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Mesophilic anaerobic co-digestion of poultry dropping and *Carica papaya* peels: Modelling and process parameter optimization study

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HIGHLIGHTS

- Biogas was maximally produced from peels of *Carica papaya* and poultry dropping.
- Response Surface Methodology and Artificial Neural Networks were used to optimize.
- RSM predicted higher biogas yield than ANNs while ANN showed higher accuracy.
- The optimized conditions for maximal biogas yield were established for the substrate.

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ABSTRACT

The study evaluated anaerobic co-digestion of poultry dropping and pawpaw peels and the optimization of important process parameters. The physic-chemical analyses of the substrates were done using standard methods after application of mechanical, thermal and chemical pre-treatments methods. Gas chromatography analysis revealed the gas composition to be within the range of 66–68% methane and 18–23% carbon dioxide. The study equally revealed that combination of the different pre-treatment methods enhanced enormous biogas yield from the digestion. Optimization of the generated biogas data were carried out using the Response Surface Methodology and the Artificial Neural Networks. The coefficient of determination (R^2) for RSM (0.9181) was lower compare to that of ANN (0.9828). This shows that ANN model gives higher accuracy than RSM model for the current. Further usage of *Carica papaya* peels for biogas generation is advocated.

1. Introduction

Anaerobic digestion is a proven technological method of converting organic matter thereby producing biogas and nutrient-rich digestate (Astals et al., 2015; Leite et al., 2016; Zou et al., 2016). It has been globally applied in the treatment of diverse wastes, agricultural residues, energy crops and is a veritable means of abating environmental pollution (Razaviarani and Buchanan, 2015; Fierro et al., 2016).

The organic fraction of poultry dropping is biodegradable and thus fitting for anaerobic digestion for methane yield (Dalkilic and Ugurlu, 2015). However, the digestion of poultry dropping is usually slowed down due to its low C/N ratio, richness in nitrogen and high total ammonia levels (Tian et al., 2015). Therefore, co-digestion with other carbon-rich substrates is often recommended to guarantee the success of anaerobic digestion and subsequent improvement in biogas yield (Khoufi et al., 2015). Co-digestion of substrates have been carried out by various researchers utilizing different biomass and waste materials and this enhanced the biodegradability and high biomethane yield from such materials (Dahunsi and Oranusi, 2013; Dareioti and Kornaros, 2015).

Pawpaw (*Carica papaya*) originated from Southern Mexico, Central and South America and is currently in many geographical locations around the world (Anon, 2010). It is known to thrive better in tropical climate and as such is abundant in countries such as Brazil, Nigeria, India, South Africa, Haiti and South East Asia and in over 50 others (Anon, 2010). Production in Nigeria reached 750,000 tons in 2011 alone and the crop is widely cultivated in several cropping

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systems across the country with the South west being its major domain (FAOSTAT, 2011).

Almost all the parts of the *C. papaya* plant are used for several purposes. The ripe and unripe fruit, leaves, latex and seed have different usage in many communities across the world (Purseglove, 1968; Anon, 2008). The only part that remains grossly underutilized is the peel/skin. This informed our choice of the pawpaw peels as a substrate for biogas generation. Being a lignocellulosic biomass with high sugar content, it has high potentials for biodegradation during hydrolysis and fermentation by hydrolytic and acidogenic microorganisms.

The optimization of bioprocess parameters is an important step for the success of the anaerobic digestion process (Kana et al., 2012; Betiku et al., 2015; Emeko et al., 2015). The aim of this research therefore was to evaluate the biogas producing potentials of *C. papaya* peels in co-digestion with poultry dropping. The process parameter optimization of the study was equally carried out using applicable models such as the Response Surface Methodology (RSM) and Artificial Neural Networks (ANN). We hope to benchmark a permanent usage for pawpaw peels and also to increase the global awareness on renewable and sustainable biofuels.

2. Materials and methods

2.1. Collection of sample

Peels of *C. papaya* and fresh poultry droppings were collected from the Landmark University Teaching and Research Farms and transported to the site of experiment. Fresh cattle's rumen content was also obtained from the Landmark University Cafeteria slaughter house and used as seed material for the digestion. The use of rumen content as inoculum has been reported in many studies (Kana et al., 2012; Alfa et al., 2014a,b). Being a lignocellulosic material, *C. papaya* peels was pre-treated using a modification of already described mechanical, thermal and chemical pre-treatment methods (Alfa et al., 2014a,b; Kim et al., 2015). The substrate was crushed using the hammer mill and was followed with heating in the water bath (CLIFTON, 88579, NICKEL-ELECTRO Ltd., ENGLAND) at 80 °C for an hour. 300 ml of 0.0 M solution of Sodium hydroxide (Na OH) was used for the chemical pre-treatment.

2.2. Digester design

Twenty-four-litre volume digesters sized according to the amount of volatile solids that must be treated daily and the period of time the material will remain in each of the digesters (Retention time) were used for this study. The digester design was according to the method of Alfa et al. (2014a). The primary structure consists of a Mild steel digestion tank which is strong enough to withstand the weight and pressures of the contained slurry, and painted to prevent corrosion. The tank is air tight and with an in-built mechanical stirrer for appropriate mixing of substrates with liquid displacement for measuring gas production.

2.3. Biochemical methane potential (BMP) test

The biomethane potential test was carried out using the method of Ghasimi et al. (2015) in order to determine the quantity of methane at standardized temperature and pressure that the substrate can potentially produce under anaerobic condition. In this study, the BMP of combination of *C. papaya* and poultry dropping was determined in-situ in two weeks batch digestion regime using four of the digesters meant for the real experiment i.e. two each for the BMP test and as blank. An inoculum to substrate ratio of 3 was adopted. Tests were performed in triplicate and background methane production from blanks (substrate-free assay) was subtracted. Biogas samples were periodically collected from the headspace of each digester for measurement of produced methane whose content was determined using gas chromatography (GC).

2.4. Residual methane potential tests

The residual methane potential was measured for the remaining solid residue collected at the end of the anaerobic digestions using the same method for the BMP test with and without added inoculum. The purpose of these tests was to evaluate the effects of chemical inhibitors. In the experiment without added inoculum, the solid residues were diluted with distilled water at a dilution factor of 2 or 4 following the method of Yap et al. (2016).

2.5. Digestion

Three and half kg each of the pre-treated peels and poultry dropping were mixed together and was further mixed with water to form slurry in the ratio 1:1 by volume and was separately introduced into each digester tank through an inlet pipe of 50 mm at the top of the digester tanks (Alfa et al., 2014a). One kg of the rumen content was also used as the inoculum. The entire slurry (16 L) was allowed to occupy three quarter of the digester space leaving a clear height space for the gas collection. Before feeding the digesters, the flexible plastic hose connecting the gas outlet from the digester to the gas holder were disconnected, such that the gas outlet from the digester is left open. This was done to prevent negative pressure build up in the digester. The gas was collected from the digester through a 10 mm diameter flexible hose connected from the digester to the bottom of the gas collection system.

2.6. Technical evaluation of the digestion and gas production measurement

The evaluation of the technical performance of the anaerobic digestion process was carried out with respect to the gas production and the treatment efficiency of the digester. Retention times of between 20 and 30 days were adopted according to experimental design during which daily measurement of gas production was carried out. In order to prevent loss of energy, the digesters were covered with polythene at night and removed in the morning. Evaluation of microbial succession at every stage of the digestion process and analysis of feedstock and effluent to evaluate the treatment efficiency of each digestion process was also done. Produced biogas measurement was done each day shortly before sunset which was taken as the total gas content of the gas holder and was computed (Alfa et al., 2014b).

2.7. Analytical methods

Prior to and after the digestion, physicochemical parameters of the inoculum and the fermenting materials were evaluated in the Environmental Engineering laboratory of Landmark University using standard methods (APHA, 2012). Parameters evaluated include Total Solids (TS), Volatile Solids (VS), pH, Ash content, Moisture content, Total Carbon, Total Nitrogen (TN), Total Phosphorus (TP), Phosphates (PO_4), Sulphates (SO_4) Potassium (K), Sodium (Na), Magnesium (Mg), Calcium (Ca), Nitrates (NO_3), Ammonium (NH_4), Iron (Fe), Copper (Cu), Zinc (Zn), Aluminium (Al) and Manganese (Mn) using the Palintest$^{(R)}$ Photometer 7100 (PHOT.1.1.AUTO.71) and Photometer 7500 (PHOT.1.1.AUTO.75) advanced digital-readout colorimeter (England). The photometer was operated at an absorbance of 0.5 and at a wave length of 450 nm in triplicates for all samples.

2.8. Microbial assessment

2.8.1. Aerobic organism's enumeration

Microorganisms associated with the biomass were characterized using standard method (APHA, 2012). Enumeration of Total Aerobic Plate Count (TAPC) were carried out for the cattle rumen content and the fermenting materials in the Microbiology laboratory of Biological Sciences Department, Covenant University, Nigeria using standard method (APHA, 2012). The media used include Nutrient agar, MacConkey agar, Potato dextrose agar, Eosin Methylene Blue agar, EMB broth, Salmonella-Shigella agar, Selenite F broth, Lactose broth and Peptone water. Analyses of individual samples were performed in triplicates and on a weekly basis. Presumptive isolates were characterized by phenotypic method and were further identified using appropriate API kits (BioMerieux).

2.8.2. Anaerobic organism's enumeration

For isolation of *Clostridium species*, samples were first cultured on Reinforced Clostridia medium (RCM) and then sub-cultured on blood agar incubated in an anaerobic jar (Oxoid) containing a moistened pack of gas generating kit (Bio-oxoid) at 37 °C for 7 days. Colonies which developed on the plates were counted and recorded (Ayandiran et al., 2014). Sub-culturing was carried out on distinct colonies until pure cultures were obtained and were transferred onto slant in bottles containing freshly prepared media. The presumptive colonies were confirmed by standard morphological and biochemical techniques and using respective rapid API kits (Ayandiran and Dahunsi, 2016). The same procedure was followed for the isolation of other anaerobes using Nutrient agar, MacConkey agar and Brain Heart Infusion agar.

2.8.3. Methanogenic bacteria enumeration

The mineral medium earlier described and used by Ghosh et al. (2014) was employed in the methanogenic bacteria evaluation and this was prepared by mixing 1 L basal medium with 10 mL supplement solution, 40 mL 1 M $NaHCO_3$, 1 mL 5% (w/v) cysteine–HCl, and 2.5 mL 36 mM $FeSO_4$ (in 50 mM H_2SO_4). The basal medium consisted of 0.5 g NH_4Cl, 0.4 g KH_2PO_4, 0.15 g $MgCl_2.6H_2O$, 0.05 g $CaCl_2.2H_2O$, 1.0 g $NaHCO_3$, 1 mL trace element solution [10×], 1 mL vitamin solution [10×], 0.001 g sodium resazurin, 0.50 g Na_2S, 0.50 g cysteine–HCl, and 0.50 g Na–thioglycolate; volume made to reach 1.0 L with double distilled water (DDW) with a final pH of 7.0. The supplement solution consisted of vitamins (5 mg cyanocobalamin, 4 mg p-aminobenzoic acid, 1 mg biotin, 10 mg nicotinic acid, 5 mg calcium pantothenate, 15 mg pyridoxamine–2HCl and 10 mg thiamine–HCl) and trace elements (1.6 mM HCl, 100 mg $FeCl_2{\cdot}7H_2O$, 7 mg $ZnCl_2$, 10 mg $MnCl_2{\cdot}4H_2O$, 0.6 mg H_3BO_3, 13 mg $CoCl_2{\cdot}6H_2O$, 0.2 mg $CuCl_2{\cdot}2H_2O$, 2.4 mg $NiCl_2{\cdot}6H_2O$, 3.6 mg $Na_2MoO_4{\cdot}2H_2O$, 0.26 mg $Na_2SeO_3{\cdot}5H_2O$ and 0.66 mg Na_2WO_4) were dissolved in DDW. The BM, $FeSO_4$, and the supplement solution were autoclaved separately. The $NaHCO_3$ and cysteine–HCl was filter sterilized and added into the medium (Stieglmeier et al., 2009). The dissolved oxygen was removed from all liquid media by sparging with N_2 gas at the rate of 10 mL/min for 30 min till the indicator (resazurin) becomes colorless. The experiments were carried out in the anaerobic chamber provided with 10% headspace in the jar.

2.9. Daily monitoring of operational parameters and gas analysis

Various physical and chemical parameters were monitored to check the status of the digesters. Monitoring was carried out every day and readings were taken to record digester and ambient temperatures using 2/1 °C Thermometers (ENGLAND). Temperature measurement was done twice daily and the average value taken. pH measurement was done weekly using pH meter model pHS-2S, (SHANGHAI JINYKE REX, CHINA) and the average of 3 replicates was computed. In order to avoid heat loss at night, each digester was completely covered with polythene nylon. Daily gas produced were collected using water displacement method earlier described (Dahunsi and Oranusi, 2013; Alfa et al., 2014b). The methane and other contents of the generated biogas were determined using a Gas Chromatography (GC) (HP 5890, Avondale, USA) coupled with a Hayesep Q column (13 m × 0.5 m × 1/800) and a flame ionization detector (FID) (Alfa et al., 2014b).

2.10. Optimization

2.10.1. Experimental design via central composite rotatable design (CCRD)

Central Composite Rotatable Design (CCRD) experimental design was employed to design the bioconversion of the biomass to biogas because of its success in improving bioprocessing systems (Betiku et al., 2015; Emeko et al., 2015). Five-level-five-factors design was applied, which generated 50 experimental runs including 42 non-centre points and 8 centre points to provide information regarding the interior of the experimental region thus making it possible to evaluate the curvature effect. The alpha value used was 2.37841. Selected factors for biogas optimization were Temperature (°C): $\mathbf{X_1}$, pH: $\mathbf{X_2}$, Retention time (days): $\mathbf{X_3}$, Total solids (g/kg): $\mathbf{X_4}$ and Volatile solids (g/kg): $\mathbf{X_5}$. These factors were selected based on their importance in biogas generation and the chosen ranges are based on report of earlier researches. The optimal temperature for most mesophilic digestions has been reported to vary between 30 and 40 °C (McKennedy and Sherlock, 2015), pH of 6.5–8 has been reported to be best for methanogenesis (Zonta et al., 2013), while the optimal retention time for mesophilic digestion has equally been reported to be within 20–30 days depending on the ambient temperature (Mao et al., 2015). For total and volatile solids, it has been documented that for efficient operation of liquid anaerobic system, the solids content must be less than 15% but not lower than 4% to avoid total failure (Jain et al., 2015). The various ranges for optimization used in this study was therefore chosen based on the above submissions in order to arrive at the very optimal condition for the most efficient anaerobic digestion of *C. papaya* peels and poultry dropping.

2.10.2. Artificial neural network (ANNs)

Neural Power version 2.5 (CPC-X software) was used in this study. Experimental data generated via Central Composite Rotatable Design was used for the ANN module. The idea was to use the data that are statistically well distributed in the input search window. A total number of 50 experimental data were divided into sets, 32 in training set, 9 in the validation set and 9 in the test set. The Tanh transfer function at hidden layer and a linear transfer function at output layer were used. The training function selected for the network is 'Tanh' and all variables and response were normalized for the reduction of network error and higher standardized results.

2.11. Statistical data analysis

2.11.1. Central composite rotatable design (CCRD)

The data obtained from biogas generation from each of the digestion regime was analysed statistically using response surface methodology, so as to fit the quadratic polynomial equation generated by the Design-Expert software version 9.0.3.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response variable to the independent variables, multiple regressions was used to fit the

Table 1
Physical and chemical characteristics of mixture of *Carica papaya* peels with poultry dropping, cattle rumen content and digestate.

Parameters	The mixture before digestion	Poultry droppings	Rumen content	Digestate
pH	7.65 ± 0.20	6.90 ± 0.22	7.91 ± 0.02	7.66 ± 0.02
Total solids (mg/kg)	10.87 ± 1.02	28.24 ± 1.02	9.52 ± 0.11	9.40 ± 0.22
Volatile solids (mg/kg)	9.60 ± 1.02	18.71 ± 1.13	9.44 ± 2.12	9.04 ± 0.10
Ash content (%)	6.90 ± 0.02	18.29 ± 2.11	5.56 ± 0.13	6.76 ± 0.12
Moisture content (%)	89.13 ± 3.22	71.76 ± 2.80	90.48 ± 2.12	90.00 ± 0.12
Total carbon (mg/L)	300.23 ± 5.12	292.10 ± 3.10	265.21 ± 4.10	254.90 ± 0.03
Total nitrogen (mg/L)	57.00 ± 1.01	61.00 ± 1.12	48.00 ± 1.12	62.00 ± 0.02
Chemical oxygen Demand (mg/L)	288 ± 1.05	228.98 ± 3.00	168.21 ± 1.12	81 ± 3.12
Total phosphorus (mg/L)	6.12 ± 0.02	7.90 ± 0.12	6.30 ± 0.13	6.14 ± 0.03
Potassium (mg/L)	8.20 ± 0.01	9.00 ± 0.00	7.20 ± 0.12	8.50 ± 0.02
Phosphate (mg/L)	3.00 ± 0.01	3.80 ± 0.10	3.00 ± 0.12	3.20 ± 0.12
Sulphate (mg/L)	142.00 ± 0.21	164.00 ± 3.02	134.00 ± 5.09	144.00 ± 0.21
Calcium (mg/L)	68.00 ± 1.20	44.00 ± 0.02	80.00 ± 1.22	60.00 ± 0.03
Magnesium (mg/L)	100.00 ± 2.02	150.00 ± 2.10	96.00 ± 2.12	110.00 ± 0.10
Manganese (mg/L)	0.028 ± 0.00	0.040 ± 0.01	0.028 ± 0.01	0.030 ± 0.11
Iron (mg/L)	1.24 ± 0.02	1.46 ± 0.02	1.18 ± 0.11	1.26 ± 0.02
Zinc (mg/L)	38.00 ± 0.12	51.00 ± 2.02	38.00 ± 0.14	39.00 ± 0.12
Aluminium (mg/L)	0.96 ± 0.02	0.62 ± 0.30	0.80 ± 0.02	1.02 ± 0.02
Copper (mg/L)	5.00 ± 0.12	5.80 ± 0.72	4.80 ± 0.05	5.10 ± 0.12

n = 35.

coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA). The fitted quadratic response model is described by:

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

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

2.11.2. QuickProp (QP) Artificial neural network ANNs

The data obtained from the CCRD obtained was also analysed statistically using ANNs. In developing ANN model, performance of ANN was heavily influenced by its network structure; therefore, the learning algorithms used was QuickProp (QP). The multilayer connection type used was multilayer normal feed forward (MNFF), three total layer numbers was used and the node number of input layer was five. For the output layer, Node Number was 1, the transfer function was Tanh and the slope of transfer function and the hidden Layer was 1, the node number was 12, transfer function was also Tanh and slope of transfer function was also 1 (Betiku and Ajala, 2014). Meanwhile, the optimum ANN structure was determined using mean square error (MSE) approach. The higher coefficient (R^2) was determined; the variable analysis also was conducted to study the effects of variables towards the biogas yield using relative importance and 3D curvature surface plots. A hybrid ANN model was used in conducting process optimization. This ANN structure was used for modelling the biogas production and the results were compared with RSM.

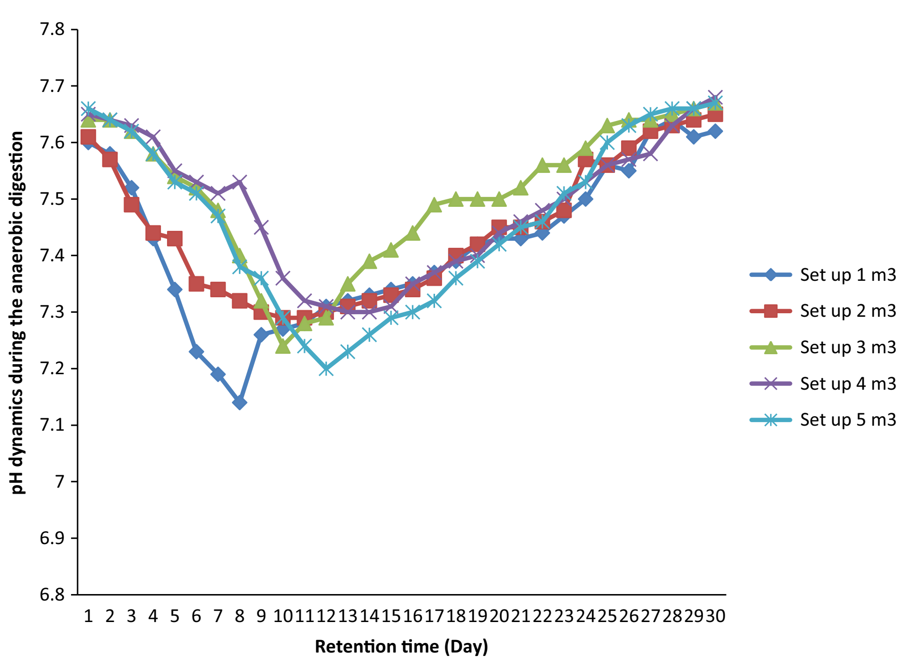


Fig. 1. Graph of pH dynamics during the anaerobic digestion (for the first five experimental set ups).

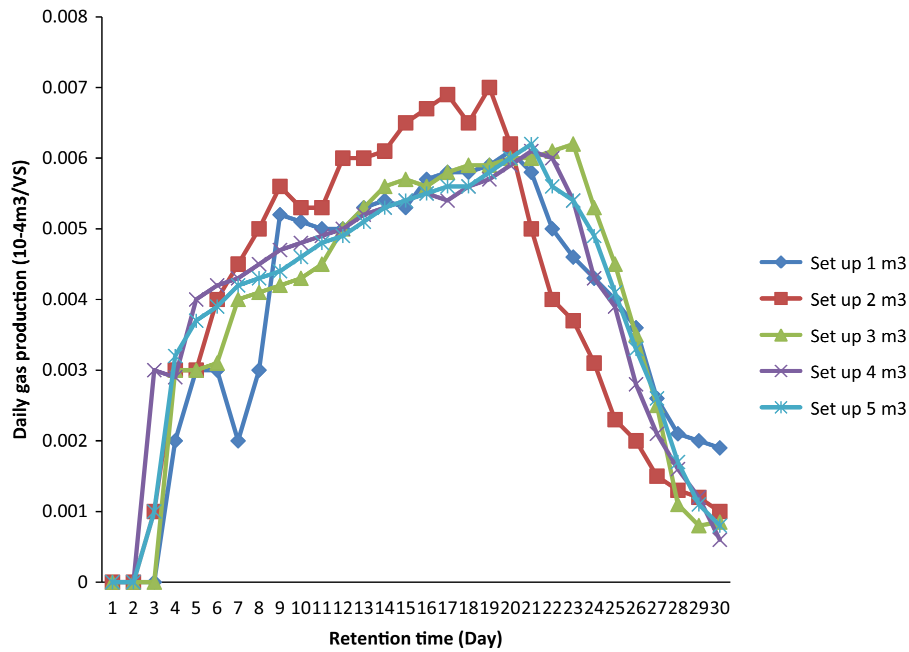


Fig. 2. Daily biogas production during the anaerobic digestion (from the first five experimental set ups).

Table 2
Factors and their levels for response surface study for biogas generation from the anaerobic digestion of *Carica papaya* peels and poultry dropping.

Variable	Symbol	Coded factor levels				
		−2	−1	0	1	2
Temperature (°C)	X_1	30	32.5	35	37.5	40
pH	X_2	6.0	6.5	7.0	7.5	8.0
Retention time (days)	X_3	20	22.5	25	27.5	30
Total solids (g/kg)	X_4	4	6	8	10	12
Volatile solids (g/kg)	X_5	4	6	8	10	12

2.11.3. Experimental validation

The model was validated with same digesters using conditions predicted by the software. The deviations of actual values from the observed values were then plotted.

3. Result

3.1. Digester stability and performance

The result of the residual methane test revealed methane production from the 2nd day of the experiment. The average methane content of the biogas was between 65 and 67%. The result of the physical and chemical analysis of substrate (before and after digestion) and that of the inoculum is shown in Table 1. As shown in Fig. 1, the pH of the substrate in all digesters was slightly alkaline throughout the digestion process and falling within the experimental design range (6.5–8) by Response Surface Methodology (RSM). The temperature of the digesters also remained within the mesophilic range (30–40 °C) throughout the experiment and according to the experimental design. All the temperature readings throughout the digestion fluctuated between 32.5 and 36 °C which was within the experimental design. The result of all physical and chemical analyses showed increase in values for, moisture content, total Nitrogen, total Phosphorus, Potassium, Sulphate, phosphate, Magnesium, Manganese, Iron, Zinc, Aluminium and Copper while there were reductions in the values of other parameters after the digestion. The table also revealed that poultry dropping alone was bulkier than the mixture of *C. papaya* peels and poultry dropping as well as the rumen content in terms of total and volatile solids. Poultry dropping was however the richest in terms of elemental composition. The COD value of the digested substrate was significantly reduced up to 71.875% for the various set ups at the end of the digestion period. In all the experiments, biogas production commenced from between the 3rd and the 4th day until between the 20th and 23rd day in most cases after which a fall was observed and remained diminishing till the end of the experiments (Fig. 2). Gas chromatography analysis revealed the gas composition to be within the range of 66–68% methane and 18–23% carbon dioxide for the digestion of *C. papaya* peels and poultry dropping.

3.2. Microbial composition

The result of the microbial analysis revealed several aerobic bacteria, anaerobes and methanogens in all the anaerobic digestion set ups. All the aerobes were isolated at the early stages (1–8 days) of the digestion processes and these include *Bacillus polymyxa*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Anaerobes implicated are *Porphyromonas assacharolyticum*, *Fusobacterium mortiferum*, *Bacteroides fragilis*, *Clostridium clostridioforme*, *Clostridium histolytica*, and *Clostridium* spp. while three different species of *Methanogens* were identified as the methane formers during the digestion process. Fungi of the genera *Aspergillus, Rhizopus* and *Mucor* were also identified at the early stages of digestion.

3.3. RSM optimization of biogas data

Table 2 shows all the five factors and their levels for response surface study for biogas generation from the anaerobic digestion of *C. papaya* peels and poultry dropping while Table 3 shows the experimental design matrix by the Central Composite Rotatable Design (CCRD) for the five-level-five-factor response surface study for biogas generation from *C. papaya* peels and poultry dropping. The experimentally observed and predicted yields as well as the residual values are shown on the table. The effects of unexplained variability in the biogas yield response due to extraneous factors were minimized by randomizing the order of experiments. Design Expert 8.0.3.1 software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. Table 4 shows the results of test of significance and that of the second-order response surface model in the form of ANOVA for every regression coefficient. Considering the large F-values (the test for comparing the variance associated with all terms with the residual variance) and low corresponding p-values (the probability value that is associated with the F-value for all terms), a good number of the model terms are remarkably significant and have very strong effects on the biogas yield with $p < 0.05$. The Model F-value of 5.05 implies the model is significant. There is only a 0.84% chance that a Model F-Value this large could occur. It was observed that the linear terms X_1, X_4, X_1X_4, X_2X_3, and X_2X_4 were the most significant model terms. The Adequate Precision of 10.461 indicates an adequate signal that the model can be used to navigate the design space.

The goodness of fit of the model was checked by the coefficient of determination (R^2). The Lack of Fit F-value of 0.81 implies the Lack of Fit is not significant relative to the pure error. There is a

Table 3
Experimental design matrix by central composite rotatable design (CCRD) for five-level-five-factors response surface study for biogas generation from *Carica papaya* peels and poultry dropping.

No	X_1	X_2	X_3	X_4	X_5	Actual biogas yield (10^{-4} m^3/VS)	Predicted biogas yield (10^{-4} m^3/VS)	Desirability
1	−1	−1	−1	−1	−1	3884.2	3991.77	1.000
2	1	−1	−1	−1	−1	3000.0	3100.42	1.000
3	−1	1	−1	−1	−1	3372.2	3384.68	1.000
4	1	1	−1	−1	−1	3475.4	3465.49	1.000
5	−1	−1	1	−1	−1	3496.4	3491.2	1.000
6	1	−1	1	−1	−1	3700.3	3701.77	1.000
7	−1	1	1	−1	−1	3573.2	3663.01	1.000
8	1	1	1	−1	−1	4201.1	4261.17	1.000
9	−1	−1	−1	1	−1	3502.8	3666.8	1.000
10	1	−1	−1	1	−1	3600.9	3663.04	1.000
11	−1	1	−1	1	−1	3834.1	3970.11	1.000
12	1	1	−1	1	−1	3572.2	3661.11	1.000
13	−1	−1	1	1	−1	3521.1	3664.36	1.000
14	1	−1	1	1	−1	3991.1	4023.49	1.000
15	−1	1	1	1	−1	3980.6	4064.71	1.000
16	1	1	1	1	−1	4600.8	4691.77	1.000
17	−1	−1	−1	−1	1	3852.2	4040.01	0.988
18	1	−1	−1	−1	1	4661.1	4633.07	0.984
19	−1	1	−1	−1	1	4190.1	4219.73	0.977
20	1	1	−1	−1	1	3932.9	4015.52	0.974
21	−1	−1	1	−1	1	3473.8	3413.87	0.973
22	1	−1	1	−1	1	3800.1	3812.21	0.972
23	−1	1	1	−1	1	4268.6	4302.77	0.967
24	1	1	1	−1	1	3891.5	3890.95	0.961
25	−1	−1	−1	1	1	3862.1	3910.99	0.916
26	1	−1	−1	1	1	3494.9	3497.34	0.908
27	−1	1	−1	1	1	4094.5	4191.04	0.904
28	1	1	−1	1	1	3905.7	3945.94	0.879
29	−1	−1	1	1	1	3722.4	3843.47	0.878
30	1	−1	1	1	1	3832.2	3932.33	0.871
31	−1	1	1	1	1	3867.9	3871.31	0.870
32	1	1	1	1	1	3771.1	3801.02	0.868
33	−2	0	0	0	0	3532.2	3621.20	0.866
34	2	0	0	0	0	3100.1	3132.41	0.865
35	0	−2	0	0	0	2981.2	2965.31	0.664
36	0	2	0	0	0	3102.2	3211.42	0.862
37	0	0	−2	0	0	2341.9	2373.03	0.861
38	0	0	2	0	0	3190.3	3212.21	0.859
39	0	0	0	−2	0	3087.1	3121.31	0.855
40	0	0	0	2	0	3021.3	3101.48	0.853
41	0	0	0	0	−2	3209.2	3243.31	0.851
42	0	0	0	0	2	2987.9	3014.15	0.847
43	0	0	0	0	0	3112.1	3167.04	0.845
44	0	0	0	0	0	2702.2	2711.14	0.843
45	0	0	0	0	0	2678.8	2712.12	0.842
46	0	0	0	0	0	2871.6	2899.03	0.841
47	0	0	0	0	0	2700.1	2761.23	0.838
48	0	0	0	0	0	2301.8	2381.90	0.836
49	0	0	0	0	0	2231.3	2241.80	0.833
50	0	0	0	0	0	2431.3	2462.02	0.831

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids.

Table 4
Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for biogas generation from *Carica papaya* peels and poultry dropping.

Source	Sum of squares	df	Mean square	F-value	p-value
X_1	3.743E+005	1	3.743E+005	10.10	0.0112
X_2	2194.98	1	2194.98	0.059	0.8132
X_3	26780.12	1	26780.12	0.72	0.4174
X_4	4.241E+005	1	4.241E+005	11.44	0.0081
X_5	94737.59	1	94737.59	2.56	0.1444
X_1X_2	20930.86	1	20930.86	0.56	0.4716
X_1X_3	1.164E+005	1	1.164E+005	3.14	0.1102
X_1X_4	1.171E+006	1	1.171E+006	31.58	0.0003
X_1X_5	124.32	1	124.32	3.353E−003	0.9551
X_2X_3	5.327E+005	1	5.327E+005	14.37	0.0043
X_2X_4	6.803E+005	1	6.803E+005	18.35	0.0020
X_2X_5	40018.00	1	40018.00	1.08	0.3260
X_3X_4	83934.78	1	83934.78	2.26	0.1667
X_3X_5	41560.94	1	41560.94	1.12	0.3173
X_4X_5	39303.06	1	39303.06	1.06	0.3301
X_1^2	2.04	1	2.04	5.498E−005	0.9942
X_2^2	4734.64	1	4734.64	0.13	0.7291
X_3^2	19018.94	1	19018.94	0.51	0.4920
X_4^2	20936.17	1	20936.17	0.56	0.4716
X_5^2	70990.59	1	70990.59	1.91	0.1998
Model	3.742E+006	20	1.871E+005	5.05	0.0084
Residual	3.337E+005	9	37077.90		
Lack of Fit	2.063E+005	6	34391.02	0.81	0.6235
Pure Error	1.274E+005	3	451.65		
R-Squared	0.9181				
Adeq Precision	10.461				

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids.

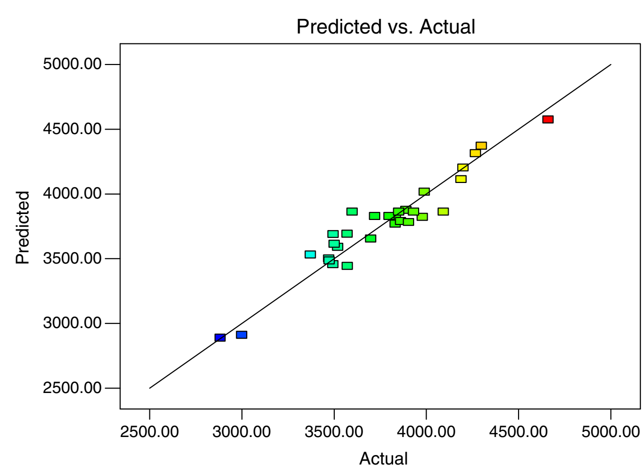
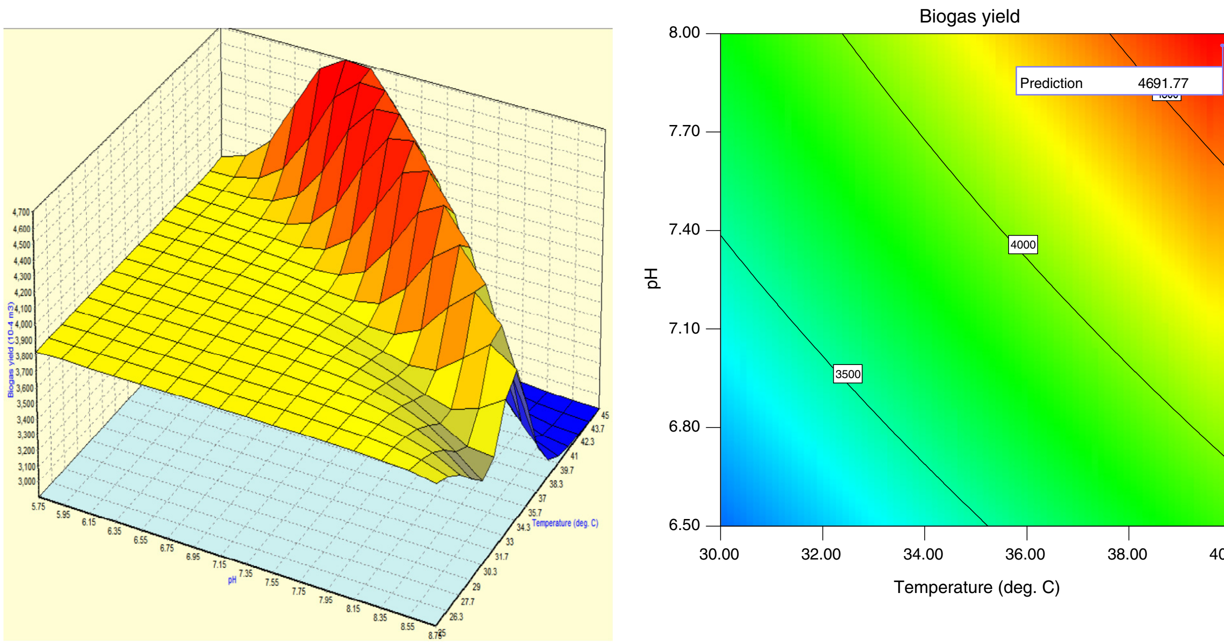
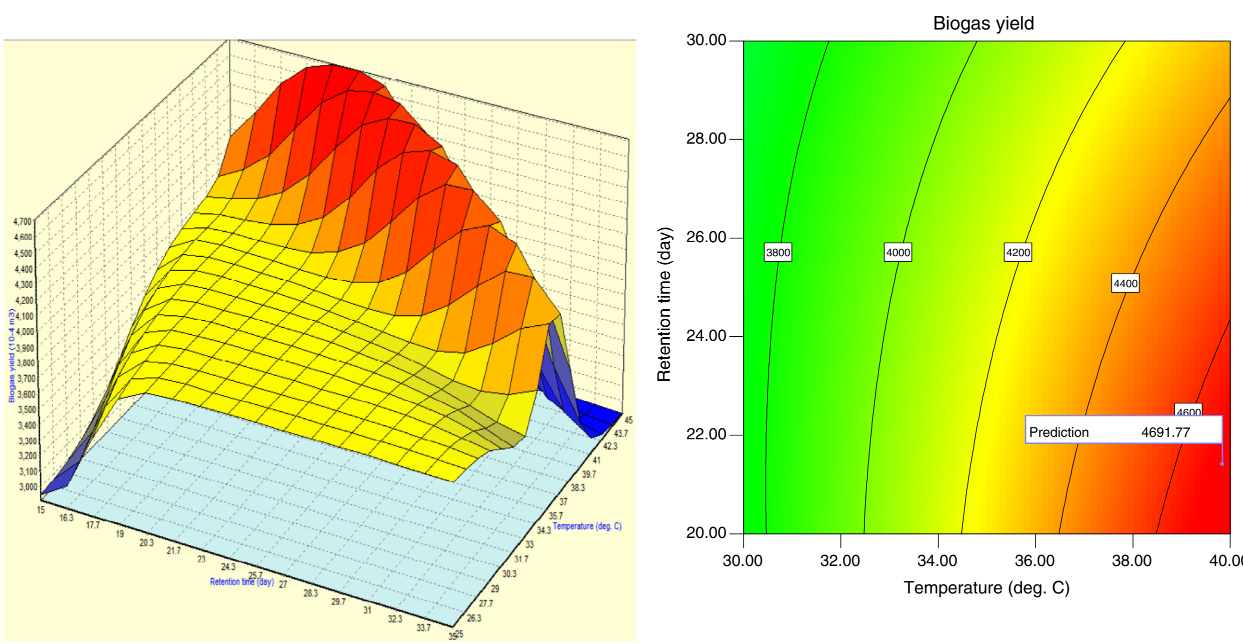


Fig. 3. Graph of predicted against the actual biogas yield for *Carica papaya* peels and poultry dropping.



(a)



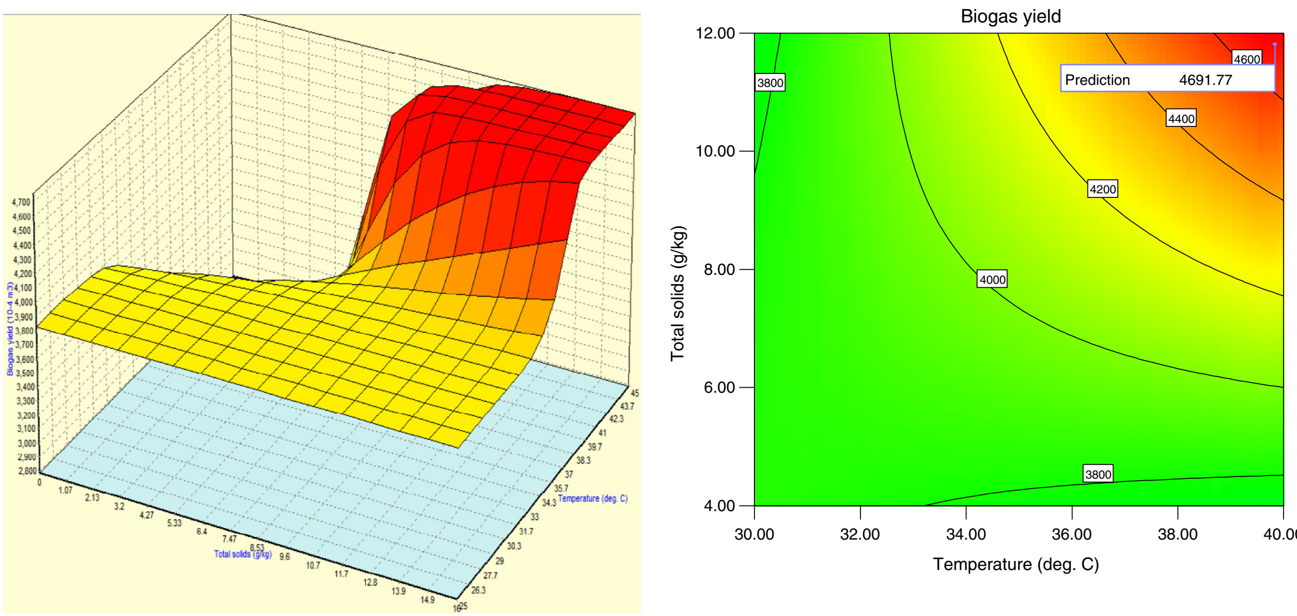
(b)

Fig. 4. (a–j): 3D curvatures' plots of ANNs optimization and their contours for biogas generation from *Carica papaya* peels and poultry dropping.

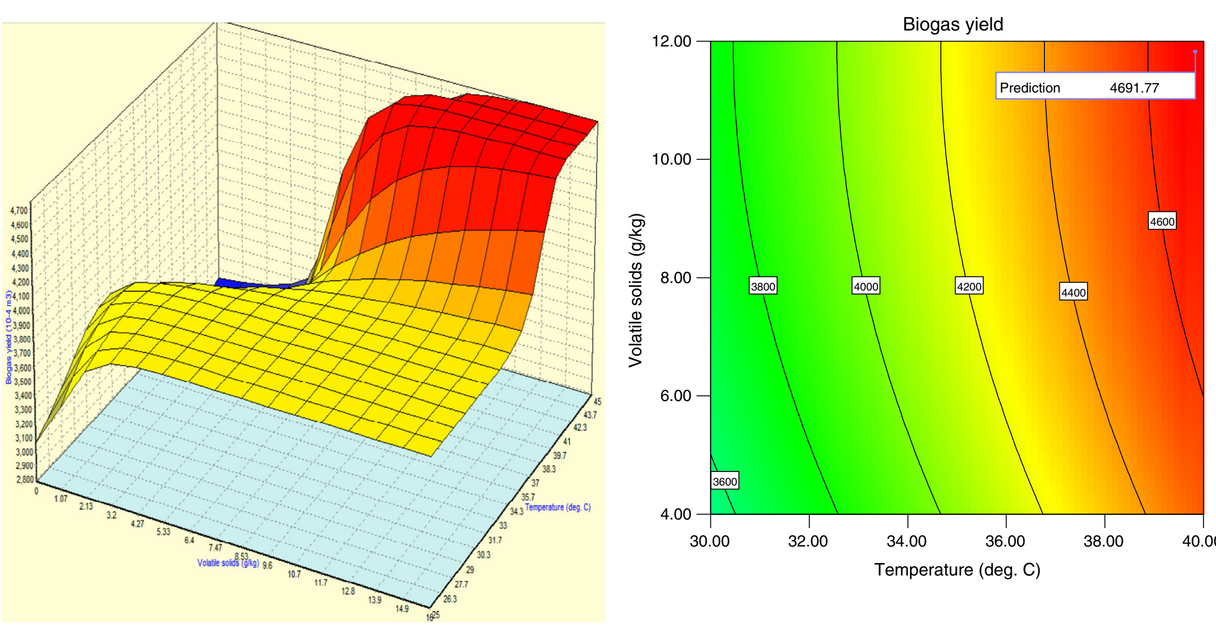
62.35% chance that a Lack of Fit F-value this large could occur. In this case, a non-significant lack of fit is good thus making the model fitting for use in theoretical prediction of the biogas production. Fig. 3 showed the graph of predicted against the actual biogas generation according to the prediction by RSM. The developed regression model equation describing the relationship between the biogas yield (Y) and the coded values of independent factors of temperature (X_1), pH (X_2), retention time (X_3), total solids (X_4) and volatile solids (X_5) and their respective interactions is described in Eq. (2).

$$\begin{aligned} Y(10^{-4}m^3/VS) = {} & 3861.63 + 124.89x_1 + 9.56x_2 + 33.40x_3 \\ & + 132.94x_4 + 62.83x_5 + 36.17x_1x_2 \\ & - 85.30x_1x_3 + 270.54x_1x_4 - 2.79x_1x_5 \\ & - 182.46x_2x_3 + 206.20x_2x_4 + 50.01x_2x_5 \\ & + 72.43x_3x_4 - 50.97x_3x_5 - 49.56x_4x_5 \\ & + 0.28x_1^2 - 13.32x_2^2 - 26.71x_3^2 - 28.02x_4^2 \\ & - 51.60x_5^2 \end{aligned} \quad (2)$$

where Y = Biogas yield (10^{-4} m^3/VS).



(c)



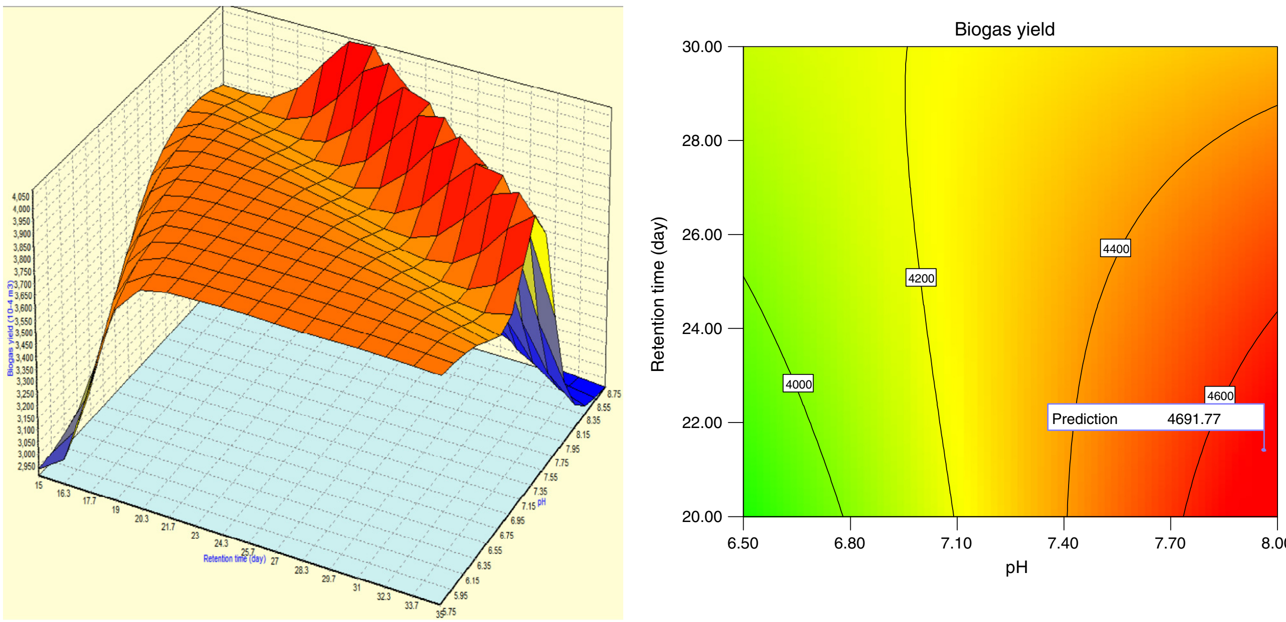
(d)

Fig. 4 (*continued*)

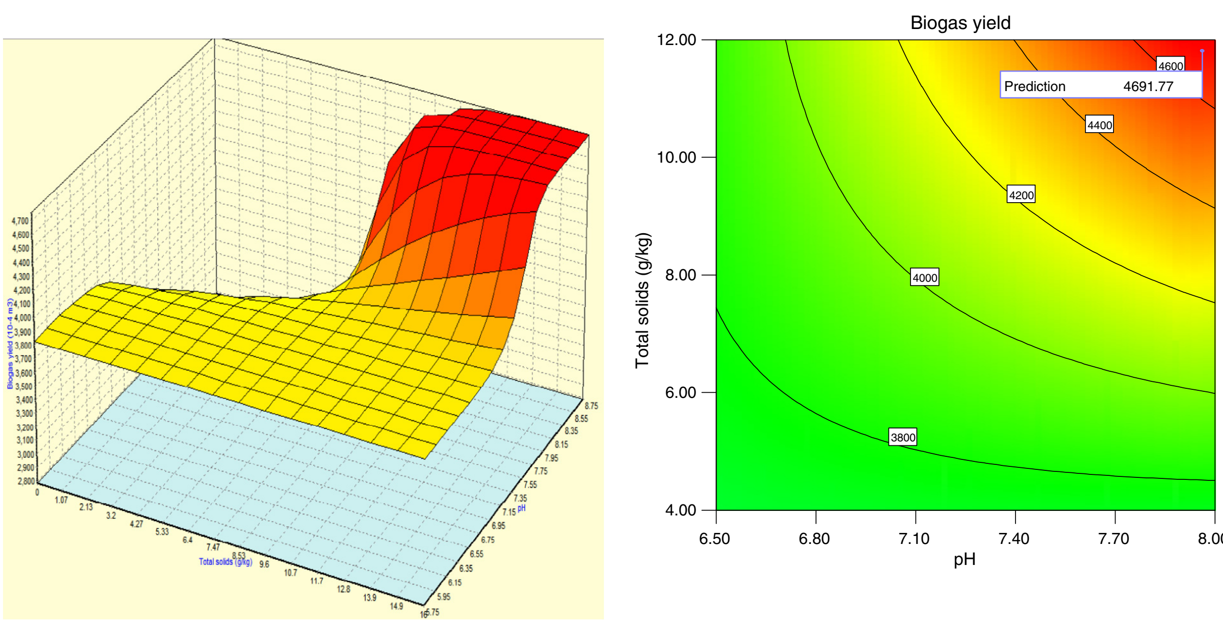
The optimal values of the independent factors selected for the biogas generation from *C. papaya* peels poultry dropping were obtained by solving the regression equation (Eq. (2)) using the Design-Expert software package. All conditions from experimental run 1–16 showed 100% desirability with the 16th run having the highest predicted value of 4691.77 (10^{-4} m^3/VS). However, the optimal conditions for this process were statistically predicted as $X_1 = 36.84$ (°C), $X_2 = 7.76$, $X_3 = 21.41$ (day), $X_4 = 11.81$ (mg/kg) and $X_5 = 11.81$ (mg/kg) with 100% desirability. The predicted biogas yield by RSM model under the above set conditions was 3991.77 (10^{-4} m^3/VS) while that of ANNs model was 3875.1 (10^{-4} m^3/VS). In order to verify the prediction of the model, the optimal conditions were applied to three independent replicates, and the average biogas yield obtained was 3979.88 (10^{-4} m^3/VS), which is well within the predicted value for the model equation.

3.4. ANN optimization of biogas data and interactions between variables

The three-dimensional (3D) response surface plots which are graphical representations of the regression equation for the optimization of the reaction variables and their contour lines are represented in Fig. 4(a–j). Fig. 5 shows the ANNs' importance level of each independent variable employed in the optimization. From the figure, Temperature is seen as the most important factor contributing to the efficiency of biogas yield from the substrate. The



(e)



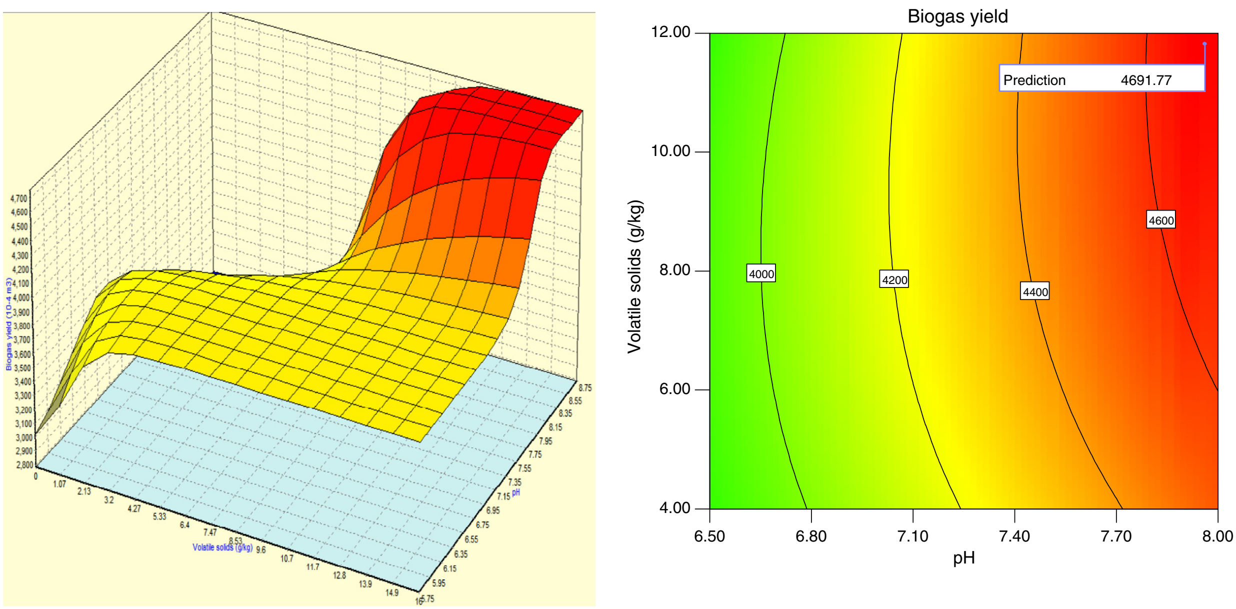
(f)

Fig. 4 (*continued*)

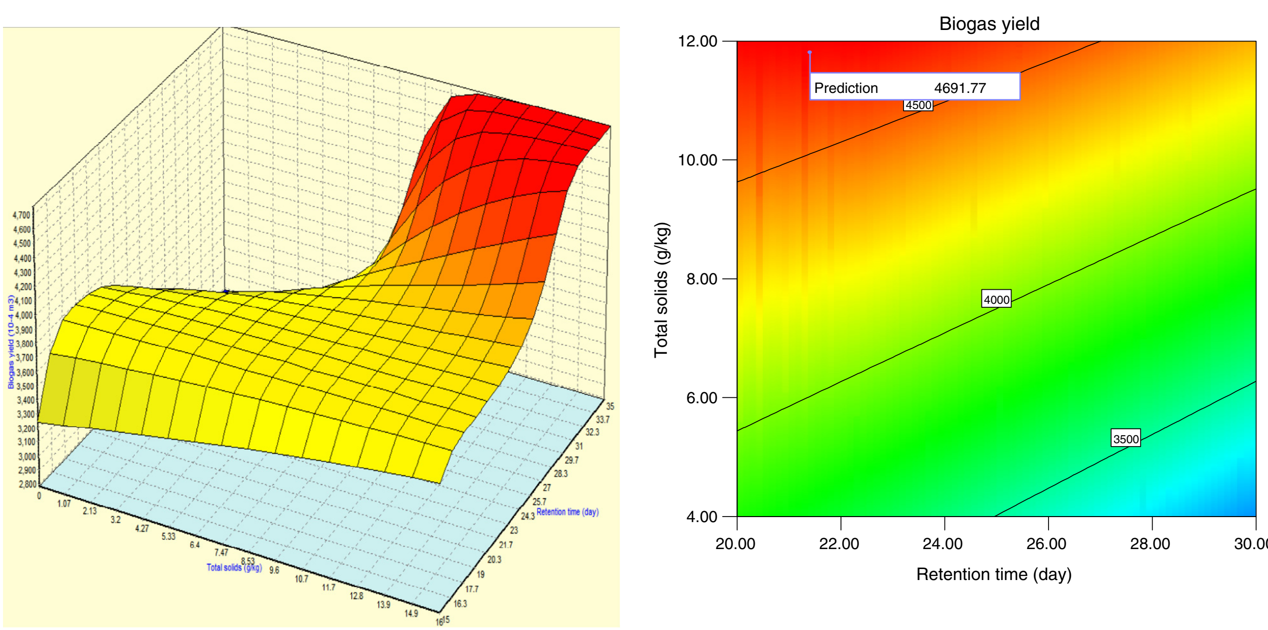
3D response surface plots for the RSM models, the schematic diagram of ANNs model and the figure showing the CPC-X Neural Power variations are in the Supplementary Materials. Table 5 shows both the RSM and ANNs design matrix for Biogas generation from *C. papaya* peels and poultry dropping with five independent variables using actual values.

4. Discussion

The pH of all the digesters throughout the digestion period remained at slightly alkaline range. There is need to maintain alkaline pH in anaerobic digesters in order to support efficient proliferation of methane producers and this in turn will hasten the bioconversion of substrates (Zahedi et al., 2016). The fall in pH after the 7th day was due to acid production thereby making the medium weakly alkaline before a rise as the population of acid producers reduces due to succession (Dahunsi and Oranusi, 2013). A pH range of 6.5–8.0 has been documented as the optimal for anaerobic organisms especially methanogens who are very sensitive to pH extremes (Zonta et al., 2013). The temperature of the digesters was also found to have remained at the mesophilic range throughout the experiment. Temperature affects the success of anaerobic



(g)

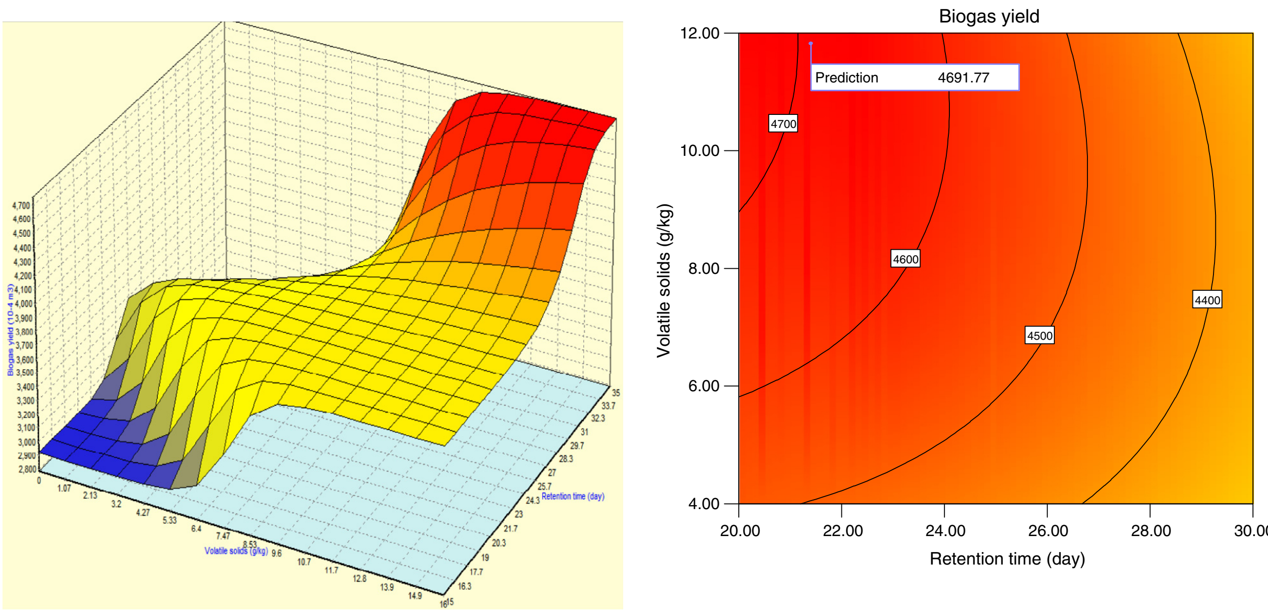


(h)

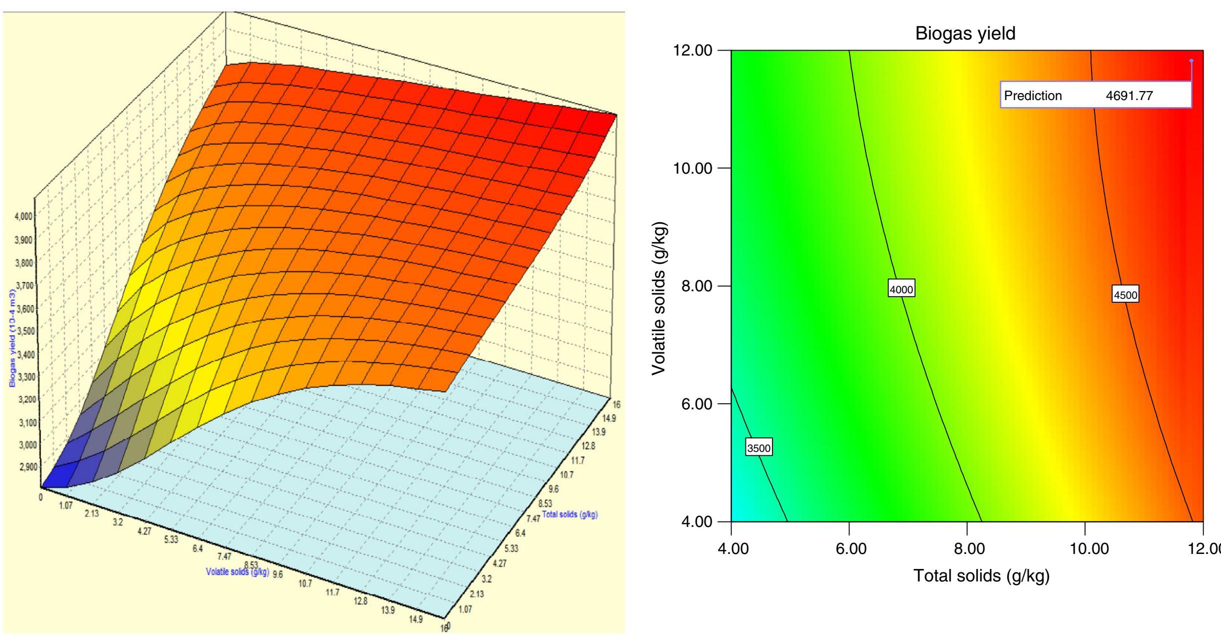
Fig. 4 (*continued*)

digestion because methanogenic bacteria operate at optimal temperature (Jain et al., 2015; Mckennedy and Sherlock, 2015). More so, anaerobic digestion at mesophilic temperature range provides stability of substrate conversion and richer bacteria population and efficiency (Mao et al., 2015).

The physical and chemical characteristics revealed that the poultry dropping was bulkier than the mixture of *C. papaya* peels and poultry dropping as well as the rumen content in terms of total and volatile solids. This could be due to the type of feed the birds were fed and also the fact that the dilution of pawpaw peels and poultry dropping has increased the moisture content of the bulky poultry dropping thereby making the mixture less bulky than the dropping alone. The anaerobic digestion process in this study was found to increase the nutrient status of the digestate as the value of most major (N P K) and minor elements (Mg, Mn, Fe, Zn, Al and Cu) increased after digestion. This is an indication that such digestate could be used as fertilizers to increase soil fertility and crop yield. One of the major issues faced in several cropping systems especially in the tropics is that of nutrient loss, pollution and toxicity to soil microorganisms due to over-reliance on chemical fertilizers. A nutrient-rich digestate such as produced in this research is a veritable option to solving this problem (Alfa et al.,



(i)



(j)

Fig. 4 (*continued*)

2014a; Pivato et al., 2015; Sun et al., 2015). The anaerobic digestion was found to be efficient in terms of COD reduction (up to 71.875%) which is higher than values reported for COD reduction in previous anaerobic digestion (Dahunsi and Oranusi, 2013; Alfa et al., 2014b). Numerous acid and methane formers were implicated in the digesters during the different stages of digestion from all the set ups. Most of the aerobes identified at the early stages are similar to those earlier reported in mesophic anaerobic digestion systems (Dahunsi and Oranusi, 2013; Jain et al., 2015) while most of the anaerobes (acidogens) are new in anaerobic system and these could be linked to the nature of the substrate and the inoculum used. The generated biogas from this experiment compared favourably with and is higher in both quantity and in methane yield than those from other substrates (Alfa et al., 2014b). This can be linked to the combination of different pretreatment methods employed prior to the actual digestion of the substrate and this proved very efficient in this study. The combination of different pretreatment methods for anaerobic digestion had been severally advocated (Jain et al., 2015). Also, the diminishing gas production observed after the 20th to 23rd days were due to the reduction in the volatile solid content as most of it has been bioconverted.

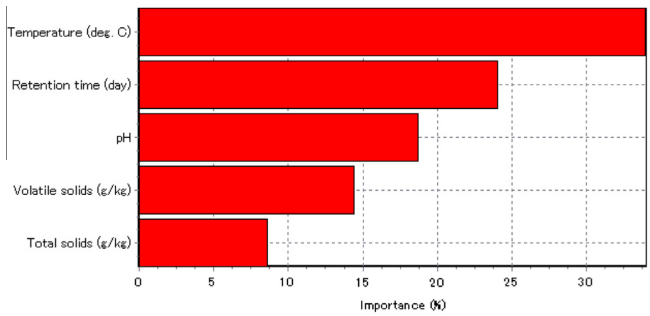


Fig. 5. ANNs' importance level of each independent variable employed in the optimization.

The model F-value with low p-value (0.0084) obtained in this study implied significance for the regression model and agreed with the submission of Yuan et al. (2008). The goodness of fit of the model was also checked by the coefficient of determination (R^2) since it had been reported that R^2 should be at least 0.80 for the good fit of a model (Pei et al., 2014). In the current study, the R^2 value of 0.9181 implied that the sample variation of 91.81% for the biogas yield is attributed to the five independent variables (temperature, pH, retention time, total solids and volatile solids) employed in the study. The Adequate Precision measures the signal to noise ratio and a ratio greater than 4 is desirable. The value of 10.461 is a good indication that the model is suitable and can be used to navigate the design space. All the p-values less than 0.05 implied that the model proved suitable for the adequate representation of the actual relationship among the selected variables. The lack-of-fit term of 0.6235 was not significant relative to the pure error. Since a non-significant lack of fit is good, the model could be used in theoretical prediction of the biogas production from the substrates used in this study. All negative and positive values in Eq. (2) shows that the variables have negative and positive effect on the yield of biogas respectively.

Table 5
ANNs Design for Biogas generation from *Carica papaya* peels and poultry dropping with five independent variables using actual values.

No	X_1	X_2	X_3	X_4	X_5	Actual biogas yield (10^{-4} m^3/VS)	RSM predicted biogas yield (10^{-4} m^3/VS)	ANNs predicted biogas yield (10^{-4} m^3/VS)
1	36.84	7.76	21.41	11.81	11.81	3884.2	3991.77	3875.1
2	36.45	7.75	20.17	12.00	11.60	3000.0	3100.42	3000.0
3	36.78	7.60	20.78	11.97	11.78	3372.2	3384.68	3371.1
4	36.99	7.79	20.05	11.99	6.10	3475.4	3465.49	3439.1
5	36.87	7.70	20.97	11.84	8.48	3496.4	3491.2	3496.3
6	35.98	7.79	20.27	11.89	10.65	3700.3	3701.77	3739.0
7	36.31	7.96	21.29	11.97	11.49	3573.2	3663.01	3573.5
8	36.99	7.99	21.63	12.00	6.89	4201.1	4261.17	3822.7
9	36.93	7.86	20.06	11.78	9.86	3502.8	3666.8	3832.7
10	35.91	8.00	20.06	11.68	7.27	3600.9	3663.04	3798.0
11	37.50	7.96	21.59	11.98	11.98	3834.1	3970.11	3813.9
12	37.00	8.00	22.01	12.00	6.97	3572.2	3661.11	3679.9
13	36.89	7.98	20.21	11.89	6.87	3521.1	3664.36	3833.4
14	36.99	7.99	20.94	11.93	8.97	3991.1	4023.49	3980.8
15	36.97	7.85	20.67	11.81	11.80	3980.6	4064.71	3959.2
16	36.89	8.00	20.52	11.11	11.99	4600.8	4691.77	4685.8
17	36.82	7.95	20.00	12.00	11.33	3852.2	4040.01	3798.0
18	38.00	7.70	24.39	12.00	8.95	4661.1	4633.07	4709.0
19	35.91	7.94	20.00	12.00	5.84	4190.1	4219.73	4195.0
20	37.00	7.60	25.07	12.00	10.00	3932.9	4015.52	3798.0
21	37.00	7.70	23.79	12.00	6.88	3473.8	3413.87	3492.4
22	37.00	7.70	24.64	11.97	8.28	3800.1	3812.21	3844.7
23	36.74	7.80	24.56	11.98	12.00	4268.6	4302.77	4254.5
24	36.99	7.95	20.00	12.00	4.90	3891.5	3890.95	3832.6
25	36.99	7.70	25.46	12.00	4.94	3862.1	3910.99	3832.6
26	37.00	7.40	28.01	12.00	9.67	3494.9	3497.34	3495.3
27	37.00	7.50	28.08	12.00	10.25	4094.5	4191.04	3798.0
28	37.00	7.70	29.22	12.00	8.29	3905.7	3945.94	3884.8
29	37.00	7.96	26.69	12.00	4.02	3722.4	3843.47	3739.6
30	37.00	7.60	28.46	11.97	5.35	3832.2	3932.33	3836.7
31	37.50	7.45	26.67	10.23	6.49	3867.9	3871.31	3832.3
32	37.50	7.50	25.56	10.10	6.52	3771.1	3801.02	3763.2
33	36.00	7.45	26.81	9.34	5.51	3532.2	3621.20	3611.0
34	36.50	7.67	23.76	9.90	8.81	3100.1	3132.41	3112.1
35	37.00	7.64	23.49	8.19	8.01	2981.2	2965.31	2943.3
36	37.00	7.90	27.01	8.08	7.98	3102.2	3211.42	3208.3
37	37.00	7.95	26.91	10.01	6.81	2341.9	2373.03	2320.3
38	37.50	7.80	24.56	9.67	5.50	3190.3	3212.21	3210.1
39	37.50	7.75	25.59	9.88	4.90	3087.1	3121.31	3113.4
40	38.00	7.67	27.02	8.18	6.12	3021.3	3101.48	3100.0
41	38.00	7.87	27.08	9.67	6.21	3209.2	3243.31	3220.6
42	36.50	7.83	26.01	10.02	6.09	2987.9	3014.15	3011.1
43	36.50	7.84	24.80	11.12	7.90	3112.1	3167.04	3132.1
44	37.00	7.89	23.40	11.31	8.12	2702.2	2711.14	2689.1
45	37.00	6.95	23.21	10.90	9.03	2678.8	2712.12	2678.1
46	37.50	6.78	22.90	9.11	9.01	2871.6	2899.03	2852.0
47	38.00	7.00	24.32	10.21	8.83	2700.1	2761.23	2698.2
48	38.00	7.98	23.41	12.00	6.54	2301.8	2381.90	2358.2
49	38.50	8.00	23.56	11.21	5.03	2231.3	2241.80	2211.2
50	37.50	7.67	23.08	10.86	4.80	2431.3	2462.02	2428.8

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids.

The entire curvatures' nature of the three-dimensional (3D) response surface plots for the optimization of the reaction variables all suggested moderate relationships for temperature, pH, retention time, total solids and volatile solids respectively. Such kind of interactions has been reported (Betiku et al., 2015; Emeko et al., 2015).

In this study, the prediction and estimation capabilities of both RSM and ANN were examined so as to know which model gives the best result. RSM and ANN were used to stimulate responses, which were then compared with actual values. The predicted biogas yield, roots mean squared error (RSME) and the coefficient of determination (R^2) were used to compare the RSM and ANN. From the results, it was noticed that the most desirable biogas yield predicted by RSM was 3991.77 (10^{-4} m^3/VS) while that of ANNs was 3875.10 (10^{-4} m^3/VS). The RSME of biogas for RSM (451.65) was higher than that of ANN (68.05). The R^2 for RSM (0.9181 i.e. 91.81%) was lower compare to that of ANN (0.9828 i.e. 98.28%). This shows that though the predictive ability of RSM was higher than ANNs, the latter gives higher accuracy and efficiency than the former for the generation of biogas from *C. papaya* peels and poultry dropping.

5. Conclusion

C. papaya in co-digestion with poultry dropping was found to be a good substrate for biogas generation. The result of modelling and optimization showed that both RSM and ANN models are efficient in the prediction of methane production from *C. papaya* peels and poultry dropping. This study has proposed a permanent usage for pawpaw peels which remained grossly underutilized till now. Due to its high biogas yield and huge potentials for biofertilizer production, the worldwide usage of pawpaw peels for energy generation is advocated even as it is found abundant around the world and available all year round.

Conflict of interest

Authors declare that there is no conflict of interest whatsoever.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.05.118.

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