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Cleaner energy for cleaner production: Modeling and optimization of biogas generation from *Carica papayas* (Pawpaw) fruit peels

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ABSTRACT

In this study, the potentials of pretreated and untreated *Carica papayas* fruit peels for biogas generation was evaluated alongside its process optimization after employing the combination of mechanical and thermo-alkaline pretreatment methods. The peel was anaerobically digested using a consortium of microorganisms from cattle rumen content as inoculum. A batch system designed by the Central Composite Design (CCD) was employed. The physicochemical and microbial characteristics of the substrates and inoculum as well as the constituent of the generated biogas were evaluated using standard methods and results showed elevated levels of most elements after anaerobic digestion. The most feasible experimental biogas yields were 0.1839 m^3/kg VS and 0.1361 m^3/kg VS for the pretreated and untreated experiments respectively. Microbiologically, members of the genus *Clostridium* dominated the microbial flora and their succession patterns were affected by temperature and pH. The methane and carbon dioxide content of biogas from both experiments were 61.5± 2.5%; 24± 1% and 52 ± 2%; 25± 1.5% respectively. The Response Surface Methodology (RSM) and the Artificial Neural Networks (ANNs) were employed in data optimization. Based on the optimized values, the predicted biogas yield for RSM was 0.1895 m^3/kg VS and 0.1839 m^3/kg VS for ANNs in the pretreated experiment. For the experiment without pretreatment, the RSM predicted yield was 0.1361 m^3/kg VS while that of ANNs was 0.1392 m^3/kg VS. In all, there was a 26.5% increase in predicted biogas yield in the pretreated experiment over the untreated. Further usage of pawpaw peels for biofuels generation is advocated.

1. Introduction

The global quest for renewable and sustainable energy generation has been incessantly increasing over the last few decades as the world's over-reliance on fossil fuels is taking its toll on humanity in terms of environmental degradation, spread of disease and climate change/global warming via Greenhouse gas (GHG) emissions (Anjum et al., 2016; Guenther-Lübbers et al., 2016; Priebe et al., 2016). To this end, there is need for the integration of cleaner production technologies and adequate policy implementation in solving the world's numerous environmental challenges most especially on the issues of energy generation and utilization (Klemes et al., 2012; Kalbar et al., 2016). Over 88% of the global energy consumption is from fossil fuels and this is usually accompanied with environmental issues such as GHG emissions and pollution of soil, air and water (Gonzalez-García et al., 2016). This situation has led to numerous studies which focused on the generation of renewable and sustainable energies from different agricultural, industrial and domestic materials and the produced fuels have been reported to be environmental friendly and also reduce GHG emissions (Mijakovski et al., 2016; Leonzio, 2016; Yong et al., 2016).

Anaerobic digestion is a biochemical process that is widely used for the treatment and energy recovery from many kinds of biomasses, especially agricultural products and agro-industrial wastes

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(He et al., 2016; Othman et al., 2017). It is an efficient method which employs diverse microbial groups for the conversion of biomasses and wastes into biogas (which serves to mitigate the energy challenges currently faced in several parts of the world) (Ismail and Talib, 2016; Chuichulcherm et al., 2017) and digestate biofertilizer which can be used to improve soil nutrients and plant growth when applied either in solid or liquid form (Shane and Gheewala, 2017; Tampio et al., 2016a).

Carica papayas (Pawpaw) originated from Southern Mexico, Central and South America from where its cultivation spread to other locations where it is currently found (Anon, 2010). It is a flowering plant belonging to the family *Caricaceae*, comprising up to 25 species of short-lived evergreen shrubs or small trees usually growing to a height of approximately 10 m. Its cultivation is currently dominated by countries with tropical climate, such as Brazil, India, South Africa, Nigeria, Mexico, Haiti and South East Asia while production occurs in more than 60 countries worldwide (Evans et al., 2012). According to the Food and Agricultural Organization (FAO), global production in year 2010 was estimated to be 11.22 million metric tons (FAOSTAT, 2012). Production in Nigeria reached 750, 000 tons in 2011 alone and the crop is widely cultivated in several cropping systems across the country with the South western region being its major area of dominance (FAOSTAT, 2011).

Several parts of the pawpaw plant have been put to efficient usage over the decades. Despite this, the skin/peel has not been efficiently used and is often regarded as wastes in most pawpaw-producing localities. The peel is often removed and thrown away or fed to domestic animals. This formed the basis of this research in using pawpaw fruit peel as a substrate for biogas generation having in mind that its high sugar content as a lignocellulosic biomass will form a suitable platform for hydrolysis and fermentation by hydrolytic and acidogenic microbes.

Use of lignocelluloses for the generation of renewable and alternative energy is popular worldwide and this is due to their abundance/availability and significant roles in GHG emissions reduction when properly utilized (Auburger et al., 2016; Shane et al., 2016). However, the high lignin contents of these biomasses have remained a major issue militating against their usage at the commercial level (Carrere et al., 2016). In order to overcome this major barrier, several pretreatment methods including biological, mechanical, thermal and chemical have been considered for improving the biodegradability of lignocellulosic biomasses (Cai et al., 2016; Lalak et al., 2016; Li et al., 2016a, b). Application of these pretreatments usually brings about efficiency of digestion, sludge reduction, digestate dewatering and rich microbial diversity leading to higher methane yield. Among other methods, alkaline pretreatment have proved more suitable for lignocelluloses especially in terms of cost and increased methane generation. In a study, Dongyan et al. (2014) pretreated wheat straw with thermo-alkaline method and obtained a 3.5 and 3.1-fold higher methane generation respectively over the ultrasonic and thermal pretreated sludge. Also, alkaline treatment holds great promise for the future of the bioenergy industry in its flexibility to be combined with other methods like thermal, ultrasound and microwave in order to maximize biomass valorization and higher methane generation over the application of a single pretreatment (Jang and Ahn, 2013).

In past researches, temperatures of between 50 and 250° C have been severally experimented in the digestion of different lignocelluloses. Excessive solubilization of lignin and production of inhibitors (phenols) have been reported at temperatures of 150° C and above. A typical example of such inhibition is the Mallaird reaction occurring between carbohydrate and protein monomers and which eventually retard the rate of anaerobic digestion as a result of formation of complex inhibiting substances via the reaction (Carrere et al., 2010; Elliot and Mahmood, 2012). Also, the loss of volatile organic acids is another major disadvantage of applying thermal pretreatment at high temperatures. Similarly, application of temperatures of 100° C and below over a longer treatment time for lignocelluloses is well reported most of which led to total failure in the degradation of complex biomasses (Protot et al., 2011) while some, especially with thermal pretreatment at 70° C yielded higher biogas production (Appels et al., 2010; Rafique et al., 2010; Chamchoi et al., 2011).

The aim of this research therefore was to evaluate the biogas producing potentials of pawpaw fruit peels via anaerobic digestion and using combination of different pretreatment methods. Though, biogas generation from the co-digestion of pawpaw fruit peels and poultry dropping has been documented (Dahunsi et al., 2016a), this is the very first reported attempt to use pawpaw fruit peels for bioenergy generation in mono-digestion. Since standardization of bioprocess parameters is an important step in biofuel/biochemical production procedure (Betiku and Ajala, 2014), the optimization of the process parameters was also carried out to establish a benchmark for pawpaw peel's usage as a bioenergy substrate.

2. Materials and methods

This section includes collection of raw materials, the pretreatment procedures, various analyses carried out and their methodologies.

2.1. Collection of sample

Carica papayas (Pawpaw) peels were collected from the Teaching and Research farm and the staff quarters of Landmark University, Omu-Aran, Kwara State, Nigeria and moved to the experimental site within a distance of 30 m in the same farm. Also, cattle rumen content was collected from the slaughter slab of Landmark University within a 50 m distance and used as inoculum in order to have adequate microbial flora in the digestion system. The use of microbial consortia in anaerobic digestion processes is well documented (Dahunsi et al., 2016a, b; Yuan et al., 2016). Considering the high lignin content of the peels, and the quest to overcome the rate-limiting challenge often encountered during the hydrolysis step of anaerobic digestion, pretreatments were applied. A combination of mechanical and thermo-alkaline pretreatments earlier described (Dahunsi et al., 2016a, b; 2017a) were employed. In doing this, the biomass was grinded into $\leq$20 mm mesh sizes using a hammer mill and was then followed with a 60 min thermal treatment at 80 °C using the CLIFTON 88579 water bath (Nickel-Electro Ltd., England). This chosen temperature was a modification of previous reports of thermal treatments which documented that higher temperatures adversely affected the overall performance of the anaerobic digestion system (Liu et al., 2012). Heating was then followed by chemical pretreatment using 3 g NaOH for 100 g TS at 55° C for 24 h and a solid loading of 35 g TS/L. NaOH was used since it has been reported as the alkali of choice by previous studies on thermo-alkaline biomass pretreatment (Dai et al., 2016; Dahunsi et al., 2017a, b). Another experiment was set up using the same substrate (*C. papayas* fruit peels) with application of mechanical breakdown but without thermo-alkaline pretreatments prior to digestion. This was meant to evaluate the effects of thermo-alkaline pretreatments on the substrate. The batch digesters used were 25 L in size each with an air-tight tank containing in-built mechanical stirrer for frequent substrate mixing. A liquid displacement apparatus was used for gas collection (Dahunsi and Oranusi, 2013; Alfa et al., 2014a).

2.2. Experimental design

Prior to the digestion of *C. papayas* fruit peels, the batch

experiment was designed using the Central Composite Design (CCD) due to the successes recorded in its usage during anaerobic digestion experimental designs (Dahunsi et al., 2016a, b). The Five-level-five-factors factorial design was applied with a total of 50 experimental runs. The five important variables selected for the modeling and optimization were Temperature (° C), pH, Retention time (days), Total solids (g/kg) and Volatile solids (g/kg) separately designated as **I, *J*, *K*, *L*** and ***M*** respectively. These factors were selected based on their importance and the need to standardize them in the anaerobic degradation of the substrate as this will have qualitative application in subsequent studies on the same substrate especially for commercialization. Most researches have reported the temperature for most mesophilic digestion systems to be between 30 and 40° C using different biomasses (Tampio et al., 2016b). For pH, accepted values usually range between 6.5 and 8.0 for efficient functioning of anaerobic bacteria (Dahunsi et al., 2016a, b; Ennouri et al., 2016). Since the ambient temperature of production usually interferes with the digestion process, most reports have suggested between 20 and 30 days as the retention time for mesophilic digestions (Hao et al., 2016; Leite et al., 2016). For solid contents, most liquid state digestions has been operated with total solids content between 4 and 15% (Tampio et al., 2016b). Based on these reports, the need arose to document the very optimal conditions required for the most efficient mesophilic digestion of *C. papayas* fruit peels as a biogas resource. The data generated via the CCD was equally used for the Artificial Neural Networks (ANNs) module with the aid of the Neural Power version 2.5 (CPC-X software) so as to select the statistically well distributed data in the input search window.

2.3. Methane potential tests

In order to determine the degradability of *C. papayas* fruit peels at standard temperature and pressure (STP) prior to field experiment, the biomethane potential and residual methane tests were carried out according to standard methods (Dahunsi et al., 2016a, b). The 30-day batch anaerobic experiment was performed using three digesters i.e. two experimental and a blank with an inoculum to substrate ratio of 2 (Experiments were done in triplicate). Collection of produced gas from the digesters was continuous and the methane content was determined by gas chromatography.

2.4. Digestion

A total of 8 kg each of the pretreated and untreated *C. papayas* fruit peels samples was made into slurry by adding water in the ratio 1:1 and to which one kg of inoculum was added thereby making a total of 17 L slurry charged into each of the digestion tanks (Dahunsi et al., 2016a, b). In order to ascertain the treatment efficiency, several important process parameters were periodically evaluated. Such include daily measurement of gas production, weekly assessment of microbial succession and chemical analyses of fermenting materials and digestates, measurement of daily temperature and pH on a weekly basis using a pH meter. The determination of methane (CH_4) and carbon dioxide (CO_2) contents of the produced biogas were achieved using a HP 5890 Gas Chromatography (Avondale, USA) with an attached Hayesep Q column (13m × 0.5m × 1/800) and a flame ionization detector (Alfa et al., 2014b; Dahunsi et al., 2016a, b).

2.5. Analytical procedure

Before the digestion of both treated and untreated samples of *C. papayas* fruit peels, chemical analyses were carried out in order to quantify their elemental/nutrients composition and other physical factors. These parameters were also evaluated for the inoculum and digestates obtained after digestion. A total of 18 different parameters (Shown in Table 1) were evaluated in the Environmental Engineering and Soil mechanics/Geotechnics laboratories of Landmark University. In these evaluations, the advanced digital-readout colorimeter (Photometer 7500 (PHOT.1.1.AUTO.75, Camlad, Cambridge, England) was used according to earlier established procedures (Dahunsi et al., 2016a, b) in which an absorbance of 0.5 and wavelength of 450 nm were used in analyzing all samples in triplicates. In order to determine the Chemical Oxygen Demand of the samples, the Standard Methods for the examination of water and wastewater (APHA, 2012) which was also used by Dahunsi et al. (2014) were used while total solids (TS) and volatile solids (VS) were analyzed following the SFS 3008 protocol of the Finnish Standard Association (1990).

2.6. Energy balance and efficiency assessment of thermo-alkaline pretreatment application

The economic feasibility of thermo-alkaline pretreatment application to *C. papayas* fruit peel was established in this study by assessing the balance between generation and consumption of energy. The cost of procuring thermal energy and alkali (NaOH) was compared with the gain from the extra energy obtained when the thermo-alkaline pretreatment was applied. By this, it was determined if the extra gas yield obtained from digesting the pretreated substrates could cover the initial cost of thermal energy and NaOH. Therefore, the thermal energy required (TER) in kWh/t TS for pretreating *C. papayas* shoot mixture from an initial temperature of 25 to a final 55° C was computed using a simple equation (Dahunsi et al., 2017b) as shown below:

$$\mathrm{TER} = \frac{w \times fh^{*}(Tfinal - Tinitial)}{3600} \quad (1)$$

where w (1000 kg) = mass of the mixture of *C. papayas* shoot and water; fh = specific heat of water which is 4.18 kJ/kg C; *T initial* (° C) = the initial temperature of substrate; *T final* (° C) = the final temperature of substrate. The US cost of NaOH was used.

2.7. Aerobic and anaerobic enumeration

Aerobic bacteria involved at every stage of the digestion process were isolated and characterized using standard methods which involved plate count and biochemical testing. Phenotypic methods were used in the identification and presumptive candidates were confirmed with the aid of appropriate rapid API kits (BioMerieux, Lyon, France) (Dahunsi et al., 2016a, b). Fungi were isolated by culturing samples on Potato dextrose agar (PDA) and the structures of the hyphae, morphology of cell spores and the nature of the fruiting bodies were considered in their identification according to the method of Tsuneo (2010). Facultative *Clostridium* species and other anaerobic acidogens were isolated anaerobically using two important media (Reinforced Clostridia medium and blood agar) at temperature of 37° C for approximately one week. Biochemical procedures were followed in the preliminary characterization and the presumptive isolates were identified using appropriate rapid API kits as reported (Ayandiran et al., 2014; Ayandiran and Dahunsi, 2017). All analyses were carried out weekly in triplicates in the Microbiology laboratory of Biological Sciences Department, Covenant University, Ota, Nigeria.

2.8. Enumeration of methanogen

A mineral-rich basal medium (BM) was compounded and used

Table 1
Physical and chemical characteristics of *Carica papayas* fruit peels and cattle rumen content (n = 60).

Parameters	Rumen content	Raw *C. papayas* peels only	Pretreated *C. papayas* peels + rumen content		Untreated *C. papayas* peels + rumen content	
			Before digestion	After digestion	Before digestion	After digestion
pH	7.91 ± 0.02	6.23 ± 1.00	7.70 ± 0.02	7.60 ± 0.03	7.75 ± 2.02	7.72 ± 1.01
Total Solids (g/kg TS)	90.52 ± 0.11	94.81 ± 1.21	110.97 ± 0.11	93.94 ± 0.02	118.05 ± 1.03	113.04 ± 1.02
Volatile Solids (g/kg TS)	80.44 ± 1.12	83.23 ± 0.22	96.22 ± 3.02	50.01 ± 2.02	88.15 ± 2.01	69.06 ± 1.00
Ash Content (%)	5.56 ± 1.02	2.54 ± 1.00	2.78 ± 0.00	5.49 ± 0.03	1.82 ± 0.02	1.09 ± 2.00
Moisture Content (%)	90.48 ± 3.02	97.26 ± 0.01	94.03 ± 4.01	96.06 ± 1.02	98.27 ± 1.01	102.17 ± 2.11
Chemical Oxygen Demand (mg/kg TS)	168.21 ± 1.12	165.11 ± 2.20	256.5 ± 4.04	83 ± 2.01	267.08 ± 2.03	98.22 ± 2.02
Total Carbon (g/kg TS)	265.21 ± 0.10	202.90 ± 4.03	214.90 ± 5.03	200.10 ± 3.03	184.13 ± 1.01	169.32 ± 2.00
Total Nitrogen (g/kg TS)	48.00 ± 2.02	37.51 ± 2.02	40.00 ± 1.01	41.60 ± 0.11	35.05 ± 1.01	31.60 ± 1.01
Total Phosphorus (g/kg TS)	6.30 ± 0.02	5.32 ± 1.02	6.12 ± 0.01	7.60 ± 1.11	4.91 ± 0.01	5.33 ± 0.11
Potassium (g/kg TS)	7.20 ± 0.11	7.32 ± 2.00	8.00 ± 0.11	10.94 ± 0.03	7.33 ± 1.01	7.53 ± 2.01
Phosphate (g/kg TS)	3.00 ± 0.02	1.03 ± 0.11	3.00 ± 0.10	4.51 ± 0.02	2.01 ± 2.51	2.50 ± 2.01
Sulphate (g/kg TS)	134 ± 2.00	112.20 ± 3.01	136.00 ± 2.03	159.49 ± 0.03	121.20 ± 0.01	133.80 ± 0.01
Calcium (g/kg TS)	80.00 ± 0.10	220.81 ± 4.41	226.00 ± 4.09	89.06 ± 2.00	173.34 ± 1.05	102.31 ± 0.01
Magnesium (g/kg TS)	96.00 ± 0.10	89.32 ± 1.02	100.00 ± 0.03	200.10 ± 5.05	82.7 ± 0.01	98.6 ± 2.00
Manganese (g/kg TS)	1.18 ± 0.22	0.021 ± 1.00	0.028 ± 0.00	0.060 ± 0.01	0.018 ± 0.01	0.022 ± 2.01
Iron (g/kg TS)	1.18 ± 0.11	1.06 ± 0.11	1.16 ± 0.21	4.60 ± 1.00	0.87 ± 1.11	1.00 ± 1.01
Zinc (g/kg TS)	38.00 ± 0.02	32.32 ± 0.01	36.00 ± 0.03	40.94 ± 1.22	31.10 ± 1.01	33.90 ± 0.01
Aluminium (g/kg TS)	0.80 ± 0.11	0.52 ± 1.02	0.76 ± 0.02	0.91 ± 0.03	0.56 ± 0.01	0.59 ± 0.01
Copper (g/kg TS)	4.80 ± 0.10	3.87 ± 0.03	4.70 ± 0.03	5.49 ± 0.03	4.27 ± 2.01	4.34 ± 1.01

for the evaluation of methanogens following earlier description by Ghosh et al. (2014). The BM contained minerals, trace elements and dyes such as NH_4Cl, KH_2PO_4, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, $NaHCO_3$, sodium resazurin dye, Na_2S, cysteine−HCl, and sodium−thioglycolate all prepared under anoxic environment with double distilled water having 7.0 final pH. To this prepared BM was added a supplement solution, $NaHCO_3$, cysteine−HCl and $FeSO_4$ in H_2SO_4· The supplement solution added to the BM also contained vitamins and trace elements which were all dissolved in double distilled water. The basal medium, $FeSO_4$, and the supplement solution were separately autoclaved and then mixed with the $NaHCO_3$ and cysteine−HCl which had earlier been filter sterilized (Stieglmeier et al., 2009). All the liquid media were rid of dissolved oxygen by flushing with nitrogen gas until the resazurin dye (indicator) turned colorless.

2.9. Statistical data analysis

The data obtained from the digestion of *C. papayas* fruit peels was statistically analyzed with the aid of the Response Surface Methodology (RSM) in order to fit the quadratic polynomial equation generated via the Design-Expert software version 9.0.3.1 (Stat-Ease Inc., Minneapolis, USA). The coefficient of the polynomial model of the response was fit using multiple regressions so as to correlate the response variable to all the five selected independent variables. The test of significance and analysis of variance (ANOVA) were used to fit the quality of the model and the fitted quadratic response model is described by:

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

where: Y = the response variable; b_o = intercept value; b_i (i = 1, 2, k) = the first order model coefficient; b_{ij} = the interaction effect; b_{ii} = the quadratic coefficients of X_i while e = the random error. Validation of the RSM model was carried out using same digesters and the predicted values after which the plots of the deviations of actual and observed values were constructed. ANNs was equally used to statistically analyze the data obtained from the CCD as earlier reported (Dahunsi et al., 2016a). The mean square error (MSE) approach was used to determine the optimum ANN structure and the higher coefficient of determination (R^2) were determined. Variable analysis was also conducted to study the effects of variables towards the biogas yield with the aid of 3-Dimensional curvature surface plots and relative importance. The ANNs structure was used for modeling the biogas production and the results were compared with those of RSM. Validation of the two models was done with same digesters using conditions predicted by the software and the deviations of actual values from the observed values were then plotted (Dahunsi et al., 2016a).

3. Results

3.1. Stability and performance of digestion

In the methane potential tests, gas generation commenced on the 4th and 7th days for the pretreated and untreated samples of *C. papayas* fruit peels respectively. Furthermore, the CH_4 and CO_2 contents of the generated biogas were 59± 2%; 24± 2% and 51± 2.5%; 25± 2% for both experiments. The results of the physical and chemical analyses of important parameters in both substrates and the inoculum before and after digestion are shown in Table 1. The pH of all digesters fluctuated around the slight alkaline range throughout though with an initial fall in the early days of experiment (Fig. 1). The same trend was observed for temperature fluctuation which was within the mesophilic range all through in both experiments. From the analytical processes carried out on both pretreated and untreated samples of *C. papayas* fruit peels, there was increase in the values of most parameters except for total and volatile solids, total carbon and calcium which were reduced afterwards. As for the values of chemical oxygen demand (COD), reductions of 67.60 and 51.69% were recorded for both experiments after the various digestions. For the experiment with pretreatments, biogas production commenced on the 4th and latest the 6th day of digestion while it started on the 6th through the 9th day in most digesters for the experiment without pretreatment. Production in both experiments was progressive until between the 18th and 22nd days when diminishing was observed for the rest of the digestions as shown in Fig. 2.

3.2. Microbial composition

At the different stages of the anaerobic digestion, several aerobic

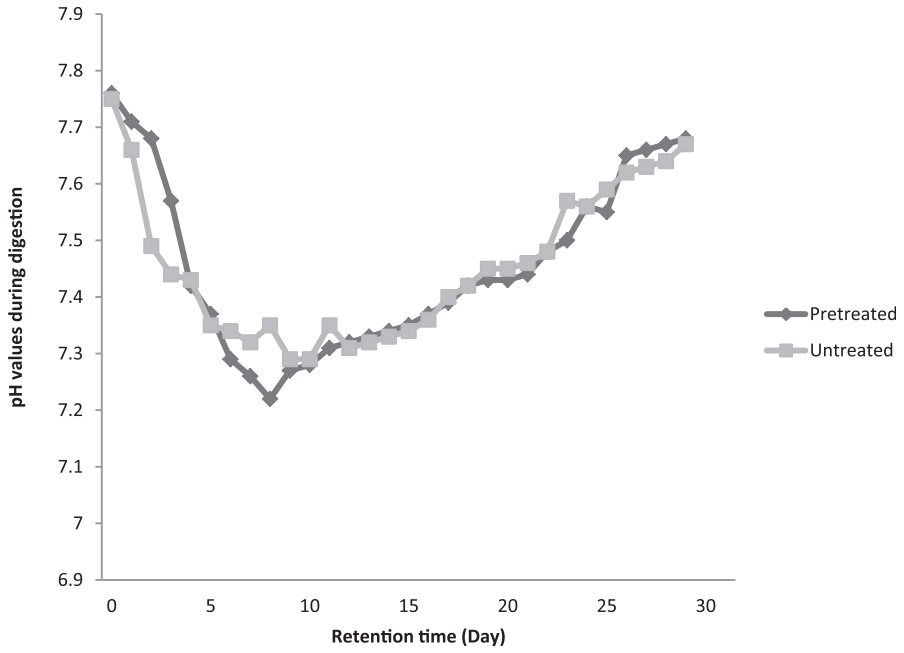

Fig. 1. pH fluctuations during the anaerobic digestion of pretreated and untreated *Carica papayas* fruit peels.

bacteria, anaerobes, methanogens and fungi were implicated and their succession pattern throughout the digestion periods are as reported in Table 2. In terms of succession, the population of aerobes and fungi increased sporadically until the third week where there highest populations were found before declining till the end of the experiments and their lowest counts were recorded during the last five days of experiments. For the facultative anaerobes and methanogens, decreases in populations were observed till the 20th day when the numbers rose and remained increasing till the end of the experiment. The isolated aerobes include *Bacillus megaterium, Bacillus circulans, Bacillus polymyxa, Bacillus licheniformis, Bacillus stearothermophilus, Proteus vulgaris, Proteus mirabilis* and *Enterococcus faecalis.* Anaerobes include *Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme Clostridium histolytica*, *Clostridium* spp and *Gemella morbillorum*. Fungal isolates include *Aspergillus niger, Mucor*, *Rhizopus stolonifer* and *Penicillum* while species of the genus *methanococcus, methanosarcinae* and *methanosaeta* were implicated as methanogens. The highest aerobic total count (TC) was 2.4×10^{11} cfu/mL while the lowest was 2.0×10^{7} cfu/mL. The highest fungal TPC was 2.4×10^{8} cfu/mL while the lowest was 2.0×10^{3} cfu/mL. For anaerobes, the highest TPC was 2.7×10^{10} cfu/mL while the lowest was 1.1×10^{10} cfu/mL.

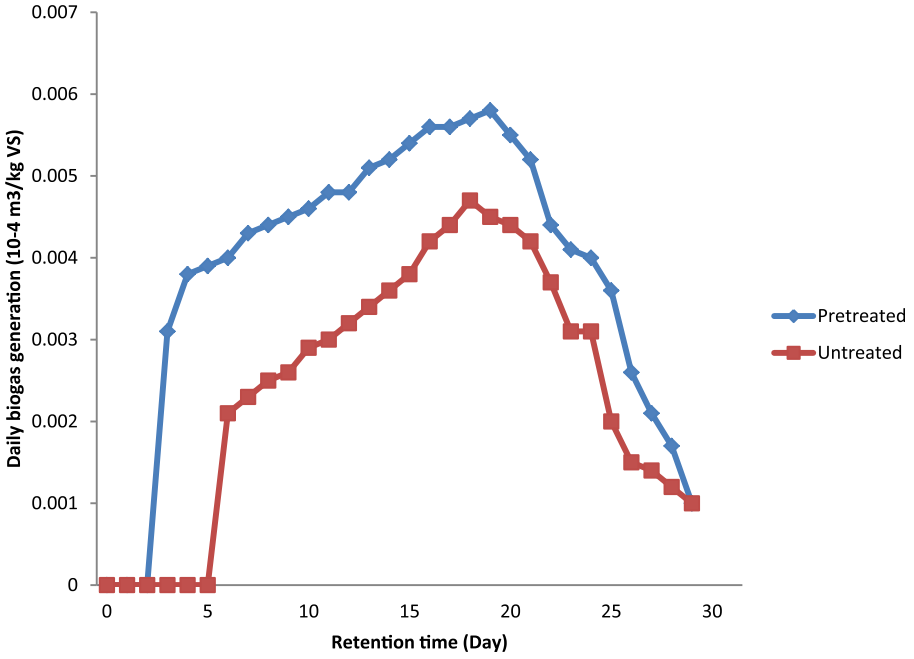

Fig. 2. Daily biogas generation during the anaerobic digestion of pretreated and untreated *Carica papayas* fruit peels.

Table 2
Microbial evaluation and succession pattern in the anaerobic digestion of *Carica papayas* fruit peels (CFU = Colony Forming Unit; TC = Total count).

Day	Aerobes (cfu/mL)		Fungi (cfu/mL)		Anaerobes (cfu/mL)		Methanogens (cfu.mL)	
	Organism	TC	Organism	TC	Organism	TC	Organism	TC
0	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	2.2×10^{10}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	1.9×10^{8}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	1.7×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	1.4×10^{10}
6	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	2.3×10^{10}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	2.1×10^{8}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	1.5×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	1.2×10^{10}
12	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	2.4×10^{11}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	2.4×10^{8}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	1.1×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	1.1×10^{10}
18	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	2.2×10^{10}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	1.6×10^{6}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	2.0×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	1.5×10^{10}
24	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	1.1×10^{10}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	7.0×10^{4}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	2.2×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	1.8×10^{10}
30	*Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*	2.0×10^{7}	*Aspergillus niger* *Mucor*, *Rhizopus stolonifer* *Penicillum*	2.0×10^{3}	*Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme* *Clostridium histolytica*, *Clostridium* spp *Gemella morbillorum*	2.7×10^{10}	*Methanococcus* spp *Methanosarcinae* spp *Methanosaeta* spp	2.4×10^{12}

The highest methanogenic TC was 2.4×10^{12} cfu/mL while the lowest was 1.1×10^{10} cfu/mL.

3.3. Optimization

The five-level-five-factor response surface design of the biogas generation from *C. papayas* fruit peels was done using a network structure by the CCD. The coefficients of the equation of the full regression model and their statistical significance were evaluated using the Design Expert 8.0.3.1 software. The test of significance and the ANOVA result for the second-order response surface model for all the regression coefficients are shown in Table 3. In the optimization of both experimental data, all the F-values are large with corresponding low p-values thus making a good number of the model terms significant ($p < 0.05$) for the studies. In both experiments, the F-values of 5.46 and 3.96 for the models with low p-values of 0.0063 and 0.0077 implies significance of the models. For the pretreated experiment, the model terms: *J*, *M*, *IK*, *IL*, *IM*, *JK*, *JL*, *JM* and M^2 are the most significant ($p < 0.05$) while *J*, *M*, *IL*, *JM*, *KL* and K^2 are the most significant for the experiment without pretreatment. The coefficient of determination (R^2) was used to check the 'goodness of fit' of the used models and as such, the F-values 0.96 and 7.19 with corresponding terms of 0.5770 and 0.4612 for both experiments respectively implies non significance hence the goodness of the fit for the experiments (see Table 4).

The probability that the models could be adequately used for the prediction was measured using the 'Adequate Precision' ratio and values of 13.883 and 10.415 were obtained indicating adequate

Table 3
Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for biogas generation from *Carica papayas* fruit peels (I = Temperature (° C); J = pH; K = Retention time (Day); L = Total solids (g/kg): M = Volatile solids (g/kg); df = degree of freedom; SS = Sum of square; MS = Mean square).

Source	df	Pretreated *C. papayas* fruit peels				Untreated *C. papayas* fruit peels			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value
I	1	68193	68193	2.91	0.1224	5224	5224	2.24	0.4205
J	1	2.202	2.202	9.39	**0.0135**	0.135	0.135	4.52	**0.0123**
K	1	4060	40599	1.73	0.2209	2209	2209	4.13	0.0744
L	1	1277	1277	0.054	0.8207	8207	8207	4.92	0.0752
M	1	3.085	3.085	13.15	**0.0055**	3055	3055	5.32	**0.0412**
IJ	1	23531	23531	1.00	0.3427	4.343	4.343	5.06	0.0721
IK	1	1.160	1.160	4.94	**0.0533**	0.053	0.053	5.42	0.1461
IL	1	5.217	5.217	22.24	**0.0011**	4.001	4.001	1.44	**0.0111**
IM	1	2.095	2.095	8.93	**0.0152**	6.152	6.152	4.54	0.1412
JK	1	1.775	1.775	7.56	**0.0225**	4.025	4.025	4.84	0.1276
JL	1	2.945	2.945	12.6	**0.0063**	7.006	7.006	6.47	0.7322
JM	1	2.278	2.278	9.71	**0.0124**	2.012	2.012	4.81	**0.0133**
KL	1	81466	81466	3.47	0.0953	1.095	1.095	5.15	**0.0218**
KM	1	55135	55135	2.35	0.1596	3156	3156	7.04	0.0507
LM	1	116.59	116.59	4.97	0.9453	2.643	2.643	1.79	0.0667
I^2	1	38693	38693	1.65	0.2311	0.411	0.411	1.39	0.2187
J^2	1	3676	3676	0.16	0.7015	1735	1735	2.33	0.2137
K^2	1	2901	2901	0.12	0.7332	1.733	1.733	2.15	**0.0225**
L^2	1	15936	15936	0.68	0.4311	3434	3434	1.66	0.3319
M^2	1	1.409	1.409	6.01	**0.0367**	2.037	2.037	4.14	0.1417
Model	20	2.564	1.282	5.46	**0.0063**	2.542	1.424	3.96	**0.0077**
Residual	9	2.111	2346			3.181	2191		
Lack of Fit	6	1.367	2278	0.96	0.5770	2.010	1210	7.19	0.4612
Pure Error	3	122	287.3			4.167	291		
R-Squared		0.9239				0.9051			
Adequate Precision		13.883				10.415			

modeling. Based on the experimental design matrix used, the actual biogas yields of choice were 0.1839 m^3/kg VS and 0.1361 m^3/kg VS with desirability values of 100 and 92% for the pretreated and untreated experiments respectively (Table 3). There was a 26% higher experimental gas yield in the pretreated experiment than the untreated. Gas chromatographic analysis revealed the CH_4 and CO_2 content of both experiments to be 61.5± 2.5%; 24± 1% and 52 ± 2%; 25± 1.5% respectively. The relationship between all the five separate variables (in coded form) employed in the modeling study (for the pretreated experiment) and the biogas yield (*Y*) is described in a single regression equation as shown:

$$\mathbf{Y} = 1762.31 - 53.30I + 95.79J + 41.13\mathrm{K} + 7.29\mathrm{L} - 113.38M + 38.35IJ + 85.14IK - 180.58IL - 114.43IM - 105.32JK + 135.67JL + 119.31JM - +71.36KL + 58.70KM + 2.70LM - 38.09I^2 - 11.74J^2 + 10.43K^2 + 24.45L^2 - 72.70M^2 \quad (3)$$

where *Y* = Biogas yield m^3/kg VS; *I* = Temperature (° C); *J* = pH; *K* = Retention time (Day); *L* = Total solids (g/kg); *M* = Volatile solids (g/kg).

The above equation was solved in order to obtain the optimal value for each independent variable employed in the modeling and optimization of biogas generation from fruit peels of *C. papayas* and the values obtained are as follows: *I* = 32.00° C, *J* = 7.50, *K* = 30.00 days, *L* = 12.00 g/kg and *M* = 12.00 g/kg. Taking these values into account, the predicted biogas yield for RSM was 0.1895 m3/kg VS and 0.1839 m3/kg VS for ANNs in the pretreated experiment. For the experiment without pretreatment, the RSM predicted yield was 0.1392 m^3/kg VS while that of ANNs was 0.1382 m^3/kg VS. There was a 26.52% increase in predicted biogas yield in the experiment with pretreatment over that of untreated *C. papayas* fruit peels. The prediction of the models were verified using the predicted values in replicate experiments. From these, average biogas yields of 0.1851 and 0.1380 m^3/kg VS were obtained from the pretreated and untreated experiments respectively. Equation (3) was further represented by 3-D response surface graphs for both RSM and ANNs and these are shown in Fig. S1 (a-j) (Supplementary materials). In all the figures, higher variable interactions were recorded in the ANNs plots than those of RSM. Also, the hierarchy of importance of each variable employed in the optimization study is shown in Fig. S2. From the figure, temperature is shown as the most important variable having the greatest effect on the biogas generation as shown in the study.

3.4. Mass balance

The mass balances of both the thermo-alkaline pretreated and untreated *C. papayas* peels are shown in Table 5. The volatile solids (VS) balance was computed by taking "*C. papayas* shoot" as the input variable while the trio of "methane", "carbon dioxide" and "anaerobic digestate" were the output variables. The degradation/consumption of VS in the thermo-alkaline pretreated experiment was 54% higher than obtained in the experiment without thermo-alkaline pretreatment.

4. Discussion

The range of pH values recorded in this study agrees with previous submissions that the most suitable pH for the efficient proliferation of anaerobic digester microflora especially members of the archaea is between 6.5 and 8 (Dahunsi et al., 2016a, b). A pH range of <6.5 or >8 has been reported to cause the total failure of the anaerobiosis/methanogenic stage in anaerobic digestion process (Ennouri et al., 2016). Therefore, maintaining appropriate pH status in anaerobic digesters is a major factor in ensuring adequate microbial and/or enzymatic substrate degradation (Dai et al., 2016; Zahedi et al., 2016). Maintenance of suitable temperature in

Table 4
Experimental Design for Biogas generation from the digestion of *C. papayas* fruit peels with five independent variables for RSM and ANNs (I = Temperature (° C); J = pH; K = Retention time (Day); L = Total solids (g/kg): M = Volatile solids (g/kg)).

Run	Independent Factors					Pretreated *C. papayas* fruit peels			Untreated *C. papayas* fruit peels		
	I	*J*	*K*	*L*	*M*	Actual biogas yield (m^3/kg VS)	RSM Predicted biogas yield (m^3/kg VS)	ANNs Predicted biogas yield (m^3/kg VS)	Actual biogas yield (m^3/kg VS)	RSM Predicted biogas yield (m^3/kg VS)	ANNs Predicted biogas yield (m^3/kg VS)
1	32.00	7.50	30.00	12.00	12.00	0.1839	0.1895	0.1839	0.1361	0.1392	0.1382
2	32.00	7.50	30.00	12.00	12.00	0.1681	0.1700	0.1680	0.1380	0.1400	0.1392
3	33.00	7.51	30.00	12.00	12.00	0.1965	0.2022	0.1967	0.1371	0.1381	0.1375
4	32.00	7.50	30.00	12.00	12.00	0.1404	0.1491	0.1404	0.1354	0.1363	0.1361
5	32.00	7.50	30.00	12.00	12.00	0.1700	0.1702	0.1702	0.1330	0.1344	0.1338
6	31.00	7.50	30.00	12.00	12.00	0.1723	0.1755	0.1723	0.1415	0.1441	0.1431
7	31.00	7.50	30.00	12.00	12.00	0.1684	0.1707	0.1685	0.1411	0.1422	0.1420
8	30.00	7.50	29.00	12.00	12.00	0.2116	0.2205	0.2202	0.1382	0.1442	0.1421
9	30.00	7.50	30.00	12.00	11.00	0.1963	0.2005	0.1743	0.1303	0.1319	0.1312
10	33.00	7.50	30.00	11.00	12.00	0.1951	0.2008	0.1951	0.1414	0.1425	0.1416
11	32.00	7.50	30.00	12.00	11.00	0.1907	0.2009	0.1910	0.1348	0.1359	0.1328
12	31.00	7.50	30.00	12.00	12.00	0.1821	0.1903	0.1821	0.1421	0.1450	0.1440
13	31.00	7.64	30.00	11.00	12.00	0.1592	0.1604	0.1743	0.1463	0.1404	0.1387
14	30.00	6.56	30.00	12.00	11.00	0.1561	0.1607	0.1561	0.1378	0.1361	0.1357
15	31.00	6.50	29.00	12.00	11.00	0.1721	0.1803	0.1719	0.1308	0.1389	0.1366
16	30.00	6.50	28.00	12.00	12.00	0.2200	0.2200	0.2202	0.1301	0.1320	0.1311
17	32.00	6.51	29.00	12.00	12.00	0.1728	0.1816	0.1727	0.1356	0.1362	0.1357
18	30.00	6.50	28.00	12.00	12.00	0.1732	0.1771	0.1732	0.1363	0.1337	0.1344
19	30.00	6.52	28.00	12.00	12.00	0.1877	0.1907	0.1870	0.1409	0.1423	0.1417
20	30.00	6.52	30.00	12.00	10.00	0.1801	0.1850	0.1801	0.1400	0.1420	0.1410
21	30.00	6.50	27.00	12.00	12.00	0.1900	0.2008	0.1890	0.1322	0.1342	0.1326
22	33.00	6.51	30.00	12.00	12.00	0.1597	0.1618	0.1597	0.1320	0.1341	0.1325
23	30.00	6.50	30.00	11.00	10.00	0.1556	0.1507	0.1555	0.1309	0.1404	0.1400
24	30.00	6.72	30.00	10.00	12.00	0.1742	0.1700	0.1743	0.1327	0.1367	0.1329
25	33.00	6.51	29.00	12.00	12.00	0.1688	0.1799	0.1743	0.1392	0.1394	0.1391
26	30.00	6.50	30.00	11.00	9.00	0.1550	0.1582	0.1551	0.1290	0.1311	0.1305
27	31.00	6.97	30.00	9.00	12.00	0.1569	0.1577	0.1570	0.1400	0.1395	0.1367
28	30.00	7.09	30.00	12.00	12.00	0.1710	0.1875	0.1719	0.1403	0.1398	0.1405
29	30.00	7.50	30.00	9.00	8.00	0.1800	0.2073	0.1809	0.1451	0.1453	0.1457
30	30.00	7.55	28.00	11.00	12.00	0.1376	0.1463	0.1375	0.1357	0.1372	0.1367
31	31.00	7.50	30.00	12.00	12.00	0.1563	0.1605	0.1751	0.1370	0.1378	0.1363
32	31.00	7.50	30.00	9.00	12.00	0.1651	0.1608	0.1610	0.1277	0.1210	0.1215
33	31.00	7.70	30.00	12.00	12.00	0.1607	0.1609	0.1601	0.1220	0.1241	0.1238
34	30.00	7.50	29.00	11.00	12.00	0.1421	0.1403	0.1403	0.1400	0.1411	0.1409
35	30.00	7.50	28.00	12.00	11.00	0.1522	0.1504	0.1500	0.1356	0.1373	0.1336
36	32.00	7.60	30.00	11.00	12.00	0.1501	0.1507	0.1502	0.1338	0.1351	0.1341
37	31.00	7.50	28.00	11.00	11.00	0.1521	0.1503	0.1402	0.1439	0.1453	0.1444
38	31.00	7.50	30.00	11.00	12.00	0.2180	0.2140	0.1927	0.1357	0.1402	0.1395
39	31.00	7.64	30.00	9.00	12.00	0.1528	0.1516	0.1502	0.1302	0.1308	0.1306
40	30.00	6.56	29.00	12.00	11.00	0.1732	0.1751	0.1670	0.1302	0.1319	0.1314
41	31.00	6.50	29.00	12.00	11.00	0.1877	0.1807	0.1801	0.1310	0.1318	0.1315
42	30.00	6.50	28.00	12.00	12.00	0.1855	0.1850	0.1810	0.1403	0.1424	0.1423
43	32.00	6.51	29.00	12.00	12.00	0.1800	0.1788	0.1697	0.1300	0.1307	0.1309
44	30.00	6.50	28.00	12.00	12.00	0.1597	0.1518	0.1505	0.1350	0.1373	0.1311
45	30.00	6.52	28.00	12.00	12.00	0.1576	0.1507	0.1503	0.1450	0.1457	0.1457
46	30.00	6.52	30.00	12.00	10.00	0.1642	0.1600	0.1583	0.1238	0.1243	0.1237
47	32.00	7.50	30.00	12.00	11.00	0.1888	0.1799	0.1751	0.1352	0.1363	0.1355
48	31.00	7.50	30.00	11.00	12.00	0.1546	0.1543	0.1530	0.1360	0.1366	0.1355
49	31.01	7.64	30.00	11.00	12.00	0.1654	0.1535	0.1519	0.1322	0.1361	0.1360
50	30.00	7.54	27.00	10.00	10.00	0.1652	0.1603	0.1609	0.1252	0.1256	0.1249

Table 5
Stoichiometry and mass balance for one ton of substrate (*Carica papayas* fruit peels) from the anaerobic digestion experiments ($ = Input–output)/input (%).

Parameter	Pretreated *C. papayas* peels + Rumen content	Untreated *C. papayas* peels + Rumen content
Input		
C. papayas peels + Rumen content (kg)	1000	1000
Volatile solids (VS) (kg)	960	880
Output		
Methane (CH_4) (%)	61.5	52
Carbon dioxide (CO_2) (%)	24	25
Digestate (kg VS)	500	690
Summation	585.5	767
$Mass balance	39	13
% Volatile solids (VS) removal	48	22

anaerobic digesters is also very important in order to ensure adequate growth and balance between acid-forming and methane-producing organisms during digestion (Priebe et al., 2016; Suksong et al., 2016). Results of the physicochemical analyses of the samples of *C. papayas* fruit peels reveals richness in important macro and micro nutrients and mineral elements required for microbial growth in a fermentation medium with the pretreated substrate enormously richer than the untreated one in terms of virtually all nutrients. This richness could be due to the nature of the peels coupled with seasonal variations and nutrients availability in their localities of collection. Previous researches has established the usage of such rich feedstock for biogas generation (Leite et al., 2016). The Nitrogen contents of the feedstock in particular falls within the range usually employed in liquid anaerobic digestion systems (Leite et al., 2016). Likewise, the two anaerobic digestates obtained in this

study had elevated nutrient compositions as against their initial values prior to digestion. This trend was recorded for elements such as nitrogen, phosphorus, potassium, magnesium, manganese, iron, zinc, aluminium and copper. Therefore, these digestates can be said to be rich in nutrient and has great potentials to increase both the microbial and nutrient status of soil when applied through the rhizosphere as fertilizers especially in nutrient-depleted soils (Tampio et al., 2016a). Plant growth and general wellbeing could also be enhanced via the use of such digestate as biofertiliser especially in Sub-Saharan Africa and other regions faced with soil nutrient depletion and erosion of top soil. Several studies has reported the potentials of anaerobic digestates as suitable replacements for inorganic chemical fertilizers which have imparted adversely on the ecosystem over the decades (Arif et al., 2016; Kantachote et al., 2016). Moreso, the digestates of this study were found to contain soil beneficial microorganism while most pathogens initially implicated have been eliminated during the digestion process. The reduction observed in the values of total and volatile solids, total carbon and calcium in the digestate is due to the uptake/usage of these component for metabolism and as precursors for microbial cell wall synthesis. Reduction in COD was a prominent characteristics of the digestates obtained in this study. The observed higher COD reduction in the pretreated experiment as against the untreated could be likened to higher microbial diversification and activities as the pretreated substrates was malleable to quick and efficient microbial/enzymatic breakdown of organic matter as a result of delignification. The bacteria (Aerobes and anaerobes) found at the different stages of digestion of the two samples of *C. papayas* fruit peels corresponds to earlier report in mesophilic anaerobic digestion processes with members of the genus *Clostridium* dominating (Ennouri et al., 2016; Dahunsi et al., 2017a, b). From the succession pattern obtained, changes in population and diversity was caused by dynamics of environmental factors especially pH and temperature as these microorganisms are very sensitive to the extremities of these factors (Dahunsi et al., 2016a, b). Also, a higher population of microorganisms was reported in the experiment without pretreatment over the pretreated due to the actions of the thermal treatment which in most cases are bacteriocidal. The dominant facultative anaerobic microorganisms including *Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme Clostridium histolytica*, *Clostridium* spp and *Gemella morbillorum* are common in anaerobic environments and have been severally implicated in anaerobic digestion studies. They are known to be active in the acetogenesis stage of digestion where they convert acids to acetone and other intermediate products that later serves as raw materials for methanogenesis (Ennouri et al., 2016). In this study, the population and diversity of these organisms contributed to the efficient biomass degradation and also led to higher biogas generation especially in the thermo-alkaline pretreated substrate. Similarly, the members of the genus *Methanococcus, Methanosarcinae* and *Methanosaeta* reported in this study are well known methanogens which converts acetone and other products of the acetogenesis stage to methane as the major product of anaerobic digestion (Ennouri et al., 2016). Earlier, Dahunsi et al. (2017b) reported that the availability of rich population and diversity of anaerobic microbes usually enhance substrate degradation, improves biogas generation and quality as well as ensuring the production of nutrient-rich digestate biofertilizer (Dahunsi et al., 2017b). Biogas generation in this study was at an appreciable level and when compared with those from other bioresources earlier utilized, *C. papayas* fruit peels has good efficiency in terms substrate interaction and microbial dynamics. The higher gas generation in the pretreated substrate over the untreated (up to 26.52%) could be said to be as a result of combination of different (Mechanical, thermal and chemical) pretreatments prior to digestion The usage of appropriate pretreatment procedures has been recommended for enhancement of biogas generation (Bolado-Rodriguez et al., 2016; Li et al., 2016a, b; Serrano et al., 2016).

Both F-values with corresponding low p-values as well as the R^2 values portrays that the regression model is significant for the studies. An 'adequate precision' value of $\geq$4 is usually recommended for the good fitting of a model. The 13.883 and 10.415 values obtained in both optimization studies signifies high fitting and suitability of the models for both experiments and this is further validated by the model terms with p-values <0.05. The lack-of-fit terms of 0.5770 and 0.4612 were not significant also validating the accuracy of the models. The configuration of the entire RSMs 3-D graphs derived from solving the model regression equation reveal low interactions among all the five variables while those of ANNs shows pronounced interactions which further reveals that the ANNs model allows for better interactions of independent variables than the RSM. Earlier researches (Dahunsi et al., 2016a, b) reported similar interactions in which ANNs showed more robustness in terms of variable interaction. In order to determine the accuracy and predictive abilities of both RSM and ANNs models, applicable parameters such as the mean squared error (RSME), R^2 values and the biogas yield prediction were employed. The RSME obtained for RSM (287.3 and 291.3) was higher than that of ANN (50.763 and 62.22) whereas the R^2 for RSM (0.9239 and 0.9051 i.e. 92.39 and 90.51%) was lower than ANNs'

Table 6
Energy and economic evaluation in the application of thermo-alkaline pretreatment to *C. papayas* fruit peels (* = difference of thermal energies produced by the pretreated experiment minus the untreated; # = difference between the thermal energy gain and the thermal energy requirement for the thermo-alkaline pretreatment; $ = difference of electrical energies produced by pretreated experiment minus the untreated).

Energy factors	Pretreated Experiment	Untreated Experiment
Electrical and thermal energies produced from combined heat and power (CHP) system	1844	1313
Thermal energy produced (kWh/t TS)	1595	464
Electrical energy produced (kWh/t TS)	755	340
Thermal balance		
*Gain in thermal energy (kWh/t TS)	1131	–
Required thermal energy (kWh/t TS)	1070	–
Required thermal energy with 80% of heat recovery (kWh/t TS)	218	–
#Net thermal energy (kWh/t TS)	61	–
Net thermal energy with 80% of heat recovery (kWh/t TS)	−913	–
Electrical balance		
$Gain in electrical energy	415	–
Energy for substrate mixing during pretreatment	–	–
Net electrical energy	415	
Economic evaluation		
Cost of NaOH (€/t TS)		

(0.9865 and 0.9801%). In all, RSMs prediction of gas was higher; ANNs' accuracy was higher in terms of R^2 value and lower error reading. ANNs is therefore a better model for predicting biogas yield from *C. papayas* fruit peels. The mass balance from both experiments in this study revealed higher VS consumption/degradation in the experiment with thermo-alkaline pretreatment over the untreated indicating adequate substrate mixture, adequate microbial activities in the breakdown and conversion of the organic matter to biogas and digestion stability/efficiency in the thermo-alkaline pretreated experiment.

As shown in Table 6, the combined heat and power (CHP) system was used to evaluate the energy balance and economic feasibility of applying thermo-alkaline pretreatment to *C. papayas* shoot using a heat and electrical efficiencies of 50 and 35% respectively (Dahunsi et al., 2017b). In determining the TER for carrying out the thermo-alkaline pretreatment procedure for *C. papayas* shoot, the energy needed to raise the temperature of 35 g TS/L mixture of *C. papayas* shoot from the initial temperature of 25° C to the final of 55° C was determined using 4.18 kJ/kg C as the specific heat of water to evaluate the specific heat of the mixture while the loss of heat was neglected (Zupancic and Ros, 2003). In the pretreated experiment, the 1131 kWh/t TS gain in thermal energy at a solid loading of 35 g TS/L was higher than the 1070 kWh/t TS thermal energy required for the pretreatment using thermal energy and NaOH as alkali. As earlier reported (Zabranska et al., 2006; Dhar et al., 2012), use of heat exchanger during the digester heating or for pretreatment is an efficient method for boosting heat recovery up to 80%. Similarly, full thermal energy integration is also a reliable tool for assessing the economic feasibility of the thermo-alkaline pretreatment (Fdz-Polanco et al., 2008; Perez-Elvira and Fdz-Polanco, 2012). In accounting for NaOH usage, the US cost of 335 dollars/ton was considered. However, this study did not evaluate the possibility of recovering the used NaOH from the pretreatment. This is an important aspect that should be considered by future studies as this will increase the economic benefits derived from the thermo-alkaline pretreatment process and this can also be applied to other lignocelluloses.

For the electrical energy required in the thermo-alkaline pretreatment, the energy consumed during substrate mixing was given consideration while the energy used for mechanical treatment was neglected since the same treatment was applied to the experiment without thermo-alkaline pretreatment (Dahunsi et al., 2017b). From the pretreated experiment, the estimated net electrical energy obtained at a solid loading of 35 g TS/L was 415 kWh/t TS which can be directly injected into the national or regional energy grid or sold at fixed price so as to obtain additional economic benefits.

5. Conclusion

This research established biogas generation from the fruit peels of *Carica papayas.* The richness of the peels in terms of minerals composition coupled with the quantity as well as methane content of the biogas suggest that it's a veritable candidate for biofuel generation. Application of thermo-alkaline pretreatment was shown to enhance the substrate's degradation as well as enhancement of biogas yield up to 26.52% over the untreated. The result of modeling and optimization showed that both RSM and ANN models are efficient in the prediction of methane production from *Carica papayas* fruit peels with ANNs being the better option. The optimal conditions for the most efficient gas generation from this substrate have equally been established and this will benchmark standard for further research on the subject. A positive energy balance was derived from the application of thermo-alkaline pretreatment and this can be increased with the use of heat exchanger during digester heating or pretreatment. Furthermore, the study revealed the huge potentials of the anaerobic digestates as biofertilizers or soil conditioner. Therefore, due to the high energy yield and huge potentials for biofertilizer production, further usage of *Carica papayas* fruit peels is encouraged especially in areas of its abundance.

Conflict of interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jclepro.2017.04.042.

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