, USA) coupled to a flame ionization detector was used for determining the volatile fatty acids (VFAs) in the samples while the total (TS) and volatile solids (VS) contents were quantified using a standard protocol of the Finnish Association [42]. In order to determine the total phenolics, a microtube test followed by a 4-amino antipyrine colourimetry [14].

2.4. Analyses of structural components

Determination of total lignin, cellulose, hemicellulose and extractives in all samples of pineapple peel was carried out following standard protocol [43]. The Soxhlet apparatus was used for the extractives for a period of 6 h while the fixed solids were

determined by burning the samples in a muffle furnace [44]. To determine the content of total lignin, cellulose and hemicellulose from the pineapple samples, the experiment was carried out in a thermostatic bath at 30 °C using 72% sulfuric acid (v.v⁻¹) (3 mL) for an hour. The resulting filtrate was used to quantify the concentration of sugars in the samples. Liquid chromatography- Mass spectrometer (SHIMADZU, Japan) was used in determining sugars and acetic acid in a refractive index detector (DIR-10A) with a specialized column (AMINEX[®] BIORAD HPX87H) with 0.005 mol L⁻¹ sulfuric acid as mobile phase and other conditions being: isocratic mode, temperature of 45 °C, 20 µL injected into the detector at a flow of 0.6 mL min⁻¹. Each compound was then determined using calibration curves benchmarked against LC-MS standards (Sigma-Aldrich) [45]. Same procedure was used to determine the concentrations of furfural and hydroxymethylfurfural (HMF) with a slight modification by attaching a diode array detector (DAD) to the LC-MS while a C18 column was used with 1:8 acetonitrile/water to which 1% acetic acid has been added as the mobile phase. Other operational conditions observed are: isocratic mode, temperature of 45 °C, 20 µL injected into the detector at a flow of 0.8 mL min⁻¹.

2.5. Structural analysis

The FTIR (IRTracer-100, SHIMADZU, Japan) was used to obtain the spectra of pineapple peel samples. This helped to determine and quantify the structural changes due to the pretreatment of the samples. This analysis was carried using the protocol of Zhao et al. [46].

2.6. Biogas potential test

This test was carried out on the pineapple peel samples so as to determine the potential biogas yield from the substrate under standard condition. The equipment for this test was 250 mL batch mini-reactors connected to 500 mL eudiometer tubes with 10% (m.v⁻¹) VS at 37 °C for 30 days [47] along with the VDI 4630 (2006) standard. Increment and stabilization of the biogas yield was achieved when biogas production per day was lower or equal to 1% of

Table 1a
Elemental composition of Pineapple peel and inoculum before and after pretreatments.

Parameter	Inoculum	Cellulose Standard	Pretreated Pineapple Peel		Untreated Pineapple Peel	
			H ₂ SO ₄	H ₂ O ₂	Sifted	Not Sifted
Ash Content (%)	5.56 ± 1.02	ND	6.20 ± 0.01	5.25 ± 1.02	4.72 ± 0.01	5.27 ± 1.00
Moisture Content (%)	90.48 ± 3.02	5	86.51 ± 2.01	91.12 ± 2.02	81.52 ± 1.01	86.8 ± 0.02
Total Carbon (g/kg TS)	265.21 ± 0.10	ND	629.52 ± 5.22	698.75 ± 2.25	425.11 ± 6.02	450.40 ± 1.20
Total Nitrogen (g/kg TS)	48.00 ± 2.02	ND	30.40 ± 0.22	32.60 ± 0.21	23.20 ± 0.02	25.30 ± 0.20
C/N	6/1	–	21/1	21/1	18/1	18/1
Acetate (g COD/g VS)	1.04 ± 0.10	ND	0.11 ± 1.10	0.12 ± 0.01	0.06 ± 0.10	0.07 ± 0.10
Propionate (g COD/g VS)	1.07 ± 0.02	ND	0.15 ± 0.03	0.13 ± 0.02	0.09 ± 0.11	0.10 ± 0.10
TVFAs (g COD/g VS)	2.44 ± 0.10	ND	1.21 ± 0.10	1.14 ± 0.10	0.12 ± 0.10	1.04 ± 0.11
Ammonia (mg/g VS)	4.97 ± 1.01	ND	2.01 ± 1.10	2.11 ± 0.02	1.21 ± 0.02	1.17 ± 0.10
Uronic acids (% VS)	1.67 ± 1.11	ND	2.82 ± 1.10	2.08 ± 0.10	1.56 ± 1.10	1.01 ± 0.10
®Soluble sugars (% VS)	4.02 ± 2.10	ND	8.14 ± 0.11	8.93 ± 1.10	4.11 ± 1.02	4.77 ± 0.10
Phenols (mg L ⁻¹)	4.71 ± 2.10	ND	0.005 ± 0.01	0.004 ± 0.10	0.001 ± 0.01	0.001 ± 0.10
Total Phosphorus (g/kg TS)	6.30 ± 0.02	ND	4.86 ± 0.12	5.94 ± 0.11	3.20 ± 0.11	3.57 ± 0.01
Potassium (g/kg TS)	7.20 ± 0.11	ND	7.6 ± 0.11	9.60 ± 0.02	4.26 ± 0.01	5.55 ± 0.01
Phosphate (g/g TS)	3.00 ± 0.02	ND	4.10 ± 0.11	4.70 ± 0.10	2.04 ± 0.01	2.10 ± 0.20
Sulphate (g/kg TS)	134 ± 2.00	ND	106.00 ± 6.10	117.10 ± 2.02	56.00 ± 2.00	66.04 ± 1.02
Calcium (g/kg TS)	80.00 ± 0.10	ND	513.0 ± 1.42	568.50 ± 4.00	339.4 ± 0.42	381.80 ± 2.03
Magnesium (g/kg TS)	96.00 ± 0.10	ND	66.00 ± 2.02	82.10 ± 1.40	39.60 ± 0.02	46.20 ± 1.10
Manganese (g/kg TS)	1.18 ± 0.22	ND	0.018 ± 0.04	0.024 ± 0.10	0.013 ± 0.01	0.014 ± 0.10
Iron (g/kg TS)	1.18 ± 0.11	ND	1.79 ± 0.03	1.54 ± 0.01	0.42 ± 0.01	0.55 ± 0.01
Zinc (g/kg TS)	38.00 ± 0.02	ND	36.00 ± 0.03	43.01 ± 0.01	19.90 ± 0.02	22.30 ± 0.01
Aluminium (g/kg TS)	0.80 ± 0.11	ND	1.44 ± 0.10	1.62 ± 0.02	0.25 ± 0.02	0.30 ± 0.12
Copper (g/kg TS)	4.80 ± 0.10	ND	4.35 ± 0.12	4.92 ± 0.11	2.11 ± 0.10	2.18 ± 0.11

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

the total production from the entire process.

2.7. Anaerobic digestion

The Computer controlled anaerobic batch reactors (EDIBON, United Kingdom) (Fig. 1) were used in the digestion process while taking into account the best responses in the design of experiment. The inoculum was collected from a reactor treating cattle manure and Sorghum stalk at mesophilic temperature. At the end of the 30-day retention time, results obtained from the pretreated samples were compared with those of the untreated while those of the sifted and not sifted samples were also compared. The components of the biogas (CH₄, CO₂ and H₂S) were determined by infrared and electrochemical sensors (BIOGASS5000, USA).

2.8. Economic feasibility assessment of pretreatments of pineapple peel

Evaluation of the viability of pretreatment was investigated for pineapple peel in this study by evaluating the balance between energy generation and consumption. The cost of thermal energy and chemicals (H₂SO₄ and H₂O₂) was compared with the gains from the extra energy obtained as a result of pretreatment application so as to establish that pretreatment of pineapple peel is economically feasible. The thermal energy required (TER) (kWh t⁻¹ TS) for pre-treating pineapple peel was evaluated as shown:

$$TER = \frac{p \times Lm \times (S_{final} - S_{initial})}{3600} \quad (1)$$

where

p (1000 kg) = mass of pineapple peel mixed with water;
 Lm = specific heat of water (4.18 kJ kg⁻¹ C⁻¹);

$S_{initial}$ (°C) = initial temperature of substrate (25 °C);

S_{final} (°C) = final temperature of substrate (55 °C);

For the purchase of H₂SO₄ and H₂O₂, the United States cost was used.

2.9. Combustion test with produced biogas

The combustibility of the generated biogas from the four experiments was evaluated using a purposely designed biogas burner. Following previous procedures with modifications in which the produced biogas was used in boiling water and rice [48] and the duration of boiling were adequately recorded as this is a function of the energy value of each biogas.

2.10. Analysis of microbial community

Rapid molecular biology method was employed in analyzing the microbial community of the digesters in each case. A total of 45 mL of each influent, mixed slurry and effluent from each digester was taken on the 6th, 12th, 18th, 24th and 30th experimental days respectively. These samples were then stored at -20 °C till further experiments was carried out. The extraction of genomic DNA was carried out using the 'NucleoSpin Soil' kit (Macherey Nagel) according to the manufacturer's manual. Afterwards, determination of the quantity and integrity of the eluted genomic DNA was determined using the spectrophotometer (NanoDrop1000, Thermo Scientific) and gel electrophoresis. The aliquoted DNA samples were stored at -20 °C. Amplifications of PCR for terminal-restriction fragment length polymorphism (T-RFLP) fingerprinting and cloning were carried out using standard protocols in a ThermalCycler (Bio-Rad) [49] by conventional PCR targeting total bacterial and archaeal communities using the total bacterial primers P338f and P518r and the *mcrA* methanogenic primer [50,51]. After



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

this, the quality and purity of the PCR products was checked by gel electrophoresis. A Real-time PCR analysis was thereafter conducted using the Applied Biosystems StepOnePlus™ Real-Time PCR System (Carlsbad, CA). With this, the total bacterial and archaeal population was analyzed [52]. The results obtained from the software were all critically examined.

2.11. Optimization study and data analysis

At the end of digestions and analyses, collected data were analyzed with the RSM based on earlier design used for the sample pretreatments. This was necessary so that the polynomial equations generated via the Design-Expert software (Version 9.0.3.1) could be properly fitted with the aid of multiple regressions and by so doing, correlation of the responses and the independent factors were done. Thereafter, test of significance and analysis of variance (ANOVA) were employed to evaluate the quality of the models. The quadratic model is represented in the equation shown below:

$$P = d_0 + \sum_{i=1}^k d_i X_i + \sum_{i=1}^k d_{ii} X_i^2 + \sum_{i < j}^k d_{ij} X_i X_j + e \quad (2)$$

where:

- P = model response;
- d_0 = value of intercept;
- d_i ($i = 1, 2, k$) = the first order model coefficient;
- d_{ij} = the interaction effect;
- d_{ii} = the quadratic coefficients of X_i ;
- e = the random error.

Validation of the model was done using the same batch reactors and the optimum values predicted by the model. After this, 3-Dimensional were constructed to show the optimized responses i.e. residual lignin, cellulose and hemicellulose. The different responses were analyzed using the STATISTICA V. 12 software (Stat-Soft, Tulsa, USA) at 95% ($p < 0.05$) confidence level before the comparison of the various means by Tukey's test.

3. Results

3.1. Characterization of untreated pineapple peel

Sieving of the ground pineapple peel reveals particle sizes of 0.869, 0.603, 0.466, 0.244 and 0.153 mm on the average which was obtained from $61.6 \pm 1.5\%$, $21.6 \pm 1.0\%$, $16.7 \pm 2.0\%$, $12.2 \pm 2.2\%$ and $6.7 \pm 0.9\%$ of sample respectively. The most abundant of all fractions however was obtained from the 0.603 mm size and was used for all subsequent tests. As shown in Table 1b, the percentage composition of total lignin, cellulose, hemicellulose and fixed solids in the not sifted untreated pineapple peel as revealed by chromatography is 19.4 ± 0.21 , 32.4 ± 0.11 , 23.2 ± 0.10 and 3.9 ± 0.10 respectively. However, values obtained for the same structural materials in the sifted untreated sample are 17.8 ± 0.11 , 26.6 ± 0.50 , 22.8 ± 1.01 and 3.6 ± 0.02 respectively. Meanwhile, about 20% of the pineapple peel's composition was not quantified and regarded as extractives. No significant difference ($p > 0.05$) was shown between the sifted and not sifted untreated pineapple peel.

Table 1b shows the elemental composition of the pineapple peel before and after pretreatment with acid and alkali. As revealed, the peel is very rich in nutrient and elements required by microorganisms for survival and proliferation in a fermentation medium

3.2. Characterization of sifted pretreated pineapple peel

3.2.1. Acidic pretreatment

The effect of applying sulfuric acid for pretreatment in this study was evident in solubilization of hemicellulose initially present in the pineapple peel (Table 3). All the bonding systems were all broken down by the action of the acid. Beside this was also the visible depolymerization of hemicellulose which was evident by the hydrolysis of xylose to simple sugars. The effect of the sulfuric acid was however different on cellulose and lignin as their contents increased in the peel after pretreatment. According to experimental design of the pretreatment, the pineapple peel was pretreated optimally at the condition: 2% (w/v) of H_2SO_4 , temperature of $121^\circ C$ and 4.01 g dry mass for 60 min. Under this condition, the lignin and cellulose contents increased by 32.3 and 43% respectively i.e. from 17.8 ± 0.11 to $26.3 \pm 1.30 \text{ m m}^{-1}$ and 26.6 ± 0.05 to $46.7 \pm 0.01 \text{ m m}^{-1}$ respectively while reduction was observed for hemicellulose from 22.8 ± 1.01 to $5.2 \pm 0.01 \text{ m m}^{-1}$ i.e. 77%.

3.2.2. Alkaline pretreatment

Similar to the result of acidic pretreatment was the high solubilization of structural component of the pineapple peel with the use of H_2O_2 for pretreatment. The different however is that lignin was the component that was greatly affected in this experiment while hemicellulose was partially solubilized. The treatment at the same time brought about the increase in composition of cellulose (Table 2b). By experimental design, the most efficient condition of pretreatment was: 7.5% (w/v) H_2O_2 , temperature of $30^\circ C$, agitation at 130 rpm and 3 g dry mass for 75 min. This condition achieved 71.34 and 61% reduction in lignin and hemicellulose respectively i.e. from 17.8 to 5.1 m m^{-1} and 26.6–43.3 m m^{-1} while there was increase in cellulosic content by 39%.

3.3. Structural changes in pineapple peel after pretreatment

The changes that occurred to the structures of the pretreated and untreated pineapple peel after pretreatment are shown in Table 3. All detected bands were between 3348 and 2900 cm^{-1} signifying that pineapple peel is composed of cellulosic chemical bonds. Absorbance for both pretreated and untreated increased to 21.3 and 54.1% which was caused by the increase in cellulosic content of the biomass due to alkaline pretreatment. A decrease in absorbance in the phenolic lignin at the 1373 and 3448 cm^{-1} peaks was however observed for the acidic treated biomass in the cellulosic O–H bonds.

3.4. Anaerobic digestion's effects on biomass structure

After digestion, further solubilization of the pineapple peel's structure. As seen in the digestates of all samples, reductions in lignin, cellulose, hemicellulose and fixed solids were: 26, 42, 30 and 15%; 47, 23, 29, 17%; 24, 18, 10 and 19% and 14, 22, 30 and 25% in the acidic pretreated, alkaline pretreated, not sifted untreated and sifted untreated pineapple peel respectively. For uronic acids, reductions to the tune of 33.12, 41.11, 17 and 26% were obtained for the four experimental set respectively. The values of soluble sugars however increased in all experiments though at different rates with values of 37.4, 46.7, 22.1 and 26.3%. Similar to the result of the structural components, the digestion process also had a meaningful impact on the chemical parameters of the substrates as enormous degradation took place resulting in significant increase in value of most elements as evident in the digestates. The values of COD also reduced in all digestions by average values of 44.9, 57.13, 34.32 and 46.4% in the four digestion regimes.

Table 1b
Characterization of Pineapple peel and inoculum before and after pretreatments.

Parameter	Inoculum	Cellulose Standard	Pretreated Pineapple Peel		Untreated Pineapple Peel	
			H ₂ SO ₄	H ₂ O ₂	Sifted	Not Sifted
pH (Sample + Inoculum)	7.86 ± 0.10	7.92 ± 0.10	7.89 ± 0.10	7.92 ± 0.10	7.80 ± 0.10 ^a	7.84 ± 0.10 ^a
Total solids (% m.m ⁻¹)	4.1 ± 0.01	95.1 ± 3.01	90.5 ± 0.51	90.1 ± 2.01	92.3 ± 2.01 ^b	93.0 ± 0.01 ^b
Volatile solids (% m.m ⁻¹)	2.4 ± 0.01	95.1 ± 2.01	88.9 ± 2.01	75.3 ± 1.01	89.4 ± 2.01 ^c	89.1 ± 0.11 ^c
Total Lignin (% m.m ⁻¹)	29.5 ± 2.01	ND	26.3 ± 1.30	5.1 ± 0.01	17.8 ± 0.11 ^d	19.4 ± 0.21 ^d
Cellulose (% m.m ⁻¹)	3.1 ± 0.01	99 ± 3.01	46.7 ± 0.01	43.3 ± 2.01	26.6 ± 0.50	32.4 ± 0.11
Hemicellulose (% m.m ⁻¹)	24.1 ± 0.01	ND	5.2 ± 0.01	8.8 ± 0.11	22.8 ± 1.01	23.2 ± 0.10
Fixed solids (% m.m ⁻¹)	1.6 ± 0.01	0 ± 0.00	1.9 ± 0.05	1.3 ± 0.01	3.6 ± 0.02	3.9 ± 0.10
Extractives (% m.m ⁻¹)	ND	ND	ND	ND	14.6 ± 0.01	14.5 ± 0.02
Solids after pretreatment (% m.m ⁻¹)	–	–	2.9 ± 0.02	2.1 ± 0.01	–	–
Added sample (g)	0 ± 0.00	1 ± 0.10	2.5 ± 0.12	2.1 ± 0.10	2.4 ± 0.10	2.3 ± 0.10
COD (g COD/g VS)	168.21 ± 1.12	ND	202.26 ± 1.40	210.92 ± 0.14	206.12 ± 3.00	209.08 ± 0.10
BP (L _N biogas. Kg VS _{ad} ⁻¹)	28.1	642.4 ± 3.02	371.5 ± 5.10	701.5 ± 1.50	498.3 ± 3.10 ^f	521.6 ± 2.10
μ _{max} (L _N biogas. Kg VS _{ad} ⁻¹).d ⁻¹	ND	161.2 ± 2.05	31.4 ± 0.01	245.5 ± 2.10	48.9 ± 0.10 ^g	56.2 ± 0.10
Day of μ _{max}	ND	3–4	5–6	1–2	4–6	3–4

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

Table 2a
Structural composition of Pineapple peel after H₂SO₄ pretreatment and actual values of the independent variables.

Run	Exposure time	Temperature	Acid Conc.	Dry mass	Lignin	Cellulose	Hemicellulose	Desirability
1	21.90	119.99	2.00	2.00	32.39	19.35	11.07	0.944
2	22.51	119.99	2.00	2.00	32.60	19.58	11.17	0.944
3	22.24	120.00	2.00	2.01	32.52	19.49	11.13	0.944
4	21.57	119.99	2.00	2.02	32.28	19.23	11.01	0.944
5	21.61	120.00	1.98	2.00	32.24	19.21	11.00	0.944
6	22.92	120.00	2.00	2.00	32.75	19.73	11.24	0.944
7	22.19	119.84	2.00	2.00	32.45	19.44	11.10	0.943
8	22.00	120.00	2.00	2.04	32.45	19.41	11.08	0.943
9	22.02	119.68	2.00	2.00	32.36	19.36	11.07	0.943
10	22.57	120.00	1.95	2.00	32.52	19.51	11.13	0.943
11	22.40	120.00	2.00	2.10	32.64	19.59	11.14	0.943
12	21.79	119.86	2.00	2.15	32.43	19.36	11.01	0.943
13	21.06	120.00	2.00	2.17	32.20	19.10	10.89	0.943
14	23.08	120.00	1.88	2.00	32.51	19.55	11.13	0.943
15	22.52	120.00	1.86	2.00	32.25	19.29	11.00	0.943
16	18.88	120.00	2.00	2.00	31.18	18.13	10.52	0.942
17	24.39	120.00	1.82	2.00	32.79	19.89	11.29	0.942
18	26.16	120.00	1.99	2.00	33.69	20.79	11.76	0.941
19	21.61	120.00	2.00	2.42	32.60	19.45	10.94	0.941
20	24.00	120.00	1.78	2.14	32.67	19.72	11.13	0.941
21	26.42	120.00	1.75	2.00	33.20	20.42	11.53	0.941
22	23.45	116.52	2.00	2.00	32.13	19.51	11.08	0.940
23	23.12	116.46	2.00	2.00	32.01	19.38	11.02	0.938
24	21.43	120.00	1.68	2.00	31.37	18.52	10.56	0.938
25	28.34	120.00	1.60	2.00	33.33	20.73	11.67	0.937
26	29.07	120.00	1.55	2.00	33.38	20.86	11.73	0.937
27	31.29	120.00	1.64	2.00	34.02	21.56	12.18	0.936
28	23.77	113.73	2.00	2.00	31.66	19.32	10.94	0.936
29	27.65	120.00	1.29	2.00	32.39	20.05	11.14	0.935
30	45.00	120.00	1.31	2.76	34.04	22.56	13.17	0.934

3.5. Microbial structure

The identified aerobes include *Bacillus pantothenicus*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Serratia ficaria*, *Serratia plymuthica* and *Proteus vulgaris*. Anaerobes include *Clostridium clostridioforme*, *Fusobacterium mortiferum* and *Porphyromonas ascharolyticum* while the methanogens include members of the genera *Methanobacteriales*, *Methanosaetaceae*, *Methanomicrobiales*, *Methanococcus* and *Methanosarcinaceae*.

3.6. Dynamics of VFAs

In most anaerobic digestion processes, VFAs accumulation is a common phenomenon which is largely caused by high population

and pronounced activities of microorganisms that produce intermediate acids which often inhibits the process. However, whenever there is balance between VFAs production and consumption by the reactors bacterial community, they are accumulated. The predominant VFAs in this study are acetate and propionate making up over 94% of all, their accumulation was minimal and was highest at the middle of the digestion process i.e. between the 13th 16th days in all reactors.

3.7. Stoichiometry and mass balance

The mass balance in terms of VS consumption was evaluated in all experiments in this study as shown in Table 4. In this regard, "Pineapple peel" was the input variable while the two major

Table 2bStructural composition of Pineapple peel after H₂O₂ pretreatment and actual values of the independent variables.

Run	Exposure time	Temperature	Agitation	Dry masss	Alkali Conc.	Lignin	Cellulose	Hemicellulose	Desirability
1	75.19	30.00	130.08	7.50	3.00	18.30	38.13	18.23	0.888
2	75.81	30.00	130.00	7.35	3.12	18.06	37.84	18.18	0.874
3	77.87	30.00	130.03	7.27	3.10	18.30	37.49	18.23	0.873
4	52.52	62.00	170.00	1.50	15.00	16.80	38.65	17.94	0.856
5	56.20	60.84	170.00	1.50	15.00	17.01	38.10	17.87	0.845
6	73.74	31.11	130.00	7.50	3.44	16.98	37.69	17.88	0.836
7	50.19	59.96	169.37	1.50	15.00	16.90	37.60	17.72	0.824
8	53.56	57.25	170.00	5.94	15.01	16.22	38.09	17.71	0.822
9	64.54	30.00	130.00	6.47	3.00	16.41	37.61	17.82	0.820
10	60.18	62.00	166.98	7.13	15.00	15.94	38.34	17.51	0.800
11	86.90	30.00	130.01	4.26	3.00	16.03	35.15	17.97	0.799
12	77.21	30.01	130.00	1.45	3.91	16.52	36.26	18.15	0.798
13	54.73	31.40	130.00	5.31	3.00	15.82	39.64	17.29	0.793
14	63.63	30.00	130.00	4.12	3.00	17.04	39.03	17.74	0.792
15	72.76	30.00	170.00	4.48	14.60	18.92	36.77	17.46	0.791
16	69.29	40.05	130.00	1.50	3.00	18.18	35.48	17.92	0.789
17	58.64	30.00	170.03	3.00	14.95	16.23	36.92	16.23	0.786
18	60.33	30.00	165.03	3.54	15.00	16.28	38.88	16.34	0.785
19	57.96	41.40	155.05	3.76	3.02	15.93	38.09	17.09	0.782
20	53.36	30.00	150.92	1.43	14.99	15.03	36.51	16.27	0.780
21	50.73	32.10	170.03	1.54	3.79	16.66	36.92	17.28	0.779
22	50.00	30.00	130.43	1.26	3.00	16.28	37.28	15.66	0.776
23	78.28	30.00	130.45	1.80	3.00	15.24	35.63	17.29	0.772
24	50.07	54.10	165.93	1.91	15.00	16.25	37.81	17.23	0.771
25	80.30	30.00	169.38	1.39	3.76	13.24	34.99	16.39	0.770
26	52.81	64.00	139.03	2.10	14.71	13.93	35.54	16.28	0.758
27	78.81	30.00	153.23	3.03	14.52	13.45	37.27	17.42	0.754
28	67.59	30.00	170.03	1.05	13.54	14.28	36.52	17.92	0.753
29	68.07	61.20	168.22	1.38	12.03	14.92	35.43	16.90	0.752
30	55.09	35.02	160.08	1.50	11.04	14.23	39.02	17.69	0.751

Table 3Wave lengths that correspond to a given functional group and respond to infrared spectroscopy, and their respective relative values for H₂SO₄ and H₂O₂ pretreatments tested for Pineapple peel.

Wavelength (cm ⁻¹)	Assignment	Untreated	H ₂ SO ₄ Pretreated		H ₂ O ₂ Pretreated	
		Absorbance/Ratio	Absorbance/Ratio	Variation (%)	Absorbance/Ratio	Variation (%)
3348	O–H stretch (Hydrogen cellulose connections bond)	0.3326	0.2617	21.3	0.4077	–22.8
2900	C–H stretch (Methyl/methylene cellulose group)	0.1235	0.1221	1.1	0.1755	–42.1
1734	Carbonyl bonds (Associated with removal of lignin side chain)	0.1002	0.0866	13.8	0.0194	80.6
1716	Carboxylic acids/ester groups	0.0931	0.0968	–4.0	0.0365	60.8
1633	Aromatic ring stretch (Associated with lignin removal)	0.1415	0.1105	22.0	0.1110	22.0
1604	Aromatic ring stretch (Changes in lignin structure)	0.1531	0.1125	27.0	0.0757	51.0
1516	Generic lignin	0.0920	0.1094	–19.0	0.0536	42.0
1516/897	Lignin/cellulose ratio	3.6	3.8	–6.0	0.8	78.0
1373	Phenolic O–H stretch (Changes in lignin structure)	0.2321	0.1227	47.1	0.1814	21.0
1319	Syringyl ring stretch (Changes in the lignin monomer)	0.1261	0.1138	10.0	0.1651	–31.0
1251	C–O absorption (Result of acetyl-lignin groups cleavage)	0.1519	0.1305	14.1	0.1071	29.5
1110	Crystalline cellulose	0.2504	0.2142	14.5	0.3324	–33.0
1059	C–O–C stretch (Cellulose and hemicellulose)	0.2852	0.2559	10.3	0.4810	–69.0
897	Amorphous cellulose	0.0244	0.0299	–22.5	0.0698	–186.0
1110/897	Crystalline/amorphous cellulose ratio	8.9	7.6	15.0	4.8	46.1
833	C–H flexion of syringyl	0.1109	0.0136	87.7	0.0100	91.0
771	Crystalline cellulose (I α)	0.0178	0.0116	35.5	0.0180	–1.1
719	Crystalline cellulose (I β)	0.0324	0.0351	–8.3	0.0572	–77.0
771/719	Ratio of crystalline cellulose polymorphs (I α /I β)	0.2	0.4	–100.0	0.3	–50.0

ND = Not determined; * % Relative variation = 100 * (Absorbance of untreated Pineapple peel – Absorbance of pretreated Pineapple peel) / Absorbance of untreated Pineapple peel; All positive values indicates decrease.

components of the generated biogas i.e. methane and carbon dioxide alongside the final product (Aanaerobic digestate) were the output variables. The values of mass balance recorded for each of the acidic pretreated, alkaline pretreated and not sifted untreated pineapple peel samples are 0.28, 0.41 and 0.22 respectively. In terms of VS consumption, the three experiments recorded removal of 38, 53 and 32% respectively.

3.8. Biogas potential (BP) test

The inoculum showed a low biogas potential by producing less than 10% of total biogas from all experiment. Similarly, the inoculum produced lower biogas than the microcrystalline cellulose standard which produced over 80% more biogas than the reference 650 LNbiogas.kg VSad⁻¹ according to the VDI 4630 standards (2006). Biogas production was highest in the alkaline hydrogen peroxide pretreated pineapple peel followed by the not sifted

Table 4
Stoichiometry and mass balance for one ton of Pineapple peel.

Parameter	H ₂ SO ₄ Pretreated Pineapple peel + Inoculum	H ₂ O ₂ Pretreated Pineapple peel + Inoculum	Untreated Pineapple peel + Inoculum
Input			
A. Pineapple peel + Inoculum (kg)	1000	1000	1000
Volatile solids (VS) (kg)	889	753	891
Output			
Methane (CH ₄) (%)	63	70	60
Carbon dioxide (CO ₂) (%)	22.4	24	25
Digestate (kg VS)	552	354	610
Sum	637.4	448	695
*Mass balance	0.28	0.41	0.22
% Volatile solids (VS) removal	38	53	32

* = $(Input - output) / input$ (%).

untreated sample and in turn by the sifted untreated sample while the lowest was obtained from the acidic pretreated sample. In comparison, biogas production was 67% more in the alkaline pretreated pineapple peel than the acidic pretreated biomass and also 51% more than the untreated samples.

Total biogas production from the acidic pretreated, alkaline pretreated, not sifted untreated and sifted untreated samples are 194.2 ± 3.0 , 587.5 ± 5.2 , 287.8 ± 2.1 and 245.4 ± 3.1 respectively. Another striking result from the alkaline pretreated biomass was that the highest biogas generation was achieved 3 days earlier than all other experiments meaning that total production was realized in only 11 out of the 30-day retention period. Gas generation started on the 2nd day of experiment and climaxed on the 12th day whereas, production started between the 3rd and 5th days in other experiments and didn't reach climax until between the 16th and 19th days. Thus, the alkaline pretreated experiment used lower retention time to achieve maximum gas production in this study (Fig. 2). Analysis of the produced gas showed the composition of the two most important gases to be in the range of 58 ± 1.2 and 70 ± 3.2 methane, 18 ± 1.3 and 25 ± 0.6 carbon dioxide.

3.9. Cooking test results

The biogas from the alkaline pretreated pineapple peel showed the highest cooking rate. The rates were 0.04L/min and 0.003 kg/min for water and rice respectively whereas, rates of 0.15L/min and 0.0045 kg/min was obtained by using the biogas from the acidic pretreated peel respectively. The rates obtained from biogas from the untreated samples were 0.13L/min and 0.0044 kg/min and 0.14L/min and 0.0048 kg/min for the not sifted untreated and sifted untreated pineapple peel respectively. The biogas flow rate based on the design of the stove was calculated to be 0.0051 m³/min. By using the biogas produced from the digestion of the alkaline pretreated pineapple peel, improvements of 51.12 and 22.75% were recorded for the cooking rates of water and rice respectively.

3.10. RSM optimization of pretreatment data

The coefficients of the model equation and their statistical significance were determined and results of the statistical analyses are as shown in Tables 5a and 5b. Most of the model terms are

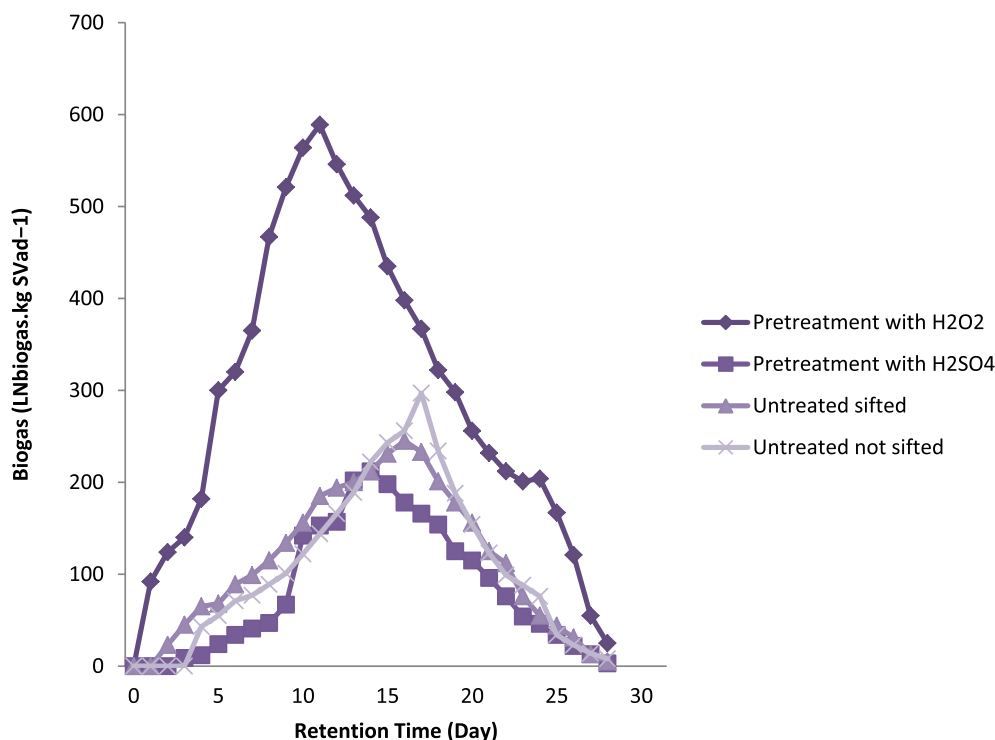


Fig. 2. Cumulative biogas production per kilogram of added volatile solids for H₂S₂ pretreated, H₂SO₄ pretreated, untreated sifted and untreated not sifted Pineapple peel.

Table 5a

Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for Sulfuric pretreatment of Pineapple peel.

Source	df	LIGNIN				CELLULOSE				HEMICELLULOSE			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value
A	1	3.043	3.043	2.33	0.0016	1.132	1.132	1.41	0.5040	3.20	3.20	3.45	0.0401
B	1	486.90	486.90	3.53	0.0797	1137.13	1137.13	1.96	0.4123	7.130	7.130	2.99	0.0123
C	1	3245.70	3245.70	23.56	0.0002	180.40	180.40	4.13	0.0014	8.402	8.402	3.58	0.0086
D	1	40.82	40.82	23.56	0.5942	15.04	15.04	2.92	0.1252	13.14	13.14	1.47	0.1252
AB	1	96.53	96.53	0.70	0.4157	18.92	18.92	3.10	0.0312	1.922	1.922	0.3717	0.0322
AC	1	38.13	38.13	0.28	0.0165	2.10	2.10	5.06	0.0721	2.10	2.10	2.06	0.0214
AD	1	17.02	17.02	0.12	0.7301	0.90	0.90	2.42	0.1461	0.04	0.04	2.22	0.1461
BC	1	21.86	21.86	0.16	0.1544	0.90	0.90	1.44	0.0110	1.08	1.08	3.06	0.1102
BD	1	65.21	65.21	0.47	0.0240	29.70	29.70	3.54	0.0412	9.81	9.81	3.41	0.0312
CD	1	0.53	0.53	3.816	0.1716	0.56	0.56	3.83	0.1276	1.56	1.56	1.34	0.1276
A ²	1	3469.50	3469.50	25.19	0.0097	1799.59	1799.59	6.47	0.0122	17.591	17.591	2.86	0.0222
B ²	1	314.17	314.17	2.28	0.0311	0.27	0.27	5.81	0.0233	3.76	3.76	5.82	0.0633
C ²	1	1609.13	1609.13	11.68	0.0049	661.92	661.92	5.15	0.0212	9.321	9.321	5.44	0.0212
D ²	1	1662.08	1662.08	12.07	0.0120	658.56	658.56	5.71	0.0107	58.516	58.516	3.77	0.0107
Model	14	11040.71	788.62	5.72	0.0009	4820.44	1.327	3.33	0.0328	230.94	1.327	2.33	0.0321
Residual	9	2066.35	137.76			1325.92	82190			125.9	82190		
Lack of Fit	6	2057.14	205.71	3.70	0.1607	1320.38	1.210	3.59	0.0612	220.08	1.210	2.92	0.1710
Pure Error	3	9.21	1.84			2.571	301.01			4.91	301.01		
R-Squared		0.9677				0.9305				0.9705			
Adequate Precision		14.822				13.192				11.561			

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

Table 5b

Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for Hydrogen peroxide pretreatment of Pineapple peel.

Source	df	LIGNIN				CELLULOSE				HEMICELLULOSE			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value
A	1	5.45	5.45	1.781	0.6014	1.50	1.50	0.026	0.0404	0.111	0.111	0.012	0.0143
B	1	52.22	52.22	1.39	0.6597	9.63	9.63	0.17	0.3003	5.24	5.24	0.60	0.0127
C	1	2.04	2.04	0.055	0.8002	0.17	0.17	2.899	0.0014	0.13	0.13	0.015	0.0023
D	1	45.28	45.28	1.21	0.0142	16.73	16.73	0.24	0.0223	4.03	4.03	0.45	0.1422
E	1	22.90	22.90	0.61	0.0102	1.59	1.59	0.028	0.0012	1.04	1.04	0.12	0.1103
AB	1	3.08	3.08	0.10	0.0023	23.04	23.04	0.40	0.0312	2.40	2.40	0.27	0.0033
AC	1	3.78	3.78	0.15	0.0103	34.81	34.81	0.51	0.1910	2.50	2.50	2.764	0.0061
AD	1	3.09	3.09	1.21	0.0118	29.16	29.16	0.61	0.0024	1.56	1.56	0.17	0.1180
AE	1	31.04	31.04	9.618	0.2212	0.30	0.30	5.261	0.6210	2.500	2.500	2.764	0.5212
BC	1	128.9	128.9	0.27	0.3218	2.72	2.72	0.047	0.0021	11.22	11.22	1.24	0.3218
BD	1	1.56	1.56	0.013	0.0157	18.92	18.92	0.39	0.2012	0.42	0.42	0.047	0.0157
BE	1	10.24	10.24	0.51	0.1162	22.56	22.56	0.063	0.1201	2.500	2.500	2.764	0.1162
CD	1	0.49	0.49	0.087	0.0111	3.61	3.61	0.21	0.1462	3.06	3.06	0.34	0.0111
CE	1	18.92	18.92	0.098	0.1577	11.90	11.90	1.44	0.0103	7.02	7.02	0.78	0.0075
DE	1	3.24	3.24	0.99	0.0300	82.81	82.81	0.95	0.0110	2.72	2.72	0.30	0.0300
A ²	1	3.65	3.65	3.07	0.3786	18.65	18.65	0.32	0.1276	4.59	4.59	0.51	0.0386
B ²	1	37.18	37.18	0.56	0.0027	11.15	11.15	0.19	0.0122	0.37	0.37	0.040	0.0067
C ²	1	114.82	114.82	2.51	0.0112	29.63	29.63	0.52	0.2911	2.37	2.37	0.26	0.0112
D ²	1	20.92	20.92	1.83	0.0049	0.43	0.43	5.947	0.0672	0.22	0.22	0.026	0.0143
E ²	1	94.11	94.11	8.01	0.0620	36.17	36.17	0.63	0.0107	6.13	6.13	0.68	0.0620
Model	14	475.61	788.62	0.64	0.0609	419.18	1.327	0.36	0.0322	57.57	2.88	0.32	0.0049
Residual	9	224.59	137.76			1325.92	82190			54.27	9.05		
Lack of Fit	6	2057.14	205.71	7.30	0.1607	1320.38	1.210	5.02	0.0612	111.84	111.02	4.71	0.1022
Pure Error	3	8.89	1.84			2.571	301.01			12.02	2.96		
R-Squared		0.9881				0.9912				0.9754			
Adequate Precision		12.662				11.921				13.643			

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

significant having large F-values and low p-values. For the acidic pretreatment, the Model F-values of 5.72, 3.33 and 2.33 for lignin, cellulose and hemicellulose shows the model's significance. In optimizing reduction of lignin, the most significant ($p < 0.05$) linear terms are A, C, AC, BD, A², B², C² and D² while those for cellulosic reduction are C, AB, BC, BD, A², B², C² and D². Similarly, the most significant terms for optimizing the reduction in hemicellulose are A, B, C, AB, AC, BD, A², C² and D². In the pretreatment by alkaline hydrogen peroxide, the F-values of 0.64, 0.36 and 0.32 for lignin, cellulose and hemicellulose all shows significance of the model. In

optimizing the reduction in lignin, the most significant model terms are D, E, AB, AC, AD, BD, CD, DE, B², C² D² and E² while those for the optimization of cellulose reduction are A, B, C, D, E, AB, AD, BC, CE, DE, B² and E². For optimizing hemicellulosic reduction, the terms A, B, C, AB, AC, BD, CD, CE, DE, A², B², C², D² are the most significant. The 'Adequate Precision' values i.e. 14.822, 13.192 and 11.561 for lignin, cellulose and hemicellulose in the acidic pretreatment shows that the model can be used for navigating the design space. The same is applied to the alkaline pretreatment with values of 12.662, 11.921 and 13.643 for the three structural components

respectively.

The fitness of the models were further checked by the coefficient of determination (R^2) values. The “Lack of Fit” F-values of 3.70, 3.59 and 2.92 for optimizing reductions in lignin, cellulose and hemicellulose during the acidic pretreatment all showed non significance thereby validating the fitness of the models since non-significant lack of fits are desirable for a model's fitness. In optimizing reductions of the three structural components during the alkaline pretreatment, the values of 7.30, 5.02 and 4.7 also showed non-significance and further validated fitness of the models. For the pretreatment with acid, the regression model equations showing the observed relationship between the responses i.e. percentage reduction in lignin, cellulose and hemicellulose and the values of each independent factor i.e. Exposure time coded as ‘A’, Temperature coded as ‘B’, Acid concentration coded as ‘C’ and Dry mass coded as ‘D’ are described below:

$$\begin{aligned} \text{Lignin} = & + 65.82 + 8.40A + 4.50B + 11.63C + 1.30D + 2.46AB \\ & + 1.54AC + 1.03AD + 1.17BC - 2.02BD - 0.18CD - 11.25A^2 \\ & - 3.38B^2 - 7.66C^2 - 7.78D^2 \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Cellulos} = & + 42.60 + 5.81A + 2.74B + 6.88C - 0.79D + 1.09AB \\ & + 0.36AC + 0.24AD - 0.24BC - 1.36BD - 0.19CD - 8.10A^2 \\ & + 0.100B^2 - 4.91C^2 - 4.90D^2 \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Hemicellulose} = & + 8.75 + 2.14A - 0.054B + 0.76C - 1.40D \\ & + 0.92AB - 0.74AC - 0.91AD + 1.54BC + 0.30BD + 0.53CD \\ & - 0.84A^2 + 0.19B^2 - 0.35C^2 + 0.20D^2 \end{aligned} \quad (5)$$

Similar equations describing the relationships during the alkaline pretreatment are shown as follows:

$$\begin{aligned} \text{Lignin} = & + 5.38 + 0.017A - 1.48B - 0.29C - 1.56D - 1.11E \\ & - 0.49AB - 0.60AC + 2.24AD + 0.15AE + 0.80BC - 0.31BD \\ & + 0.80BE + 0.17CD + 1.09CE - 0.45DE - 0.41A^2 + 1.30B^2 \\ & + 2.29C^2 + 1.24D^2 + 2.63E^2 \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Cellulose} = & + 29.54 - 0.25A - 0.63B + 0.083C - 0.86D \\ & + 0.29E + 1.20AB + 1.48AC + 1.35AD + 0.14AE + 0.41BC \\ & - 1.19BD - 0.48BE - 0.86CD + 2.27CE - 1.85DE - 0.92A^2 \\ & + 0.71B^2 + 1.16C^2 + 0.16D^2 + 1.63E^2 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Hemicellulose} = & + 14.67 + 0.067A - 0.48B + 0.075C - 0.47D \\ & - 0.24E - 0.39AB - 0.013AC + 0.31AD - 0.012AE + 0.84BC \\ & + 0.16BD + 0.013BE - 0.44CD + 0.66CE - 0.41DE - 0.46A^2 \\ & + 0.13B^2 + 0.33C^2 - 0.13D^2 + 0.67E^2 \end{aligned} \quad (8)$$

The above equations are also represented in the form of 3-dimensional (3D) plots (Figs. 3 and 4) and they diagrammatically display the relationships between all independent factors in

optimizing the pretreatment procedures involving both acid and alkali.

4. Discussion

The result of analyses revealed that Pineapple peel contains important nutrients and mineral elements which the microorganisms in the anaerobic digesters that utilize for their survival and which will equally bring about high microbial population and diversity culminating in higher yield of products [53]. Some researcher have applied pretreatments to different biomass and obtained results similar to those obtained in the current study. Su et al. [54] obtained values of 17.4 ± 0.3 , 36.5 ± 0.3 , 28.9 ± 0.3 and $5.2 \pm 0.8\%$ for lignin, cellulose and hemicellulose respectively after applying pretreatment to corn stalk. Cai et al. [18] also studied the pretreatment of whole corn parts and obtained values of 21.4, 43.4 and 19.5% for lignin, cellulose and hemicellulose respectively. In a similar study, Li et al. [55] evaluated the structural composition of the different parts of corn and obtained 20 ± 2 , 34 ± 3 and 24 ± 2 total lignin, cellulose and hemicellulose respectively. Venturin et al. [12] in their own study investigated the chemical properties of corn stalk and obtained values of 18.9 ± 1.4 , 32 ± 0.5 and 23.5 ± 0.5 for the three structural components respectively. The relevance of all these studies to the current one is that both pineapple peel and corn stalk are very similar in their structural compositions.

Pretreatment of pineapple peel using the two chemicals in this study both resulted in significant solubilization of the structural components of the biomass. The optimum values used for the acidic treatment according to the experimental design was able to achieve high solubilization of the hemicellulosic in the peel. In previous studies [12,19,55], prominent hemicellulose reduction was achieved when corn stalk was treated with acid. In the current study, lower quantity of acid and shorter time period was used to achieve enormous solubilization of hemicellulose over the results of previous studies. Another observation in the acidic pretreatment as carried out in this study was the increased lignin and cellulose component after the acidic pretreatment. This result is in agreement with a previous submission [18] in which 25% increase was observed for both lignin and cellulose after acidic treatment whereas, 87% reduction was recorded for hemicellulose. Other authors [20,21,24,55–57] have all reported similar findings. Not only did the cellulosic content increased after acidic pretreatment in this study, it was also modified due to the influence of the acid, elevated temperature and pressure in agreement with previous reports [18,46,55]. Sulfuric acid as seen in this study also affected lignin as there was reduction in important chemical bands i.e. 1734, 1716, 1633 and 1604 cm^{-1} bands all associated with lignin after acidic treatment of the biomass.

The results obtained for the alkaline pretreatment of pineapple peel using hydrogen peroxide is quite different from the result of treatment with acid in that the alkali acted more on the lignin component of the biomass bringing about its enormous solubilization because virtually all the peaks linked with lignin (1734, 1716, 1633, 1604 and 1516 cm^{-1} bands) were ruptured or completely disappeared. This phenomenon was documented in some earlier researches that pretreated biomass using alkalis such as H_2O_2 , NaOH and KOH [5,51–53]. By using hydrogen peroxide to pretreat corn straw, Cai et al. [18] obtained 19.6 and 32.8% reduction in lignin and cellulose respectively. In yet another study on the use of hydrogen peroxide on corn straw [57], obtained 38.9% reduction in lignin.

In comparison, there was gross decrease in the lignin-cellulose ratio obtained from the hydrogen peroxide pretreatment whereas, acidic pretreatment with H_2SO_4 caused the increase in this ratio. Most previous studies involving acidic pretreatment have

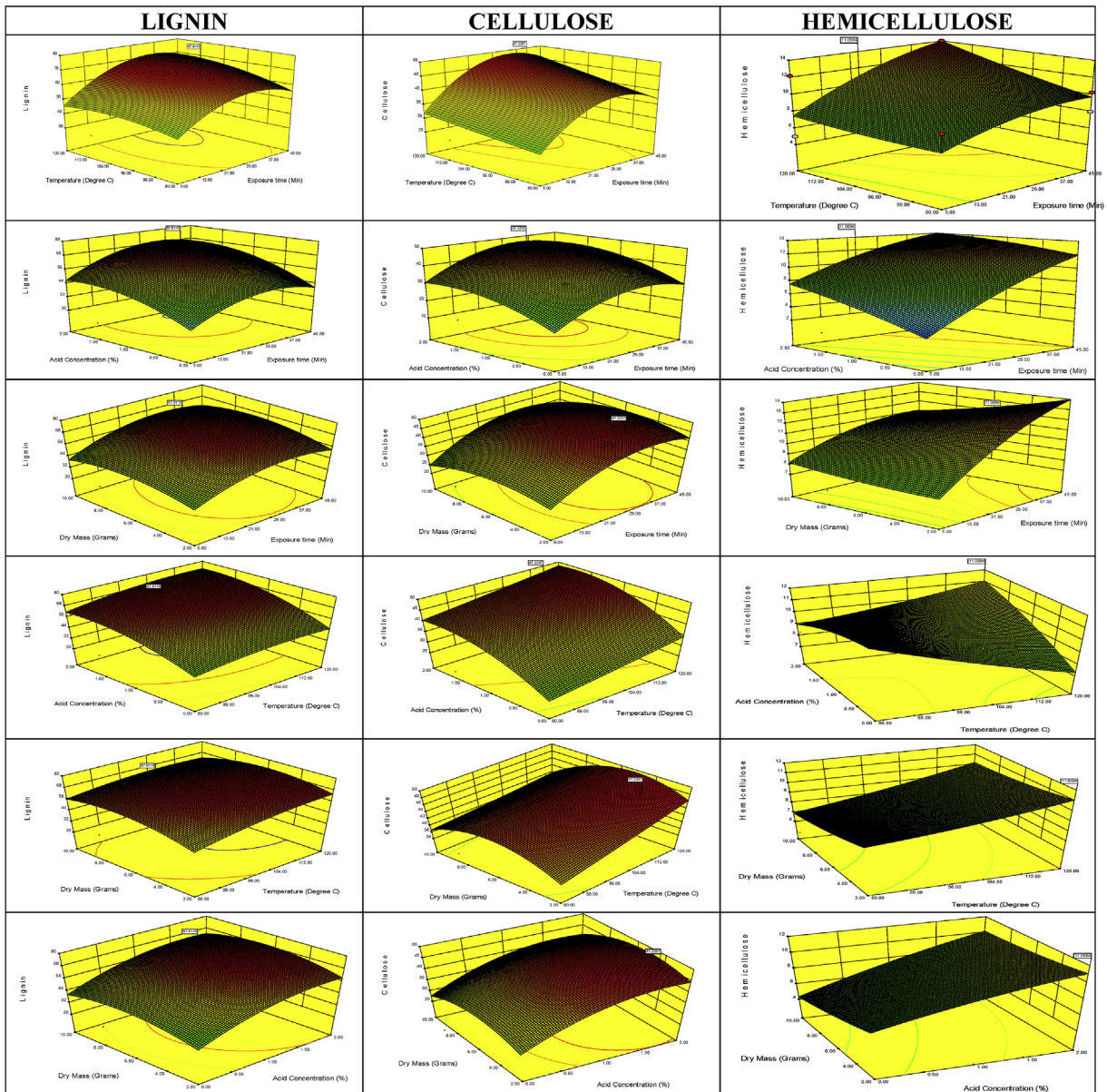


Fig. 3. 3-Dimensional curves for the optimization of sulfuric acid pretreatment for Pineapple peel.

reported increase in lignin and formation of pseudo lignin and other forms of lignin including phenolic, syringyl at the 1329 cm^{-1} band and the acetyl lignin at the 1251 cm^{-1} band as a result of acidic pretreatment which caused inhibition to the anaerobic digestion process and often leads to total failure [11,23,55,59]. As seen in this study, the action of sulfuric acid was preferential on phenolic lignin causing modification of the lignin whereas the hydrogen peroxide acted on the whole lignin besides causing an increase in the percentage of the syringyl ring of lignin [60]. However, both pretreatments caused reduction in the content of acetyl lignin with higher effects seen in the alkaline pretreated biomass [59,60]. Similar to lignin composition, pineapple peel was also shown to contain crystalline and amorphous cellulose ($1110\text{ cm}^{-1}/897\text{ cm}^{-1}$) and the ratio between them was 51.3 and 19.4% decrease for alkaline and acidic pretreatment respectively [18,55].

Similarly, the anaerobic digestion process had enormous effect on the pretreated biomass as further solubilization was recorded

after the digestion. This was characterized by increase in the values most elements as against their initial values before digestion whereas, carbon and calcium recorded reduced values as these have been used up by the microorganisms for metabolism and cell wall synthesis. The nitrogen content of pineapple peel was moderate thereby eliminating the usual inhibition caused by nitrogenous compounds during anaerobic digestion. A similar report has been documented for food wastes and different spent animal beddings [61].

Concentrations of VFA's as seen in the current study is not very different from earlier submissions [61]. Due to the huge bacterial population and diversity in the anaerobic reactors used in this study, the two last stages of digestion i.e. acetogenesis and methanogenesis were pronounced. Prominent among the bacterial species were members of the genera *Clostridium* which have been reported to have high efficiency in degrading amino-acids thereby producing acids coupled with the end-product which is usually ammonia [53]. Similarly, the high microbial population and

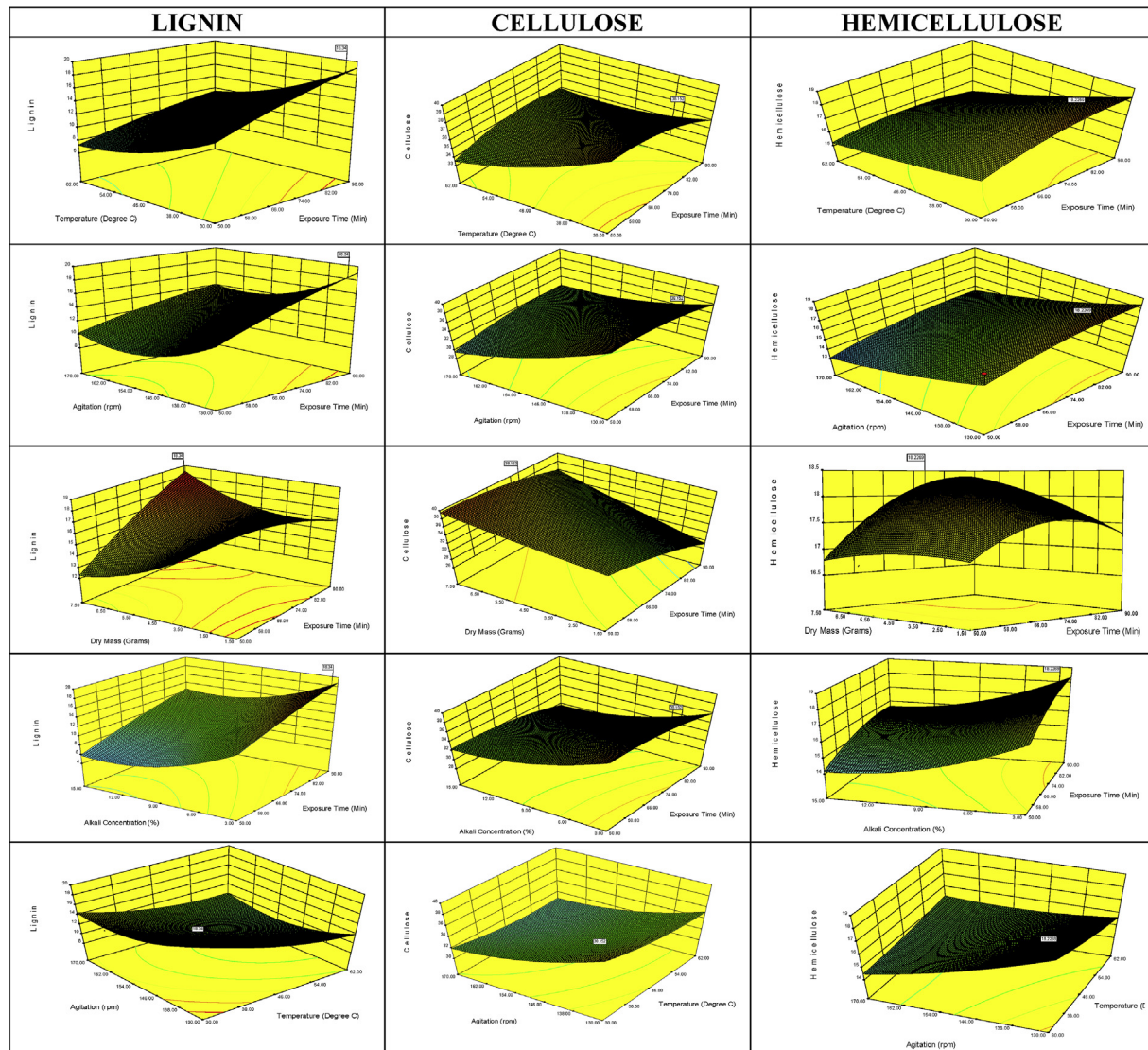


Fig. 4. 3-Dimensional curves for the optimization of alkaline hydrogen peroxide pretreatment for Pineapple peel.

activities brought about a higher volatile solid consumption indicating efficiency in organic matter conversion especially in the biomass pretreated with alkaline hydrogen peroxide leading to higher biogas.

As shown in Fig. 3, more biogas was produced from the biomass with alkaline pretreatment validating the effectiveness of alkali in biomass pretreatment and this was further demonstrated in the production rate ($\text{LNbiogas.kg VSad}^{-1}.\text{d}^{-1}$) which was also highest in the alkaline treated experiment. There is therefore substantial justification for biomass pretreatment before anaerobic digestion [62].

In the optimization studies for both pretreatment methods used in this study, the models are significant after being validated with tools such as the F-values and their p-values, the R^2 and the 'adequate precision' values. In this study, the adequate precision values of 14.822, 13.192 and 11.561 for lignin, cellulose and hemicellulose in the acidic pretreatment and 12.662, 11.921 and 13.643 in the alkaline pretreatment indicates fitting and significance

($p < 0.05$) of the models. Similarly, the "lack-of-fit" terms of 3.70, 3.59 and 2.92 for the optimization of lignin, cellulose and hemicellulose reductions in the acidic pretreatment and 7.30, 5.02 and 4.7 for the alkaline pretreatment showed non-significance which further validated the model's fitness. All the 3-D surface plots constructed to show the optimized reductions in lignin, cellulose and hemicellulose in the acidic pretreatment shows that all the model parameters interacted significantly while those for the alkaline treatment displayed moderate interactions. These results agrees with earlier reports [9,10].

Examination of the anaerobic digestates resulting from all the reactors in this study shows high quality which makes them idea candidates for use as organic fertilizers for soil properties ammendments and nutrients restoration. The digestates are very rich in soil beneficial microorganisms and nutrient elements for crop plant' growth and noutishment if added to the soil. Most countries across Africa are currently having gross issues of soil nutrient loss and infertility, soil pollution, toxicity to soil

microorganisms and other menaces ascribed to the over-dependence on inorganic fertilizers. An investment into organic agricultural practices including the use of digestate biofertilizers will help boost food security in this region [63–65].

As shown in Table 6, computation of the energy balance in the pretreatment of pineapple peels was carried using 50% thermal and 35% electrical energy efficiencies respectively [34]. The required heat for increasing the temperature of pineapple peel to 55 °C was then determined while neglecting loss of heat [35,36]. For the alkaline treated experiments, the gain in thermal energy by 1504 kWh t⁻¹ TS exceeded the 921 kWh t⁻¹ TS thermal energy employed for pretreatment. By this, a net thermal energy of 583 kWh t⁻¹ TS was recovered. This net energy could be higher if heat exchangers are used for pretreatment or reactor heating [66,67]. The thermal could also be fully integrated [68,69]. On the other hand, the acidic pretreatment of pineapple peel seems economically futile since a total of -200 kWh t⁻¹ TS was gain as thermal energy and this is much lower than the 1236 kWh t⁻¹ TS thermal energy that was used in the pretreatment giving a negative net value of -1436 kWh t⁻¹ TS.

For the electrical energy, only the energy used for substrate

mixing was considered since mechanical breakdown of biomass was done for all samples prior to chemical treatment [34,36,70,71]. A net energy of 874 kWh t⁻¹ TS was obtained from the alkaline pretreatment which surpasses the -633 kWh t⁻¹ TS from the acidic pretreated experiment. This further confirms the economic feasibility of using alkaline pretreatment over the acid.

5. Conclusions

This study shows that pretreatment of pineapple peel using the alkali enhanced enormous lignin reduction and enhanced biogas production. In comparison, biogas production was 67% more in the alkaline pretreated pineapple peel than the acid treated peel and also 51% higher than the untreated samples. The use of this alkali on lignocelluloses has remained unpopular prior to this study. However, its usage in this study yielded better result than all the conventional treatments yet at low cost. Also, the 583 kWh t⁻¹ TS net thermal energy obtained by applying alkaline H₂O₂ showed that the investment is economically feasible while the acidic pretreatment of Pineapple peels is not profitable due to a net thermal energy of -1436 kWh t⁻¹ TS that was obtained. Therefore, the use of

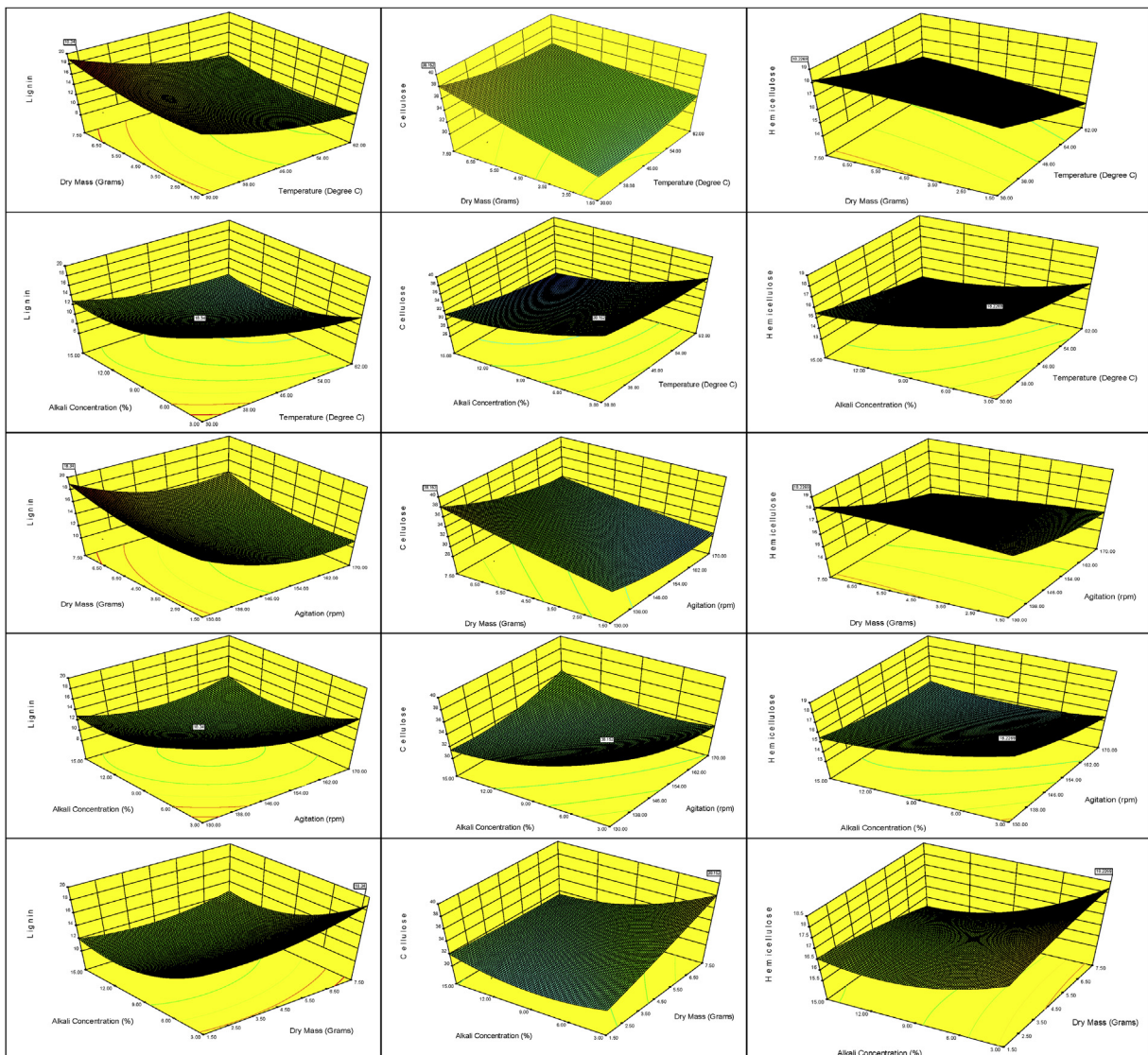


Fig. 4. (continued).

Table 6
Energy and economic evaluation for the digestion of Pineapple peel.

Energy parameters	H ₂ O ₂ pretreated	H ₂ SO ₄ pretreated	Not Sifted (Untreated)	Sifted (Untreated)
Produced electrical and thermal energy from combined heat and power (CHP)	3335	1251	1503	1372
Produced thermal energy (kWh t ⁻¹ TS)	2687	983	1183	1101
Produced electrical energy (kWh t ⁻¹ TS)	1477	663	893	776
Thermal balance				
^a Thermal energy gain (kWh t ⁻¹ TS)	1504	-200	-	-
Thermal energy requirement (kWh t ⁻¹ TS)	921	1236	-	-
Thermal energy requirement with 80% of heat recovery (kWh t ⁻¹ TS)	184	247	-	-
^b Net thermal energy (kWh t ⁻¹ TS)	583	-1436	-	-
Net thermal energy with 80% of heat recovery (kWh t ⁻¹ TS)	466	-1148	-	-
Electrical balance				
^c Electrical energy gain	1832	-252	-	-
Energy for mixing during pretreatment	958	411	-	-
Net electrical energy	874	-633	-	-
Economic evaluation				
Cost of H ₂ O ₂ and H ₂ SO 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.

mild alkaline pretreatment is advocated in biogas generation from pineapple peel and also for quality biofertilizer production. This will be more beneficial for renewable and sustainable energy generation.

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