Biochemical conversion of fruit rind of *Telfairia occidentalis* (Fluted Pumpkin) and poultry manure

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This study evaluated the potentials of Fluted pumpkin fruit rind and poultry manure for biogas generation. Mechanical and thermo-alkaline pre-treatments were applied to two samples labelled ‘O’ and ‘P’ while the third sample (Q) had no thermo-alkaline treatment. The physicochemical characteristics of the substrates revealed richness in nutrients and mineral elements. The modelling was done using the Response Surface Methodology and Artificial Neural Networks and statistical prediction showed the process optimal conditions to be 30.02 ^{o} C, 7.90, 20.03 days, 5.94 g/kg and 4.01 g/kg for temperature, pH, retention time, total solids and volatile solids. Using the above set values, the biogas yield was predicted to be 2614.1, 2289.9 and 1003.3 $10^{-3}m^3$/kg VS for digestions ‘O’, ‘P’ and ‘Q’ respectively. The results showed that use of combination of pre-treatment methods enhanced the biogas yield in the pre-treated substrates. Analysis of the gas composition showed 66.5 ± 2.5 % Methane, 25 ± 1% Carbon dioxide; 58.5 ± 2.5 % Methane, 26 ± 1% Carbon dioxide; 54.5 ± 1.5 % Methane, 28 ± 2% Carbon dioxide for the three experiments respectively. All the obtained values show the models had a high predictive ability. However, the coefficient of determination (R^2) for RSM was lower compared to that of ANN which is an indication that ANNs model is more accurate than RSM model in predicting biogas generation from the anaerobic co-digestion of rind of Fluted pumpkin and poultry manure. The substrates should be further used for energy generation.

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Abstract

This study evaluated the potentials of Fluted pumpkin fruit rind and poultry manure for biogas generation. Mechanical and thermo-alkaline pre-treatments were applied to two samples labelled 'O' and 'P' while the third sample (Q) had no thermo-alkaline treatment. The physicochemical characteristics of the substrates revealed richness in nutrients and mineral elements. The modelling was done using the Response Surface Methodology and Artificial Neural Networks and statistical prediction showed the process optimal conditions to be 30.02 ^{o} C, 7.90, 20.03 days, 5.94 g/kg and 4.01 g/kg for temperature, pH, retention time, total solids and volatile solids. Using the above set values, the biogas yield was predicted to be 2614.1, 2289.9 and 1003.3 $10^{-3}m^3$/kg VS for digestions 'O', 'P' and 'Q' respectively. The results showed that use of combination of pre-treatment methods enhanced the biogas yield in the pre-treated substrates. Analysis of the gas composition showed 66.5 ± 2.5 % Methane, 25 ± 1% Carbon dioxide; 58.5 ± 2.5 % Methane, 26 ± 1% Carbon dioxide; 54.5 ± 1.5 % Methane, 28 ± 2% Carbon dioxide for the three experiments respectively. All the obtained values show the models had a high predictive ability. However, the coefficient of determination (R^2) for RSM was lower compared to that of ANN which is an indication that ANNs model is more accurate than RSM model in predicting biogas generation from the anaerobic co-digestion of rind of Fluted pumpkin and poultry manure. The substrates should be further used for energy generation.

Keywords: Biogas, Methane, Microorganisms, Optimization, Pre-treatment, Rumen content

1. Introduction

The global increase in the generation of organic wastes from animal husbandry and other agricultural activities is phenomenal, thus culminating in huge environmental pollution problems in many nations (Zou et al., 2016). Also, the improper management of these wastes has been reported to cause several environmental challenges such as water, land and air pollution and the spread of pathogenic organisms which causes diverse diseases within the human population (Fierro et al., 2016). However, the proper and adequate management/utilization of these solid wastes can improve human's living standards as well as ensuring environmental protecting via the production of environmental-friendly biofuels and nutrient-rich digestate biofertilizers (Dahunsi et al., 2016a, b).

A veritable way to achieve this is by employing anaerobic digestion (AD) systems which has the capacity for reducing greenhouse gas (GHG) emissions by producing clean fuels such as biogas (Dahunsi et al., 2017; Dahunsi, Oranusi & Efeovbokhan, 2017a, b, c). The AD technology for methane generation has been reported to be a more efficient method for energy generation from biomasses in contrast to other biological and thermo-chemical conversion systems (Zahedi et al., 2016). AD is equally known to be particularly famous in developing countries where mostly the produced gas is used as fuel for cooking while the digestate is used as fertilizer or soil conditioner (Dahunsi & Oranusi, 2013; Abudi et al., 2016).

Biogas generation from the mono-fermentation of poultry manure has been extensively reported in energy literature. However, the major challenges encountered were low C/N ratio and high total ammonia levels of the substrate (Dalkilic & Ugurlu, 2015). It has therefore been opined that for the best digestion and adequate gas yield, poultry dropping is better co-digested with other high energy-yielding substrates such as grasses, silage and other green biomass (Pagliaccia et al., 2016). The advantages of co-digestion over the conventional AD include adjustment/balances of C/N ratio and nutrient, pH buffering capacity increase, decreases in ammonia toxicity and accumulation of VFAs and upgrading of biochemical conditions for microbial proliferation (Serrano et al., 2016).

T. occidentalis, Hook. f. (Fluted pumpkin) originated from Southern Nigeria (Akoroda et al., 1990; Schippers, 2002) where it is regarded as an important leaf and seed vegetable all over West and Central Africa. Its major dominance is in Nigeria, Ghana and Sierra Leone (Okoli & Mgbeogwu, 1983). It is a large perennial plant of the family *Cucurbitaceae* with the ability to grow to height of up to 20 m. It is known for climbing with the use of bifid and tendrils and produces drooping and ellipsoid berry fruit (40 to 95 cm × 20 to 50 cm) usually weighing up to 6 kg, with 10 prominent ribs, pale green and covered with white bloom wax, fruit pulp yellow and many embedded seeds (Eseyin, Sattar & Rathore, 2014). *T. occidentalis*is majorly cultivated in different agricultural systems because of its nutritious leaves which have been shown to have ≥21 % protein content, and is very high in vitamins, calcium, phosphorus and iron. Other uses of the leaf include generation as concoction for anemic patients due to its hematinic abilities (Eseyin, Sattar & Rathore, 2014). However, despite the huge biomass production from the fruit rind of fluted pumpkin,it has remained grossly under-utilized in its different producing localities. It is often thrown into the garbage bin or left in stock piles where they decay and serve as vehicle for breeding and transmitting disease-causing microorganisms.

Thestructural and chemical properties of lignocellulosic biomass make them resistant/recalcitrant to anaerobic degradation (Naran, Toor & Kim, 2016) and this factor is also responsible for their limited commercial usage (Kim, Lee & Park, 2015; Menon et al., 2016). Application of pretreatments therefore is a sure way to enhance the microbial degradation of feedstock before anaerobic digestion (Monlau et al., 2015). Several pre-treatment methods have and are still being investigated as a way of combating the initial recalcitrance often encountered in the usage of lignocellulosic biomass. These methods includes ultrasound, high pressure and lysis, thermal, ozonation, dilute acids, alkali, use of microorganisms, enzymes etc. Mechanical pre-treatments are suitable for substrate particle size reduction and they are widely applied in the treatment of animal wastes/manure, lignocellulosic materials and sludge from wastewater treatment plant (Barakat et al., 2014). However, these methods are poor at pathogen removal/reduction besides causing clogging of equipment (Zheng et al., 2014). Thermal pre-treatments are efficient at pathogen reduction, high dewatering and viscosity reduction of digestate and these accounts for their successful industrial application (Naran, Toor & Kim, 2016). Different temperature ranges have been experimented for lignocellulosic biomass among which temperatures > 100° C resulted in the pronounced/excessive lignin solubilisation and the subsequent production of inhibitory phenolic compounds (Liu et al., 2012). More common is thermal treatment at temperatures between 70 and 90° C and this has yielded positive results in terms of biogas productionin several experiments (Appels et al., 2010; Liu et al., 2012; Dahunsi et al., 2017a, b, c).

Preference for alkali pre-treatment is high in anaerobic digestion process due to the fact that a successful digestion usually requires a pH buffering by increasing alkalinity (Naran, Toor & Kim, 2016). In some recent studies, alkaline treatment was reported to enhance methane production fromthe mono-digestion of substrates like sunflower stalks and sorghum forageand from the co-digestion of *Carica papayas* fruit peels and poultry dropping, *T. occidentalis* fruit peels and poultry manure, *Chromolaena odorata* and poultry manure and *Arachis hypogaea* hulls (Liu et al., 2012; Dahunsi et al., 2016a, b; Dahunsi et al., 2017; Dahunsi, Oranusi & Efeovbokhan, 2017a). Generally, chemical pre-treatments are not suitable for easily biodegradable biomass due to their higher rate of degradation coupled with production and accumulation ofvolatile fatty acids (VFA's) which in turn may cause the total failure of methanogenesis. The methods are however very idea for lignocellulosic biomass due to the complex lignin-cellulose-hemicellulose matrix presents in them (Sambusiti et al., 2013). Usage of combined treatment methods such as thermo-chemical,chemo-mechanical and others are also widely reported especially in the mono-digestion of sludge from wastewater treatment plants and these helped to improve on the limitations of single pre-treatment methods (Modenbach & Nokes, 2012; Barakat et al., 2014; Yuan et al., 2016).

T. occidentalis is novel in biofuel research because this is the first reported study that established the appropriate pre-treatment methods, optimized the important process parameters (Montingelli et al., 2016), and assessed the mass and energy balance of the in co-digestion with poultry manure as well as evaluating the economic feasibility of pre-treatments (Monlau et al., 2015). Though biogas generation from the mono-digestion of *T. occidentalis* fruit rind has been documented (Dahunsi et al., 2016b), there is gap in knowledge as the potentials of this biomass for biogas generation in co-digestion alongside the standardization of its process parameters is yet to be reported despite it abundance and year-round availability. In this research therefore, the anaerobic co-digestion of *Telfairia occidentalis* fruit rind and poultry manure was carried out after the application of different pre-treatment methods. Despite the massive biomass production and year-round availability of fluted pumpkin, its fruit's rind remains largely unused indicating the need for a permanent and sustainable solution for this menace. Also, the optimization of the process parameters, mass, energy and economic balances (Betiku et al., 2015; Dahunsi, Oranusi & Efeovbokhan, 2017a) was evaluated in this research in order to set a future benchmark for the use of fluted pumpkin's fruit rind as a biomass for bioenergy production.

2. Materials and methods

2.1. Sample collection and digester design

Fruits of *Telfairia occidentalis* were collected from the farms at Landmark University, Omu-Aran, Nigeria after which the seeds of the fruits were removed after cutting with a knife and the rind to be used in this study was carefully separated and taken to the site of the experiment after which it was air-dried to constant weight. Collection of fresh poultry manure was done at the Teaching and Research Farms of the University while the Bovine rumen content to be used as inoculums was obtained from the slaughter slab of the University's cafeteria. Since the rind is a lignocellulosic biomass and the need for pre-treatment arose, three different methods were employed in order to establish the best pre-treatment procedure for the biomass prior to anaerobic digestion. Pre-treatment of the first sample labelled 'O' was done using a combination of mechanical, thermal and NaOH alkaline pre-treatment earlier reported (Dahunsi et al., 2016a, b). To achieve the mechanical treatment of the biomass, a hammer mill was used for crushing until a mesh size of ≤ 20 mm was obtained. The crushed biomass was then thermally treated (By heating) in the Clifton, 88579, Nickel-Electro Ltd., England water bath at 80^{o} C for an hour. Prior to choosing the suitable temperature, duration of thermal treatment and quantity of alkali to be used, the Central Composite Design (CCD) was used for the experimental design according standard method (Dahunsi, Oranusi & Efeovbokhan, 2017a, b, c). In the design, a four-factor model was used i.e. (i) Temperature for thermal pre-treatment (ii) Time/duration of thermal pre-treatment (iii) Quantity of alkali for alkaline pre-treatment (iv) Time/duration for alkaline pre-treatment. The pre-treatment temperature was varied between 70 and 200^{o} C while a pre-treatment time between 50 and 80 min was considered. For the quantity of alkali, a variation of 2 g/100 g TS to 5 g/100 g TS was used while a time variation of between 18 and 36 h was used for the alkaline pre-treatment.

Immediately following the thermal procedure was alkaline pre-treatment with 3 g NaOH/100 g TS at 55 ^{o} C for a 24 h period and at a solid loading of 35 g TS L^{-1}. The second sample labelled 'P' was pre-treated using the above mechanical and thermal methods but with KOH alkaline also using 3 g KOH/100 g TS at 55^{o} C for a 24 h. The choice of NaOH and KOH was premised on earlier reports that among other widely used alkalis, they produced the best result for thermo-alkaline pre-treatment (Li, Champagne & Anderson, 2015). The third sample 'Q' was treated mechanically but without thermal and alkaline pre-treatment and served as control. The digester earlier described (Alfa et al., 2014a; Dahunsi et al., 2016a, b) was used with the collection of produced gas via liquid displacement method (Dahunsi & Oranusi, 2013; Alfa et al., 2014b).

2.2. Methane potential tests and experimental design

Prior to digestion, the potential methane production from the co-substrates was anaerobically determined using already described standard method (Dahunsi et al., 2016a, b) while the same method was used to evaluate the solid digestate for its residual methane content (Yap et al., 2016). The CCD used in designing the pre-treatment procedures was also employed in the experimental design of the anaerobic digestion of all the pre-treated and untreated samples of *T. occidentalis* fruit rind and poultry manure due to the reported high efficiency of the model in product optimization (Betiku et al., 2015; Zou et al., 2016). As shown in Tables S1 and S2 (Supplementary materials), 50 experimental runs were generated using the five-level-five-factors design. Five importance process parameters: (Temperature (^{o} C), pH, Retention time (days), Total solids (g/kg) and Volatile solids (g/kg) were selected for the modelling and optimization and each was designated as T_1, T_2 T_3 $T_4$and T_5respectively. Temperature as reported in most previous mesophilic digestion studies has been varied between 30 and 40^o C (Naran, Toor & Kim, 2016). Similarly, the common pH values reported in literature for anaerobic digestion ranges between 6.5 and 8 (Liao et al., 2016; Naran, Toor & Kim, 2016) while 20 to 30 days retention time is the usual practice in most experiments (Naran, Toor & Kim, 2016; Saha et al., 2016). For the total solids, a composition of < 15% and ≥ 4% has been severally proposed for liquid digestion (Bayrakdar et al., 2016; Zhang et al., 2016). These were critically considered in the optimization value ranges used in this study in order to obtain the optimal condition for the digestion of *Telfairia occidentalis* fruit rind and poultry manure. The experimental data generated via the CCD was also employed in the ANN module in which the determination of the optimum ANN structure was done using mean square error (MSE). The higher coefficient R^2 was also determined and the effect of each optimized variable in the biogas yield was evaluated using relative importance and 3-Dimensional curvature plots. The results of the ANN were then compared with RSM while validation of both models was done under the set conditions as predicted by the software (Dahunsi et al., 2016a, b).

2.3. Digestion

Anaerobic digestion was carried out for the three samples of *T. occidentalis* fruit rind in addition with poultry manure. Since dilution of feedstock is a necessary step to eliminating ammonia inhibition during digestion (Bayrakdar et al., 2016; Sun et al., 2016), *T. occidentalis* fruit rind and poultry manure was mixed with water to form slurry at a solid loading of 35 g TS L^{-1} and was introduced into each digester tank through an inlet pipe (Alfa et al., 2014a). This was carried out for each of the pre-treated sample of *T. occidentalis* fruit rind and poultry manure. One (1) kg of the rumen content was added to each of the pre-treated substrate before loading. In each case after loading, the slurry occupied three quarter of the digester space and leaving one quarter space for collection of produced gas. Measurement of daily biogas production was done daily, while evaluation of microbial diversity and succession was done weekly. Temperature of the digesters was measured twice daily for the average values while pH measurement was on weekly basis using pH meter model pHS-2S, (SHANGHAI JINYKE REX, CHINA). Analyse of the produced biogas for its constituents determination was done using a Gas Chromatography (GC) (HP 5890, Avondale, USA) coupled with a Hayesep Q column (13m x 0.5m x 1/800) and a flame ionization detector (FID) (Alfa et al., 2014b; Dahunsi et al., 2016a, b).

2.4. Analytical procedures

Substrates for anaerobic digestion must be adequately characterized prior to digestion (Lalak et al., 2016). With this fact in mind, all the samples of *T. occidentalis* fruit rind, the poultry manure and inoculums were analysed in order to quantify their important physical and chemical parameters. These analyses were also carried out on the digestates at the end of the digestions. The analyses were done in the Environmental Engineering laboratory (Civil Engineering Department), Landmark University, Omu-Aran, Nigeria. Prior analyses, centrifugation was carried out in order to separate the liquid from the solid portion in each sample and the latter was used for all analyses except those of total phenol. All the chemical parameters were evaluated in triplicates using the Palintest(R) Photometer 7500 (PHOT.1.1.AUTO.75) advanced digital-readout colorimeter (Camlad, Cambridge, United Kingdom)which was operated at 0.5 absorbance and 450 nm wavelength as earlier described (Dahunsi et al., 2016a, b). These parameters include total carbon, total nitrogen, total phosphorus, phosphates, sulphates, potassium, sodium, magnesium, calcium, nitrates, ammonium, iron, copper, zinc, aluminium and manganese. The APHA, (2012) method subsequently used by Dahunsi et al. [2014] was used to determine COD of all samples. Determination of total and volatile solids was done using the SFS 3008 protocol of the Finnish

Standard Association, (1990). For TS, samples were dried at 105º C until constant weight was achieved while for VS, known weights of the dried samples were ignited at 575 ± 25º C to constant weight. Amicrotube test (Spectroquant, Merck) closely followed by a 4-aminoantipyrine colorimetric measurement was used for total phenolic contents determination (Monlau et al., 2015). A mild acid hydrolysis protocol with further quantification by the anthrone method was used for soluble sugars i.e. sucrose and inulin extraction (Monlau et al., 2012). For the quantification of structural carbohydrates i.e. glucose, xylose and arabinose anduronic acids i.e. galacturonic and glucuronic acids, a strong acid hydrolysis protocol (Monlau et al., 2015; Dahunsi et al., 2017a, b) was used. In determining the lignin content of the samples, 100 mg dried samples was hydrolysed with 12 M H_2SO_4 for 1 h at room temperature. The solution was then diluted to reach a 1.5 M final acid concentration and was kept at 100º C for 2 h before centrifuging at 10000 rpm for 10 min. The Klason lignin content was thereafter determined as the weight of the residue. The monomeric sugar content of the samples was used for cellulose and hemicelluloses content determination (Barakat et al., 2015).

2.5. Preliminary energy balance and assessment of thermo-alkaline pre-treatment efficiency

There is need to justify the investment into the thermo-alkaline pre-treatment applied in this study. In doing this, an assessment was carried out to compare the energy generation and consumption. The cost of obtaining heat energy and alkalis (NaOH and KOH) was compared with the gain accrued from the sale of the additional energy obtained when thermo-alkaline pre-treatments were applied to experiments 'A' and 'B'. This helped to determine if the gain from the sale of the extra gas (Obtained from the digestion of the pre-treated substrates) was enough to cover the initial expenses on heat energy and alkalis. A simple computational equation was used to first determine the thermal energy required (TER) in kWh t^{-1} TS for raising the temperature of one ton TS of *T. occidentalis* fruit rind from 25 to 55 ° C during pre-treatment. The equation is shown thus:

$$TER = \frac{m \, x \, Sh * (Qfinal - Qinitial)}{3600} \quad (1)$$

where *m*(1000 kg) = mass of the mixture of *T. occidentalis* fruit rind and water (kg); *Sh* = specific heat of water i.e. 4.18 kJ kg^{-1} C^{-1}; *Q initial* (° C) is the initial temperature of substrate i.e. 25 ° C; *Q final* (° C) is the final temperature of substrate i.e. 55 ° C. The United States cost of NaOH and KOH were used.

2.6. Microbial enumeration

The aerobic organisms (Bacteria and fungi) associated with the fermenting substrates were isolated and enumerated weekly using standard methods for total aerobic plate enumeration and presumptive isolates confirmed with the aid of appropriate rapid Analytical Profile Index (API) kits (BioMerieux, Leon, France) (Tsuneo, 2010; Dahunsi et al., 2016a, b). Members of the genera *Clostridium* and other facultative anaerobes were serially isolated using specialized media like Reinforced Clostridia medium, blood agar and Brain Heart Infusion agar in an anoxic condition at 37° C for 5 to 7 days as earlier reported (Ayandiran et al., 2014). Confirmation of the presumptive isolates was done with corresponding rapid API kits (Ayandiran & Dahunsi, 2017). For members of the Achaea (Methanogens), a mineral-rich basal medium earlier described by was compounded and used for the evaluation of members of the achaea following earlier description by Ghosh, Jha & Vidyarthi (2014) was used. The medium was fortified with minerals, trace elements and dyes and prepared according to standard prescription with resazurin as the indicator dye (Stieglmeier et al., 2009).

2.7. Statistical data analysis

The RSM was used to statistically analyse all data obtained from each of the three experiments using the Design-Expert software version 9.0.3.1 (Stat-Ease Inc., Minneapolis, USA) while using multiple regressions to fit the coefficient of the polynomial model of the responses. Fitting of the model was afterwards done using the test of significance and analysis of variance (ANOVA) as shown in the quadratic response model below:

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

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.

3. Results

3.1. The effects of thermo-alkaline pre-treatment on the chemical composition of *T. occidentalis* fruit rind

The results of the structural parameters analyses carried out on the raw *T. occidentalis* fruit rind, the thermo-alkaline pre-treated and the untreated substrates used in the digestion process are shown in Table 1. In both thermo-alkaline pre-treated samples i.e. 'A' and 'B', pronounced solubilization of cellulose, hemicelluloses and klason lignin were reported compared to sample 'C' which had no thermo-alkaline pre-treatment. After the pre-treatments, there were 56 and 43% reduction in cellulose concentration for experiments 'A' and 'B' respectively. For hemicelluloses, the observed reductions were 47 and 32.28% while for klason lignin concentration, reductions of 36 and 29% were reported respectively. There were changes in the concentrations of uronic acids as reductions of 51.4 and 36.25 were reported respectively. For the concentration of soluble sugars, there were increases of 68.03 and 65.18% as a result of the pronounced solubilisation due to the application of thermo-alkaline pre-treatment to fruit rind of

T. occidentalis. Overall, higher solubilisation of components was reported in experiment 'O' as against 'P'.

3.2. Anaerobic digestion performance and stability

In the test for methane potential, production of biogas commenced on the 3rd, 4th and 7th experimental days respectively in digestions 'O', 'P' and 'Q'. Estimated average methane content of the produced gas ranged from 64 to 68%, 58 to 61% and 53 to 58% respectively. Table 1 further shows the results of samples of *T. occidentals* fruit rind and poultry manure based on analyses of physical and chemical parameters prior to and after digestion and that of the inoculum used. At the end of the digestion of the three samples, further solubilization of structural components of the biomass was recorded. In experiment 'A', there were 36, 50.14, 31, 23 and 95.44% decrease in the values of cellulose, hemicelluloses, klason lignin, uronic acids and phenol while the soluble sugar content increased by 40% at the end of the anaerobic digestion. For experiment 'B', the record shows 31, 33.03, 19, 32 and 95% decrease in the values of cellulose, hemicelluloses, klason lignin, uronic acids and phenol while the increase in soluble sugar content was by 32.06%. Similarly, for experiment 'C', there was 20, 22.29, 25, 59 and 99% decrease in the values of cellulose, hemicelluloses, klason lignin, uronic acids and phenol while soluble sugar content increased by 46% after the digestion. As shown in Figure 1, pH values in all experiments remained at the slightly alkaline range throughout digestion thus aligning with the values considered for experimental design (6.5 to 8). Similarly, temperature in all experiments remained at the mesophilic range (30 to 40° C). In the chemical analyses results after the termination of experiments, several parameters such as ash content, moisture content, total nitrogen, total phosphorus, potassium, sulphate, phosphate, magnesium, manganese, iron, zinc, aluminium and copper recorded increased values while others had reduction in values for the three experiments. In terms of bulkiness, the table further revealed that the rumen content alone was bulkier than the mixtures of *T. occidentalis* fruit rind and inoculums with respect to total and volatile solids. Also, the results showed reduction in the average values for COD in all experiments i.e. 67.29, 62.21 and 59.72 % reduction for experiment 'O', 'P' and 'Q' respectively. The raw *T. occidentalis* fruit rind recorded low C/N ratio with value of 10/1 whereas samples 'A', 'B' and 'C' had values of 17/1 and 16/1 and 18/1 respectively.

3.3. Volatile Fatty Acids (VFAs) dynamics and mass balance

Depending on the production and consumption rates, VFAs can accumulate in an anaerobic system where they serve as inhibitors. In this study, the raw sample of *T. occidentalis* fruit rind and poultry manure recorded low concentrations (0.06 g COD/g VS) for both acetate and propionate. After the thermo-alkaline pretreatment, VFAS concentration of both treated samples increased. For acetate, concentrations of 0.11 g COD/g VS and 0.10 g COD/g VS were recorded for both experiments 'A' and 'B' while for propionate, values were 0.13 g COD/g VS and 0.11 g COD/g VS. As the digestion progressed, accumulation of VFAs was also progressive till their highest concentrations were recorded between 14th and 16th experimental days and this is indicative of imbalance between the first two stage of anaerobic digestion i.e. hydrolysis and acidogenesis and the last two stages i.e. acetogenesis and methanogenesis. Similarly, accumulation of TVFAs reached its peak between the 14th and 15th experimental days in both experiments 'A' and 'B' and the 13th day in experiment 'C'. For concentration of Ammonia (NH_3), the peak was reached between the 13th and 16th days of digestion in the three experiments.

3.4. Optimization of pretreatment and biogas generation

According to the experimental design used for the thermo-alkaline pre-treatment procedure in this study, the optimal condition for the treatment was: temperature of 80 ^{o} C, thermal treatment duration of 60 min, alkali concentration of 3g/100 g TS and alkaline treatment for 24 hr. Among all the tested experimental runs, the above stated condition gave the highest biogas yield of 1659.9010^{-3}m^{3}/kg VS in the mono-digestion of *Telfairia occidentalis* fruit rind as shown in table 2. Production of biogas in the three experiments commenced from between the 2nd and 4th, 5th and 7th and 7th and 9th days in digestions 'O', 'P' and 'Q'. Steady production continued till between the 17th and 26th day before gradual decline till the end of the experiments as shown in Figure 2. Table 4 (Supplementary materials) shows the biogas generation design matrix for both RSM and ANNs with five independent variables using actual values. As shown in the table, the most desired actual/experimental biogas yield for digestion 'A' was 2539.2 10^{-3}m^{3}/kg VS which was higher than the 2239.2 10^{-3}m^{3}/kg VS and 0995.5 10^{-3}m^{3}/kg VS values obtained for digestions 'P' and 'Q' respectively. The optimal value of each independent factor selected for the biogas generation was obtained by solving the regression equation with the aid of the Design-Expert software. The optimal value of each variable employed in this process was statistically predicted as temperature (T_1) = 30.02^{o} C, pH (T_2) = 7.90, retention time (T_3) = 20.03 days, total solids (T_4) = 5.94 g/kg and volatile solids (T_5) = 4.01 g/kg. Using these values, the biogas yield was predicted to be 2614.1, 2289.9 and 1003.3 10^{-3}m^{3}/kg VS for digestions 'O', 'P' and 'Q' respectively as shown in Table 3. For verification of the predictive abilities of the RSM and ANNs model, the optimal values were applied to three independent replicates for each of experiment 'O', 'P' and 'Q', and the average biogas yield was 2612.58, 2245.71 and 0989.7 10^{-3}m^{3}/kg VS, all of which are within the range of the predicted values. The composition of the produced biogas as shown by chromatography was within the range of 66.5 ± 2.5% Methane, 25 ± 1% Carbon dioxide; 58.5 ± 2.5% Methane, 26 ± 1% Carbon dioxide; 54.5 ± 1.5% Methane, 28 ± 2% Carbon dioxide for experiments 'O', 'P' and 'Q' respectively.

3.5. Microbial composition

Aerobic bacteria implicated at the early period of anaerobic digestion in all three experiments include *Bacillus pantothenticus, Bacillus licheniformis, Bacillus stearothermophilus, Serratia ficaria, Serratia plymuthica* and *Proteus vulgaris.* Fungal isolates include *Aspergillus niger, Mucor*, *Rhizopus stolonifer* and *Penicillum.* Facultative anaerobes include *Fusobacterium mortiferum*, *Bacteroides fragilis, Clostridium clostridioforme, Clostridium histolytica, Clostridium spp, Clostridium barattii* and *Porphyromonas assacharolytica* while methanogens of the genera *Methanosarcinales, Methanosaeta* and *Methanomicrobiales* were implicated. The highest count for aerobic bacteria was 2.6 x 10^{11}cfu/mL recorded in the first week that of fungi was 1.5 x 10^{8}cfu/mL also recorded in the first week. For the anaerobes, the highest count of 1.9 x 10^{11}cfu/mL was recorded in the fourth week while that of methanogens was 2.1 x 10^{12}cfu/mL obtained in the sixth week of digestion.

3.6. Stoichiometry and mass balance

The mass balances of all the digested samples of *T. occidentalis* fruit rind and poultry manure in terms of volatile VS degradation are shown in table 4. In computing the mass balance, ''*T. occidentalis* fruit rind'' was considered to be the input variable while the ''methane'', ''carbon dioxide'' contents of the gas and ''the anaerobic digestate'' were the output variables. In all three digestions, mass balances of 39, 31 and 12 were recorded. Also, experiments 'O' and 'P' had 69.23 and 61.29% higher mass balance than experiment 'Q'. In terms of VS degradation, the three experiments recorded VS reduction of 51, 41 and 21% respectively. Also, there were 59 and 49 higher VS removal in experiments 'O' and 'P' respectively over 'Q'.

3.7. RSM optimization of biogas data

Table S1 (Supplementary materials) shows all the five factors and their levels for response surface for biogas generation. Similarly, Table S2 (Supplementary materials) show the experimental design matrix by the CCD for the five-level-five-factor response surface study for biogas generation. The table reveals the experimentally observed and predicted yields as well as the residual values while the coefficients of the full regression model equation and their statistical significance were also determined. Table 5 shows the results of test of significance and that of the second-order response surface model's fit as ANOVA for every regression coefficient. Considering the F-values and their corresponding low p-values, a good number of the model terms are significant with $p< 0.05$. In experiments 'O', 'P' and 'Q', the Model F-values of 4.03, 4.06 and 4.08 all shows significance of the model. Similarly, the 'Adequate Precision' values of 8.009, 9.017 and 10.006 for experiments 'O', 'P' and 'Q' suggests that the model is suitable for the design.

The goodness of fit of the model was checked by the coefficient of determination (R^2) and the "Lack of Fit" F-values of 3.36, 3.52 and 3.44 obtained in the three experiment respectively implied that the "Lack of Fit" are not significant. This further substantiated the accuracy of the model since non-significant "Lack of fit" values are appropriate for experimental prediction. The relationship/interaction between the biogas yield (Y) and the coded values of the five variable i.e. temperature (T_1), pH (T_2), retention time (T_3), total solids (T_4) and volatile solids (T_5) was described by a regression model equation 3 below:

$$Y = 1770.17 + 13.16T_1 - 2.51T_2 - 13.62T_3 + 50.41T_4 + 3.64T_5 + 15.19T_1T_2 + 71.23T_1T_3 + 52.31T_1T_4 + 14.24T_1T_5 - 9.47T_2T_3 - 26.60T_2T_4 - 25.73T_2T_5 + 0.23T_3T_4 + 17.33T_3T_5 - 1.79T_4T_5 + 21.42T_1^2 + 16.89T_2^2 - 20.48T_3^2 - 55.72T_4^2 + 7.04T_5^2 \quad (3)$$

Where Y = Biogas yield (m^3/kg VS)

When the above equation was represented in figure forms, the three-dimensional (3D) plots formed are shown in Figure 3(a-j). Figure 4 shows the importance level of each independent variable as shown by the ANNs' architecture (Experiment 'O').

4. Discussion

The use of mechanical grinding, thermal and alkaline (NaOH and KOH) for pre-treatment brought about enormous solubilisation of all tested structural components of the biomass (*T. occidentalis* fruit rind). Similarly, the optimized conditions obtained via the CCD contributed to the breakdown of these structural materials and the subsequent high biogas yield obtained especially in the thermo-alkaline treated samples. Cellulose and hemicellulose breakdown as a result of thermo-alkaline pre-treatment (steam explosion, dilute acids, ammonia fibre expansion, ionic liquids, thermal, thermo-alkaline, alkaline thermo-mechanical and the fenton process) applications is well reported in literature and such treatments usually led to higher biogas yield (Dahunsi et al., 2016a, b; Mahdy, Ballesteros & Gonzalez-Fernandez, 2016; Mustafa, Poulsen & Sheng, 2016; Tufaner & Avsar, 2016). Similarly, lignin solubilization as a result of pre-treatment application to different biomasses has been reported. Notable is the work of Naran, Toor & Kim (2016) where high lignin breakdown was reported when NaOH alkaline-thermal treatment was applied. Similar results have been obtained from other studies (Sambusiti et al., 2013; Monlau et al., 2015; Dahunsi et al., 2017a, b, c). Another major observation caused by the application of thermo-alkaline pre-treatments in this study was the higher soluble sugar yield in the pre-treated experiments and this compares favourably with the results of Monlau et al. (2015). These sugars

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are beneficial to acidogenic and hydrolytic bacteria who utilize them during substrate degradation and this usually boost microbial population, activities as well as diversity. When these happen, intermediate acids are produced serving as raw materials for the subsequent acetogenesis and methanogenesis stages of digestion. Production of phenols was another evidence of structural breakdown in this study and this is further evident in the concentrations of these compounds recorded in experiments 'A' and 'B'. Such trend has been reported with the application of alkaline pre-treatment (Monlau et al., 2015; Dahunsi et al., 2017a, c). The 17 and 16 C/N ratios obtained for experiments 'A' and 'B' after pre-treatment further confirms the potency of the method for treating the biomass and this agree with the 17 C/N obtained by Degueurce et al. (2016) from the digestion of spent cow beddings.

The pH range recorded in this study is in tandem with previous studies which reported values between 6.5 and 8 as the most suitable for efficient methanogenesis (Dahunsi & Oranusi, 2013; Dahunsi et al., 2016a, b; Zahedi et al., 2016). Similarly, temperature has been reported to be an important factor in anaerobiosis especially for the anaerobic bacteria to function efficiently (Jain et al., 2015; Mao et al., 2015). All the three samples of *T. occidentalis* fruit rind used in this study were shown to be rich in nutrients and mineral elements required for microbial growth and functioning in a fermentation process as shown by the analysis in Table 2. The nutrient status of the three anaerobic digestates were found to be higher after the various digestion compared to the levels prior to digestion with digestion 'O' being the highest followed by 'P' and 'Q' was the least. The increased nutrient content of the three anaerobic digestates suggests the usefulness of such preparation as efficient fertilizers in order to increase fertility of soils as well as enhancing yield of crop. In most cropping systems in the tropics, there is over-dependence of the use of chemical inorganic fertilizers which has brought untold environmental hardships such as depletion/loss of soil nutrients, pollution of soil water, toxicity to soil microorganisms, eutrophication and many others. One of the ways to overcome these challenges is the use of organic manure/fertilizer such as produced in this study. The possibility of using nutrient-rich anaerobic digestates as biofertilizers or soil conditioners has been demonstrated in few studies while many others are ongoing (Alfa et al., 2013a, b; Pivato et al., 2015; Sun et al., 2015). In this study, the COD removal was higher than was reported in previous anaerobic digestion studies (Alfa et al., 2014b; Dahunsi et al., 2016a, b). The *Clostridium* species which dominated the microflora in the three digestions are well reported in anaerobic digestions processes. They are known to converts acids to acetone and other intermediate products which will usually serve as raw materials for the methanogenesis stage of digestion. Similarly, the diversity and high population of these organism was instrumental to the high biomass degradation and subsequent biogas generation obtained in this study especially in the thermo-alkaline pretreated substrates. Members of the genera *Methanosarcinales, Methanosaeta* and *Methanomicrobiales* are also very important and well reported in anaerobic digestion systems ecause they are efficient in converting acetone and other products to methane in the metanogenesis stage. Abundance of microbial species and population has been reported to enhance enormous substrate degradation ultimately leading to higher biogas production (Dahunsi et al., 2017a, b, c). The quantity and quality (methane contents) of the biogas produced in this study is higher than those from other substrates previously utilized in anaerobic digestions (Dahunsi & Oranusi, 2013; Alfa et al., 2014b). The highest biogas yield obtained in experiment 'O' could be as a result of the combined use of mechanical, thermal and alkaline (NaOH) pretreatments and this proved more effective in the substrate degradation than experiment 'P' where KOH was used instead and this was also better than experiment 'Q' which was mechanically treatment only. Application of combination of pretreatments as earlier proposed therefore is a promising alternative to achieving biomass degradation and higher biogas generation (Dahunsi et al., 2016a, b; Mthews, Grunden & Pawlak, 2016; Dahunsi et al., 2017). This already reflected in the results of the stoichiometry and mass balance i this study which shows pronounced substrate interactionsand VS consumption which was highest in experiment 'A" followed by 'B' and then 'C'.

Considering the concentrations of VFAs reported in this study,there is an indication of pronounced synergy between the two last stages of digestion i.e. acetogenesis and methanogenesis which is caused by the high population and diversity of anaerobes especially the *Clostridium* species coupled with favorable pH and temperature (Riggio et al., 2017). These bacteria are efficient in amino acids degradation leading to the release of acids and ammonia as end-products of the acetogenesis stage (Degueurce et al., 2016). The concentration of ammonia reported in this study shows there was buffering of process leading to the maintenance of neutral pH and process stability.

The regression model used in this study was proved to be significant by the low p-value (0.0183, 0.0150 and 0.0190) of the model F-value with in experiments 'O', 'P' and 'Q') respectively. The goodness of fit of the model was checked by the coefficient of determination (R^2). Pei et al. (2014) have reported that R^2 value should be at least 0.80 for a model to be fit. In this study, the R^2 value of 0.8996, 0.9067 and 0.8993 showed that the sample variation of 89.96, 90.67 and 89.93% obtained for biogas yield in experiments 'O', 'P' and 'Q' is a function of the five independent variables (T_1, T_2, T_3, T_4, and T_5) employed in the modelling. The 'Adequate Precision' is a measure of the signal to noise ratio and a value greater than 4 is desirable for the good fitting of a model. In this study, values of 8.009, 9.017 and 10.006 were obtained in experiments 'O', 'P' and 'Q' which further validated the suitability of the model. The 'Lack-of-fit' values of 0.174, 0.169 and 0.176 obtained for the three experiments were not significant and this means that the model is very suitable in theoretical prediction of the biogas generation from the anaerobic co-digestion of *T. occidentalis* fruit rind and poultry manure since a non-significant lack of fit is desirable.

All the 3D plots for the expression of the model's regression equation revealed different curvatures' nature brought about by the variable interactions. Plots a, d, g and i of RSM showed low interactions between the concerned variables; plots b, e and h displayed moderate interactions while plots c, f and j all showed pronounced relationships between T_1, T_2, T_3, T_4, and T_5. However, all the ANNs plots showed pronounced interactions revealing that ANNs model accommodated more variable interactions than RSM and this phenomenon had earlier been documented (Betiku et al., 2015). In all, the ANNs model proved more accurate than RSM with respect to the roots mean squared error (RSME) and the coefficient of determination (R2) values in all experiments.

The combined heat and power (CHP) system was used to assess the energy balance as well as the economic feasibility of thermo-alkaline pre-treatment application to *T. occidentalis* fruit rind. In doing this, a 50% thermal efficiency and 35% electrical efficiency was adopted as shown in table 6. In using this system, the possibility that the profit obtained from the sale of the extra thermal and electrical energies will be sufficient to replenish the cost of procuring heat for thermal pre-treatment and chemicals (NaOH and KOH) used for the alkaline pre-treatment. In determining the TER for thermo-alkaline pre-treatment of *T. occidentalis* fruit rind therefore, the energy needed to raise the temperature of 35 g TS L^{-1} *T. occidentalis* fruit rind mixture from 25 to 55 ^{o}C was determined using 4.18 kJ kg^{-1} $^{o}C^{-1}$ as the specific heat of water in order to evaluate the specific heat of the mixture while heat loss was neglected (Zupancic & Ros, 2003). The result show that for experiment 'O', the 1147 kWh t^{-1} TS thermal energy gain at a solid loading of 35 g TS L^{-1} was higher than the TER for the thermo-alkaline pre-treatment which was 1088 kWh t^{-1} TS when heat and NaOH were used. For experiment 'P' the thermal energy gain of 1049 kWh t^{-1} TS was lower than the TER of 1109 kWh t^{-1} TS needed for pre-treatment using heat and KOH. Earlier researches have proposed the use of heat exchanger during digester heating and/or biomass pre-treatment as a way of boosting up to 80% heat recovery (Dhar, Nakhla & Ray, 2012; Zabranska et al., 2006).

For the electrical energy assessment, only the electric energy used for the substrate mixing was considered while the energy used during mechanical grinding was neglected since this was also done for experiment 'Q' which had no thermo-alkaline pre-treatment (Menardo, Airoldi & Balsari, 2012). The result show that the estimated net electrical energies at a solid loading of 35 g TS $L^{-1}$was 430 kWh t^{-1} TS and 223 kWh t^{-1} TS for experiments 'O' and 'P' respectively. The possibility of injecting these energies into the energy grid or being sold for a fixed cost is high as this will generate extra income and also compensate for the resources used for the pre-treatment. In accounting for the economic value of the used alkalis, the 335 and 100 dollars ton $^{-1}$ US cost of NaOH and KOH were used.

Table 7 shows heat balance of different biomasses anaerobically digested with prior thermal and thermo-alkaline pre-treatments. In all, substrate degradation and higher biogas generation were achieved due to the pre-treatments. Most of these researchers studied ways of minimizing the TER for carrying out pre-treatments and reported that the rate of solid loading and heat recovery from pre-treatment are the major factors responsible for a high TER why some of the studies emphasised the use of low solid loadings (Fdz-Polanco et al., 2008; Monlau et al., 2013; Passos, Garcia & Ferrer, 2013), others supported high solid loading of 15% solids w/w or above when thermo-alkaline pre-treatments are employed (Schell et al., 2003; Modenbach & Nokes, 2012).

Conclusion

As shown in this study, the richness of the co-substrates (*T. occidentalis* fruit rind and poultry manure) in terms of minerals and elemental composition showed them as suitable materials for biogas and biofertilizer generation. Result of optimization and modeling study showed that both RSM and ANNs models are suitable and very efficient in predicting gas production from *T. occidentalis* fruit rind and poultry manure. It was equally showed that the combination of mechanical and thermo-alkaline pretreatment produced higher biogas quantity and methane content as well as higher mass, energy and economic balances. *T. occidentalis* is a crop that is well adapted to several geographical locations especially in the tropics whereas poultry manure is generally available as an environmental scourge in most locations around the globe. Therefore, further usage of *T. occidentalis* fruit rind and poultry manure as energy feedstock is proposed.

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Conflict of interest

Authors declare no conflict of interest.

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Figure Captions

Figure 1: The figure shows the graph of pH fluctuations during the anaerobic digestion of *Telfairia occidentalis* fruit peels

Figure 2: The figure shows the graph of average daily biogas yield for the anaerobic digestion of *Telfairia occidentalis* fruit peels

Figure 3 (a-j): The figure shows the RSM and ANNs curvatures' nature of 3D surfaces plots for biogas generation from *Telfairia occidentalis* fruit peels

Figure 4: This figure show the importance level of all the five independent variables used in the optimization study

Table 1 (on next page)

Physical and chemical characteristics of *Telfairia occidentalis* fruit rind, poultry manure and cattle rumen content

Each value indicates the composition of the respective parameter in the tested samples

Table 1: Physical and chemical characteristics of *Telfairia occidentalis* fruit rind, poultry manure and cattle rumen content

Parameters	*T. occidentalis* fruit rind	Poultry droppings	Rumen content	Experiment A		Experiment B		Experiment C	
				Substrate	Digestate	Substrate	Digestate	Substrate	Digestate
Acetate (g COD/g VS)	0.06±0.12	1.16±0.01	0.92±0.12	0.11±1.10	0.005±0.01	0.10±0.01	0.006±0.10	0.06±0.12	0.007±0.10
Propionate (g COD/g VS)	0.06±0.10	1.17±0.10	0.94±0.10	0.13±0.03	0.003±0.02	0.11±0.01	0.007±0.11	0.06±0.10	0.009±0.01
TVFAs (g COD/g VS)	0.17±0.02	3.33±0.12	2.31±0.02	1.23±0.10	0.12±0.10	0.21±1.10	0.15±0.01	0.17±0.02	0.09±0.11
Ammonia (mg/g VS)	0.08±0.11	16.23±2.00	8.31±0.11	2.20±0.10	1.45±0.02	3.88±1.01	2.85±0.20	0.08±0.11	0.69±0.11
COD (g COD/g VS)	142.21±1.02	228.98±3.00	168.21±1.12	239.02±5.01	88.30±3.20	255±3.20	156.77±5.01	142.21±1.02	110.75±2.11
Cellulose (% VS)	30.77±1.10	4.11±1.10	12.30±0.10	13.60±0.11	08.72±1.10	17.57±3.10	12.12±1.02	30.77±1.10	24.65±1.22
Hemicelluloses (% VS)	13.32±0.10	1.51±1.11	7.71±1.10	07.10±1.01	03.54±0.50	09.02±2.10	06.04±1.10	13.32±0.10	10.35±1.10
Klason lignin (% VS)	28.04±2.10	7.08±1.05	17.17±1.12	18.00±1.05	12.46±0.11	20.02±2.01	16.23±0.01	28.04±2.10	21.16±1.02
Uronic acids (% VS)	2.51±1.10	0.51±1.10	1.67±1.11	1.22±1.10	0.94±0.02	1.60±1.10	1.09±0.11	2.51±1.10	1.04±0.10
[&]Soluble sugars (% VS)	2.11±1.02	2.65±1.05	4.02±2.10	6.60±0.01	10.92±0.11	6.06±0.10	8.92±0.10	2.11±1.02	3.88±0.10
Phenols (mg L^{-1})	0.08±0.01	1.00±0.01	4.71±2.10	0.41±1.10	09.01±1.01	0.58±1.00	11.16±1.10	0.08±0.01	07.16±0.10
pH	5.98±0.12	6.90±0.22	7.91±0.02	7.55±0.20	7.75±0.31	7.55±1.02	7.69±0.11	5.98±0.12	7.75±0.31
Total Solids (g/kg)	71.91±1.02	281.24±1.02	91.52±0.11	128.01±0.02	81.40±3.21	133.11±6.02	127.62±0.10	141.91±1.02	128.11±0.10
Volatile Solids (g/kg)	62.71±1.02	229.71±1.13	84.44±2.12	99.63±2.21	47.74±3.21	118.47±3.22	72.46±0.02	122.71±1.02	92.70±0.03
Ash Content (%)	4.00±2.01	18.29±2.11	5.56±0.13	6.36±0.01	4.26±0.10	4.01±1.02	4.09±1.10	4.00±2.01	3.98±0.10
Moisture Content (%)	95.52±0.11	71.76±2.80	90.48±2.12	91.89±3.02	94.19±0.01	88.41±4.02	91.44±0.02	75.52±0.11	83.31±0.11
Total Carbon (g/kg TS)	243.20±3.02	292.10±3.10	265.21±4.10	678.60±2.01	449.00±3.01	612.01±1.02	398.00±.10	443.20±3.02	313.20±1.00
Total Nitrogen (g/kg TS)	25.12±0.21	61.00±1.12	48.00±1.12	48.01±2.11	45.60±5.10	37.61±2.21	39.25±3.21	25.12±0.21	35.21±2.02
C/N Ratio	10/1	5/1	6/1	17/1	10/1	16/1	10/1	18/1	10/1
Total Phosphorus (g/kg TS)	3.21±1.02	7.90±0.12	6.30±0.13	4.56±0.20	6.18±1.01	4.01±1.30	5.84±1.01	3.21±1.02	4.63±1.01
Potassium (g/kg TS)	5.61±0.22	9.00±0.00	7.20±0.12	6.12±0.12	8.0±1.01	5.87±2.01	7.7±1.01	5.61±0.22	6.30±1.01
Phosphate (g/kg TS)	1.81±0.10	3.80±0.10	3.00±0.12	2.30±0.01	3.10±0.01	2.11±1.02	2.70±0.01	1.81±0.10	2.40±0.01
Sulphate (g/kg TS)	101.11±1.02	164.00±3.02	134.00±5.09	118.00±3.12	132.00±4.50	104.31±3.02	112.23±2.20	101.11±1.02	101.10±2.00
Calcium (g/kg TS)	257.09±4.02	44.00±0.02	80.00±1.22	160.00±2.11	96.00±3.10	266.46±5.02	84.00±1.10	257.09±4.02	80.00±2.11
Magnesium (g/kg TS)	52.21±2.02	150.00±2.10	96.00±2.12	70.00±1.22	100.0±0.21	52.41±2.04	91.0±0.20	52.21±2.02	82.0±0.21
Manganese (g/kg TS)	0.016±0.01	0.040±0.01	0.028±0.01	0.020±0.01	0.030±0.01	0.019±1.00	0.026±0.01	0.016±0.01	0.024±0.01
Iron (g/kg TS)	0.62±1.23	1.46±0.02	1.18±0.11	0.92±0.01	1.16±0.01	0.51±0.22	1.02±0.01	0.62±1.23	0.62±0.01
Zinc (g/kg TS)	24.02±1.03	51.00±2.02	38.00±0.14	29.00±1.20	38.00±3.00	25.41±1.12	29.00±2.00	24.02±1.03	24.03±1.01
Aluminium (g/kg TS)	0.45±2.00	0.62±0.30	0.80±0.02	0.58±0.01	0.74±0.11	0.61±1.02	0.66±0.10	0.45±2.00	0.63±0.10
Copper (g/kg TS)	2.81±0.11	5.80±0.72	4.80±0.05	3.80±0.02	4.70±0.41	3.17±0.02	4.22±0.21	2.81±0.11	4.16±0.11

N = 120; *COD = Chemical Oxygen Demand; C/N = Carbon/Nitrogen ratio; TVFAs = Total Volatile Fatty Acids; The solid portion was dried at 60 ^{o} C for 24 h after thermo-alkaline pretreatment;* [&]*= sum of initial soluble sugars and the solubilization of cellulose and hemicelluloses.*

Table 2 (on next page)

Experimental design of *Telfairia occidentalis* fruit rind's pretreatment prior to digestion

Each value indicates the range of values considered during the optimization of the biomass pretreatment prior to anaerobic digestion

Table 2: Experimental design of *Telfairia occidentalis* fruit rind's pretreatment prior to digestion

Sample	**Pretreatment temperature (^{O} C)**	**Pretreatment time (Min)**	**Quantity of alkali for pretreatment (g/100 g TS)**	**Time/duration for pretreatment (h)**	**Biogas Produced from mono-digestion of *Telfairia occidentalis* fruit rind ($10^{-3}m^3$/kg VS) (Dahunsi et al. 2016b)**	**Biogas Produced from co-digestion of *Telfairia occidentalis* fruit rind and poultry manure ($10^{-3}m^3$/kg VS)**
UTO	0	0	0	0	1003.30	2134.06
$TO_{70,70}$	70	70	2	24	1166.22	2237.31
$TO_{80,60}$	**80**	**60**	**3**	**24**	**1659.90**	**2614.14**
$TO_{90,60}$	90	70	3	28	1622.17	2600.20
$TO_{100,60}$	100	60	5	32	1592.12	2543.12
$TO_{110,60}$	110	70	3.5	30	1561.13	2403.31
$TO_{120,60}$	120	60	2.5	26	1432.36	2231.11
$TO_{130,50}$	130	50	4	24	1575.23	2163.05
$TO_{140,70}$	140	70	4.5	24	1483.26	2231.91
$TO_{150,50}$	150	50	5	28	1323.24	2521.51
$TO_{160,70}$	160	70	4	34	1149.24	2145.55
$TO1_{70,50}$	170	50	3	36	1509.21	2311.11
$TO_{180,50}$	180	50	3.5	28	1199.21	2401.11
$TO_{190,60}$	190	60	2.5	36	1581.70	2090.00
$TO_{200,50}$	200	50	3	30	1600.03	2311.04

Note: TO = Telfairia occidentalis; UTO = Untreated Telfairia occidentalis

Table 3 (on next page)

Experimental Design for Biogas generation from the co-digestion of *Telfairi aoccidentalis* fruit rind and poultry manure with five independent variables for RSM and ANNs using actual values

Each value shows the range of values chosen for the experimental design of the study

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Table 3: Experimental Design for Biogas generation from the co-digestion of *Telfairi aoccidentalis* fruit rind and poultry manure with five independent variables for RSM and ANNs using actual values

	Independent Factors					Digestion O			Digestion P			Digestion Q		
Run	T_1	T_2	T_3	T_4	T_5	**Actual biogas yield (10^{-3} m^3/kg VS)**	**RSM Predicted biogas yield (10^{-3} m^3/kg VS)**	**ANNs Predicted biogas yield (10^{-3} m^3/kg VS)**	**Actual biogas yield (10^{-3} m^3/kg VS)**	**RSM Predicted biogas yield (10^{-3} m^3/kg VS)**	**ANNs Predicted biogas yield (10^{-3} m^3/kg VS)**	**Actual biogas yield (10^{-3} m^3/kg VS)**	**RSM Predicted biogas yield (10^{-3} m^3/kg VS)**	**ANNs Predicted biogas yield (10^{-3} m^3/kg VS)**
1	30.02	7.90	20.03	5.94	4.01	2539.2	2614.1	2540.3	2239.2	2289.9	2249.5	0995.5	1003.3	0997.4
2	39.98	7.90	29.88	11.45	11.83	2480.9	2462.5	2484.3	2260.9	2290.9	2221.2	0990.6	1008.3	0921.5
3	30.43	7.99	20.05	6.64	4.11	2365.1	2408.1	2368.5	2265.1	2201.6	2203.4	0988.7	1001.6	0979.8
4	39.85	6.59	25.46	11.79	11.60	2473.3	2540.8	2459.6	2203.9	2220.8	2203.6	1000.5	1007.5	0977.2
5	39.98	6.53	29.57	11.98	7.08	2600.1	2612.1	2597.0	2200.1	2211.6	2200.5	0950.6	0978.3	0967.2
6	39.52	6.52	25.39	10.86	11.51	2523.1	2606.2	2523.5	2280.1	2211.3	2285.6	0986.5	1001.0	0985.4
7	40.00	7.72	29.99	11.03	10.89	2484.2	2486.2	2484.4	2241.2	2200.2	2240.3	0964.6	0979.9	0980.5
8	39.93	7.08	29.23	11.89	9.23	2435.9	2481.8	2435.9	2225.9	2201.9	2226.3	0945.3	0979.5	0952.6
9	39.68	6.68	29.68	9.99	11.24	2563.3	2572.9	2560.2	2263.3	2283.9	2263.1	0943.6	1007.4	0952.4
10	39.56	7.41	29.89	11.42	11.77	2851.1	2872.6	2836.2	2251.1	2201.7	2251.2	0958.8	1002.4	0929.5
11	39.77	6.74	29.92	8.40	11.45	2907.1	3065.6	2588.3	2207.1	2252.9	2207.1	0937.4	0920.3	0904.5
12	30.22	7.92	20.09	7.46	4.05	2681.0	2664.9	2588.2	2221.0	2252.5	2219.5	1002.4	1002.2	0978.2
13	39.17	6.68	26.24	10.69	11.97	2591.6	2608.6	2591.5	2291.6	2206.4	2290.2	1001.2	0997.2	0951.5
14	39.96	6.63	25.40	11.30	11.62	2551.1	2557.3	2553.7	2209.1	2216.6	2266.5	1002.1	0959.5	0937.8
15	39.97	6.99	29.35	11.91	9.24	2501.2	2556.3	2503.3	2221.2	2208.1	2221.6	0941.1	0967.5	0950.1
16	39.96	6.55	27.00	11.29	10.30	2511.9	2555.9	2509.9	2204.9	2226.5	2266.5	0984.5	1001.7	0976.1
17	39.21	6.74	27.19	11.70	11.23	1002.5	1054.9	1002.5	2228.0	2209.2	2266.5	0938.3	1001.7	0949.4
18	39.97	7.74	29.72	10.86	11.42	2732.0	2749.8	2731.6	2232.0	2201.6	2266.5	0996.3	1004.7	0952.2
19	40.00	7.70	29.65	11.89	11.58	2727.3	2749.4	2734.6	2277.3	2201.4	2277.1	0977.6	1000.9	0957.3
20	39.99	7.19	29.94	11.53	9.40	2700.9	2743.7	2700.4	2203.9	2204.7	2201.5	0990.4	0964.1	0971.2
21	39.95	7.42	29.84	10.21	10.96	2700.1	2733.3	2705.6	2291.1	2202.9	2285.4	0931.5	0982.5	0919.9
22	40.00	7.75	30.00	10.57	9.57	2597.2	2610.9	2600.5	2297.2	2202.9	2294.9	0907.9	0992.8	0940.7
23	40.00	8.00	28.83	10.84	4.00	2556.1	2504.6	2555.7	2256.1	2287.9	2255.8	0955.6	0998.3	0990.1
24	40.00	8.00	29.55	10.73	4.00	2642.1	2701.3	2643.5	2242.1	2287.6	2242.1	0942.8	0971.3	0984.7
25	30.00	8.00	20.00	7.95	5.56	2398.1	2377.9	2397.5	2288.1	2207.8	2289.3	0968.1	0983.1	0959.7
26	40.00	8.00	29.82	11.05	4.01	2350.1	2476.6	2588.2	2250.0	2287.4	2250.0	0901.7	0977.6	0951.4
27	40.00	8.00	29.53	11.26	5.38	2569.0	2673.6	2567.5	2269.0	2385.5	2281.5	0966.7	1005.1	0950.1
28	40.00	8.00	29.18	9.85	5.07	2410.0	2473.3	2404.4	2210.0	2383.6	2210.3	0950.6	1000.4	0978.7
29	30.00	7.53	20.00	6.58	4.00	2400.0	2457.9	2588.2	2250.0	2383.1	2250.7	0940.8	1003.1	0951.6
30	40.00	8.00	26.91	10.30	4.45	3456.0	3429.5	3456.3	2276.0	2382.9	2276.3	0979.3	1002.6	0955.8
31	38.00	7.82	28.99	10.03	10.19	2681.02	2540.8	2836.1	2201.1	2332.2	2221.3	0948.1	1003.1	0959.3
32	37.93	7.08	29.23	11.89	9.03	2691.62	2612.1	2588.3	2307.1	2316.3	2214.3	0904.6	1006.1	0950.7
33	38.68	6.58	28.68	9.29	10.24	2551.14	2606.2	2588.2	2351.0	2398.9	2243.2	0941.9	1001.2	0963.2
34	38.56	7.41	29.89	10.42	10.17	2601.25	2486.7	2591.5	2292.6	2301.2	2289.4	0977.3	1001.3	0975.8
35	37.77	6.74	29.92	8.40	11.45	2531.97	2581.8	2553.8	2310.1	2322.3	2203.8	0941.5	1002.1	0956.6
36	36.22	7.62	20.09	7.46	4.05	1902.58	2572.9	2503.3	2211.2	2323.9	2221.1	0901.3	0983.3	1001.6
37	39.17	6.58	26.24	10.69	10.97	2742.63	2872.6	2509.9	2234.9	2343.2	2240.0	0984.5	1003.6	1004.8

38	38.96	6.63	25.40	11.30	10.62	1037.32	1265.6	1002.5	2223.0	2300.1	2254.6	0913.7	1000.4	0964.4
39	38.97	6.69	29.65	10.91	9.24	2700.91	2964.9	2731.5	2262.0	2301.3	2269.7	0989.9	1001.2	0959.7
40	37.96	6.55	27.00	10.29	10.30	2710.14	2618.6	2735.6	2207.3	2312.9	2209.0	0904.6	1002.4	0969.3
41	39.21	6.75	27.19	11.70	10.23	2457.25	2657.3	2730.4	2211.9	2343.3	2224.8	0981.9	1002.3	0929.4
42	39.97	7.74	29.42	10.86	11.42	2456.13	2506.3	2705.6	2231.1	2376.3	2242.2	0981.7	1006.4	0950.7
43	40.00	7.71	29.45	11.89	10.58	2652.12	2585.9	2600.6	2297.2	2302.3	2299.9	1007.8	1002.3	0947.1
44	39.99	7.19	29.94	11.53	9.40	2693.31	2554.9	2535.8	2259.1	2311.2	2263.2	1005.8	1006.8	0926.2
45	38.95	7.45	29.64	10.21	10.96	2450.58	2749.8	2643.5	2242.1	2393.2	2256.6	0949.1	1003.8	0956.6
46	40.00	7.55	30.00	10.57	8.57	2569.34	2749.4	2497.5	2288.1	2301.2	2296.6	0999.6	1005.6	1006.2
47	38.00	8.00	29.08	9.85	6.07	2410.33	2743.6	2588.2	2250.0	2362.2	2258.8	0987.0	1002.5	1008.7
48	30.00	7.53	20.00	6.58	4.00	2400.62	2733.3	2567.4	2229.0	2325.3	2231.1	0929.7	1005.6	1001.6
49	37.00	8.00	26.91	10.30	5.45	3245.92	2620.9	2504.3	2220.0	2316.5	2219.6	0907.7	1001.6	1002.2
50	38.00	7.52	27.59	10.03	10.89	3215.42	2534.6	2553.8	2251.4	2361.6	2259.4	0958.5	1001.2	0968.2

T_1 = Temperature; T_2 = pH; T_3 = Retention time; T_4 = Total solids: T_5 = Volatile solids

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Table 4(on next page)

Stoichiometry and mass balance for one ton of *Telfairia occidentalis* fruit rind and poultry manure from the anaerobic digestions

Each value is the result of stoichiometry and mass balance between the substrates employed in the co-digestion study

Table 4: Stoichiometry and mass balance for one ton of *Telfairia occidentalis* fruit rind and poultry manure from the anaerobic digestions

Parameter	Experiment O	Experiment P	Experiment Q
Input			
T. occidentalis fruit rind + Rumen content + Poultry manure(kg)	1000	1000	1000
Volatile solids (VS) (kg)	768	825	923
Output			
Methane (CH_4) (%)	66.5	58.5	54.5
Carbon dioxide (CO_2) (%)	25	26	28
Digestate (kg VS)	377	485	727
Cumulative output	468.5	569.5	809.5
*Mass balance	39	31	12
$% Volatile solids (VS) removal	51	41	21

*= *(Input–output)/input (%)* $ = *(Input-Digestate)/Input (%)*

Table 5 (on next page)

Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for biogas generation from *Telfairia occidentalis* fruit rind and poultry manure

Each value indicates the corresponding result of the analysis of variance carried out on the data generated during the anaerobic co-digestion processes

Table 5: Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for biogas generation from *Telfairia occidentalis* fruit rind and poultry manure

		Digestion O				Digestion P				Digestion Q			
Source	**df**	**SS**	**MS**	**F-value**	**P-value**	**SS**	**MS**	**F-value**	**P-value**	**SS**	**MS**	**F-value**	**P-value**
Model	20	3.65	183.68	4.03	**0.018**	3.84	158.4	4.11	**0.015**	3.91	187.15	4.08	**0.019**
T_1		4159	4159	0.92	0.363	5183	5.83	0.06	**0.038**	4946	4946	0.045	0.281
T_2		151.3	151.3	0.033	0.859	1.508	1.558	1.33	0.574	5408	5408	1.29	0.706
T_3		4452	4452	0.98	0.347	7.362	5.362	7.69	0.069	6.033	6.033	6.64	0.061
T_4		6099	6099	13.47	**0.005**	8215	8.151	0.78	0.516	8.371	8.371	0.91	0.396
T_5		317.5	317.5	0.070	0.797	6468	6768	0.65	0.447	7267	7267	0.71	0.034
T_1T_2		3691	3691	0.82	0.390	4.006	4506	6.02	0.236	5.405	5.405	4.09	0.037
T_1T_3		8118	8118	17.93	**0.002**	5.229	5.229	4.98	**0.016**	6181	6181	5.63	**0.015**
T_1T_4		4379	4379	9.67	**0.013**	7442	7.442	5.66	**0.115**	6.289	6.289	0.055	0.526
T_1T_5		3243	3243	0.72	0.419	3657	3657	3.07	0.173	4189	4189	0.42	0.716
T_2T_3		1435	1435	0.32	0.587	2968	2.068	1.24	0.766	3.594	3.594	0.40	0.573
T_2T_4		1132	1132	2.50	0.014	5.049	5.049	5.10	**0.025**	6.104	6.104	3.96	**0.041**
T_2T_5		1059	1059	2.34	0.160	5.498	5.498	7.78	**0.020**	4.966	4.966	6.02	**0.011**
T_3T_4		0.85	0.85	1.869	0.989	2.015	2.015	2.90	0.119	1.033	1.033	1.84	0.199
T_3T_5		4805	4805	1.06	**0.029**	1.589	1.589	5.87	0.063	1095	1095	10.01	**0.031**
T_4T_5		51.05	51.05	0.011	0.918	1.013	1.013	9.93	**0.015**	1.161	1.161	8.96	0.133
T_1^2		1224	1224	2.70	0.135	1651	1.651	3.13	0.555	1657	1657	0.19	0.500
T_2^2		7603	7603	1.68	**0.027**	5.733	5733	4.72	0.108	3.899	3.899	6.06	**0.044**
T_3^2		1118	1118	2.47	0.151	3158	3158	4.23	0.655	3.258	3.258	0.23	0.534
T_4^2		8281	8281	18.29	**0.002**	1156	1.156	1.63	0.625	1188	1188	0.012	0.813
T_5^2		1322	1322	0.29	0.602	82.93	8.293	9.05	0.660	80.93	80.93	7.028	0.581
Residual	9	407.9	453.00			413.9	460.00			404.2	460.03		
Lack of Fit	6	355.1	591.19	3.36	0.174	405.1	651.8	3.52	0.169	353.1	583.13	3.44	0.176
Pure Error	3	27.87	157.62			28.37	149.07			24.57	161.60		
R-Squared		0.8996				0.9067				0.8993			
Adequate Precision		8.009				9.017				8.006			

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

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Table 6(on next page)

Energy and economic evaluation for the anaerobic co-digestion of *Telfairia occidentalis* fruit rind and poultry manure

Each value shows the result of energy and economic evaluation for the anaerobic co-digestion of *Telfairia occidentalis* fruit rind and poultry manure

Table 6: Energy and economic evaluation for the anaerobic co-digestion of *Telfairia occidentalis* fruit rind and poultry manure

Energy parameters	**Experiment O**	**Experiment P**	**Experiment Q**
Produced electrical and thermal energy from combined heat and power (CHP)	1785	1699	1155
Produced thermal energy (kWh t^{-1} TS)	1645	1547	498
Produced electrical energy (kWh t^{-1} TS)	770	563	340
Thermal balance			
*Thermal energy gain (kWh t^{-1} TS)	1147	1049	-
Thermal energy requirement (kWh t^{-1} TS)	1088	1109	-
Thermal energy requirement with 80% of heat recovery (kWh t^{-1} TS)	218	210	-
#Net thermal energy (kWh t^{-1} TS)	59	-60	-
Net thermal energy with 80% of heat recovery (kWh t^{-1} TS)	-929	-839	-
Electrical balance			
$Electrical energy gain	430	223	-
Energy for mixing during pretreatment	-	-	-
Net electrical energy	430	223	
Economic evaluation			
Cost of NaOH (є t^{-1} TS)			

** = 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 electricity energies produced by pretreated experiment minus the untreated.*

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Table 7 (on next page)

Energy balances of thermal and thermo-chemical pretreatment procedures as applied to different substrates

Each value indicates the result of e**nergy balances of thermal and thermo-chemical pretreatment procedures as applied to different substrates**

1 **Table 7: Energy balances of thermal and thermo-chemical pretreatment procedures as applied to different substrates**

Substrate	Condition of pretreatment	Increase in Methane yield (m3 t^{-1} TS)/ operation mode	Biogas Conversion	Surplus thermal energy (kWh t^{-1} TS)	Thermal pretreatment requirements (kWh t^{-1} TS)	Net Heat Energy (kWh t^{-1} TS)	References
Telfairia occidentalis fruit rind	Thermo-alkaline (55 ^{o} C; 4% NaOH (w/w); 24 h) Solid load: 35 g TS L^{-1}	40/Batch mode	CHP: 35% electricity; 50% heat	1147	1088	59	Current study
	Thermo-alkaline (55 ^{o} C; 4% KOH (w/w); 24 h) Solid load: 35 g TS L^{-1}	35/Batch mode	CHP: 35% electricity; 50% heat	1049	1109	-60	Current study
Tithonia diversifolia shoot	Thermo-alkaline (55 ^{o} C; 4% NaOH (w/w); 24 h) Solid load: 35 g TS L^{-1}	53/Batch mode	CHP: 35% electricity; 50% heat	1176	1068	108	Dahunsi et al. 2017c
	Thermo-alkaline (55 ^{o} C; 4% KOH (w/w); 24 h) Solid load: 35 g TS L^{-1}	30/Batch mode	CHP: 35% electricity; 50% heat	862	1150	-288	Dahunsi et al. 2017c
Peanut hull	Thermo-alkaline (55 ^{o} C; 4% NaOH (w/w); 24 h) Solid load: 35 g TS L^{-1}	70/Batch mode	CHP: 35% electricity; 50% heat	761	1173	-412	Dahunsi et al. 2017b
Sunflower stalks	Thermo-alkaline (55 ^{o} C; 4% NaOH (w/w); 24 h) Solid	36/Continuous mode	CHP: 35% electricity;	185	1034	-849	Monlau et al. 2015

	load: 35 g TS L^{-1}		50% heat				
	Thermo-alkaline (55 ° C; 4% NaOH (w/w); 24 h) Solid load: 50 g TS L^{-1}	36/Continuous mode	CHP: 35% electricity; 50% heat	185	733	-548	Monlau et al. 2015
	hermo-alkaline (55 ° C; 4% NaOH (w/w TS); 24 h) Solid load: 200 g TS L^{-1}	36/Continuous mode	CHP: 35% electricity; 50% heat	185	210	-25	Monlau et al. 2015
	Thermo-alkaline (55 ° C; 4% NaOH (w/w); 24 h) Solid load: 50 g TS L^{-1} 80% of heat recovery from pretreatment	36/Continuous mode	CHP: 35% electricity; 50% heat	185	147	38	Monlau et al. 2015
Sunflower Oil Cake	Thermal (170 ° C; 1 h) Solid load: 50 g TS L^{-1}	32/Batch mode	CHP: 35% electricity; 50% heat	161	3535	-3375	Monlau et al. 2013
	Thermal (170 ° C; 1 h) Solid load: 200 g TS L^{-1}	32/Batch mode	CHP: 35% electricity; 50% heat	161	1010	-849	Monlau et al. 2013
	Thermal (170 ° C; 1 h) Solid load: 200 g TS L^{-1} 80% of heat recovery	32/Batch mode	CHP: 35% electricity;	161	152	9	Monlau et al. 2013

	from pretreatment		50% heat				
Ensiled Sorghum Forage	Thermo-alkaline (100 º C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L^{-1}	92/Batch mode	CHP: 40% electricity; 41% heat	378	547	-169	Sambusiti et al. 2013
	Thermo-alkaline (100 º C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L^{-1} 80% of heat recovery from Pretreatment	92/Batch mode	CHP: 40% electricity; 41% heat	378	109	269	Sambusiti et al. 2013
Wheat straw	Thermo-alkaline (100 º C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L^{-1}	137/Batch mode	CHP: 40% electricity; 41% heat	577	547	30	Sambusiti et al. 2013
	Thermo-alkaline (100 º C; 30 min, 10% NaOH w/w) Solid load: 160 g TS L^{-1} 80% of heat recovery from Pretreatment	137/Batch mode	CHP: 40% electricity; 41% heat	577	109	468	Sambusiti et al. 2013
Microalgae	Thermal (75 º C; 15 min) Solid load: 11.7 g TS L^{-1} 85% of heat recovery	32/Batch mode	100% heat conversion	316	458	-142	Passo et al. 2013

from Pretreatment						
Thermal (75 ^{o}C; 15 min) Solid load: 20 g TS L^{-1} 85% of heat recovery from Pretreatment	32/Batch mode	100% heat conversion	316	268	48	Passo et al. 2013
Thermal (75 ^{o}C; 15 min) Solid load: 30 g TS L^{-1} 85% of heat recovery from Pretreatment	32/Batch mode	100% heat conversion	316	173	143	Passo et al. 2013

Figure 1(on next page)

pH dynamic during the anaerobic digestion process

Each data point indicates the daily pH value obtained during the anaerobic digestion process

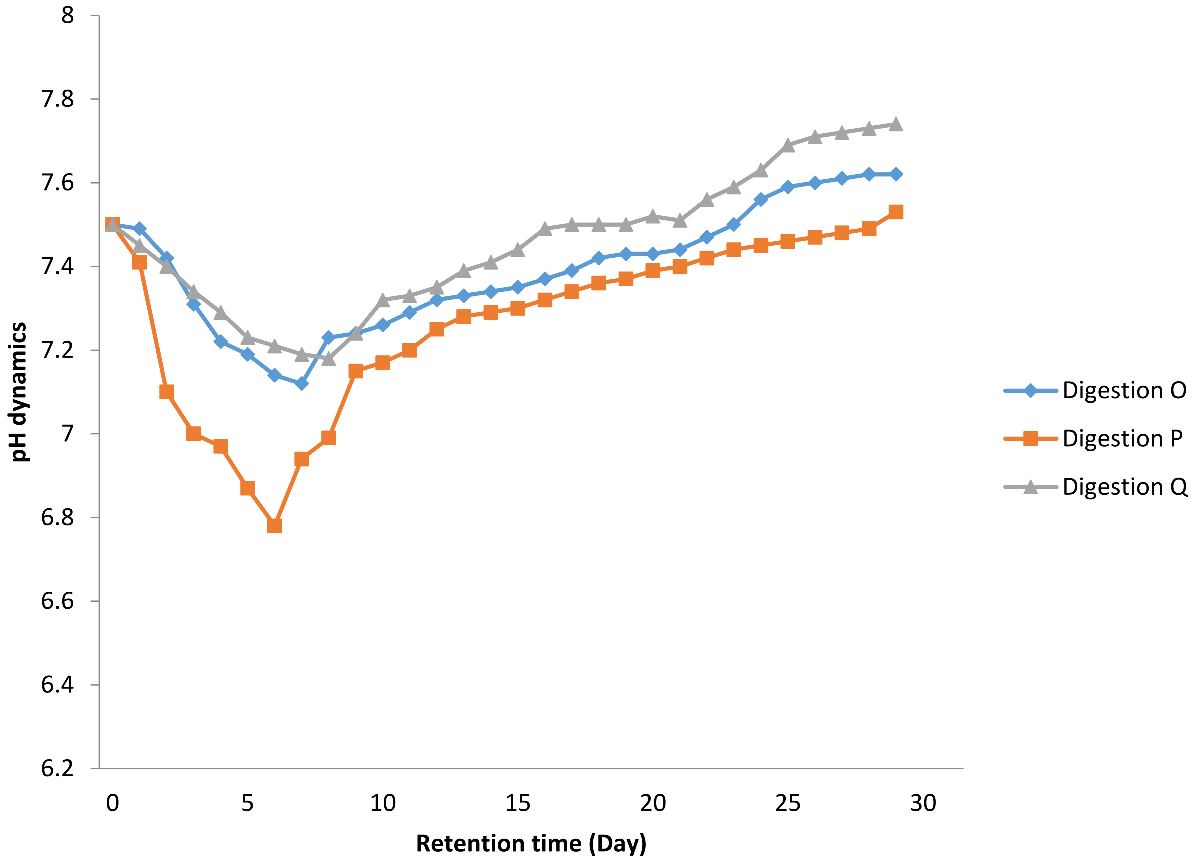

Figure 1: pH fluctuations during the anarobic digestion of *Telfairia occidentalis*fruit rind and poultry manure (Digestions O, P and Q)

Figure 2 (on next page)

Daily biogas generation during the anaerobic digestion process

Each data point indicates the daily biogas generation during the anaerobic digestion processes

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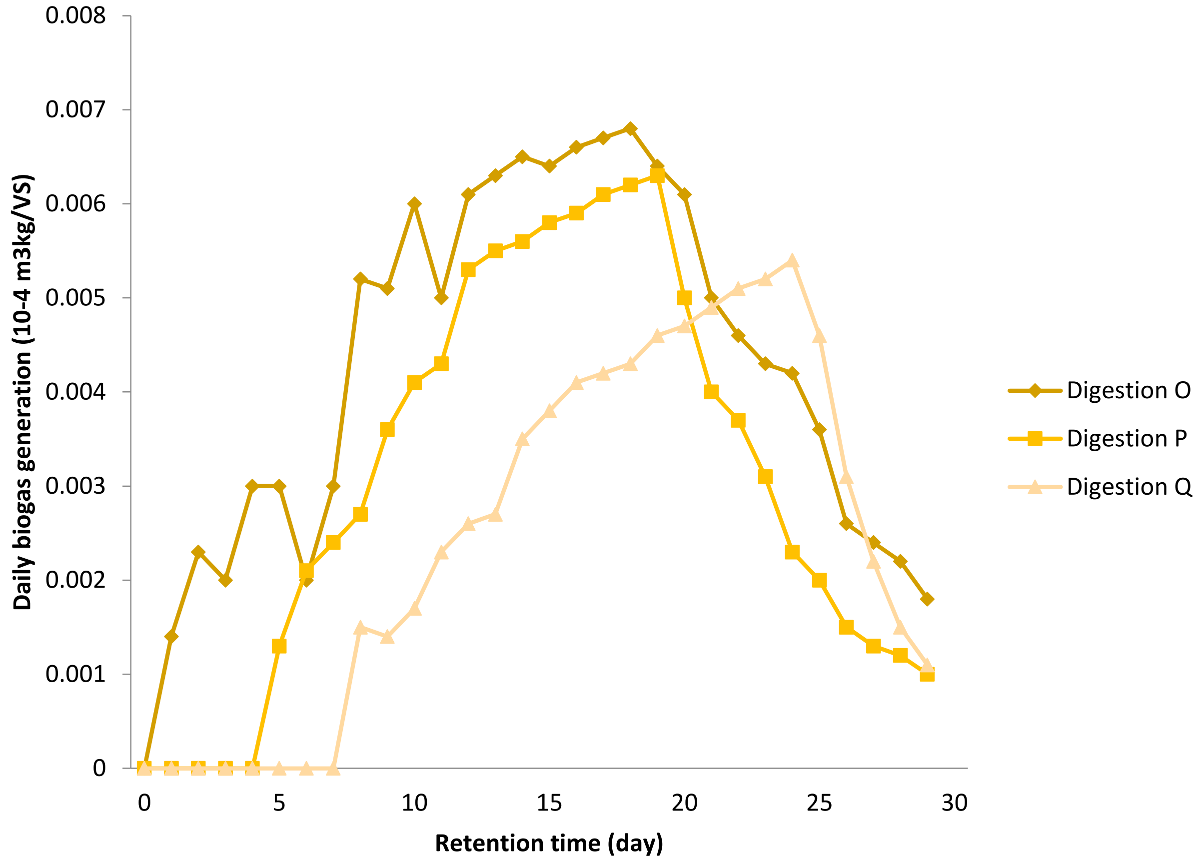

Figure 2: Average biogas generation during the anarobic digestion of *Telfairia occidentalis*fruit rind and poultry manure (Digestions O, P and Q)

Figure 3(on next page)

RSM and ANNs surface plots for the optimization of data during the digestion processes

Each figure shows the RSM and ANNs surface plot showing the interactions between the variables employed in the optimization study

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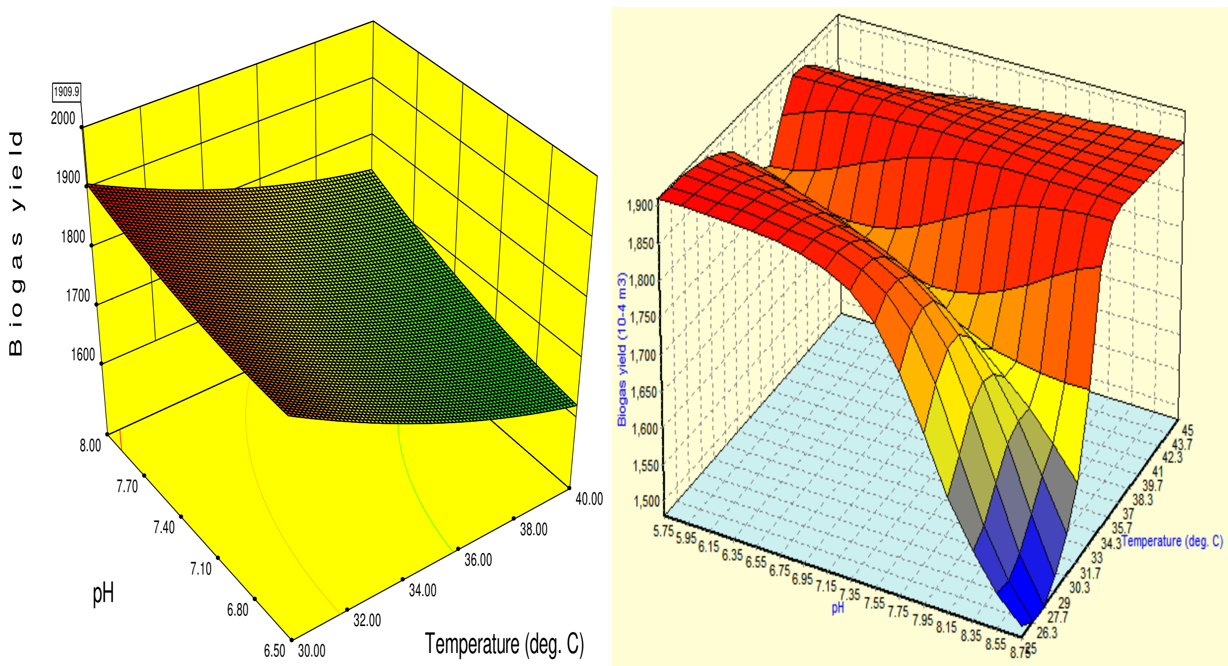
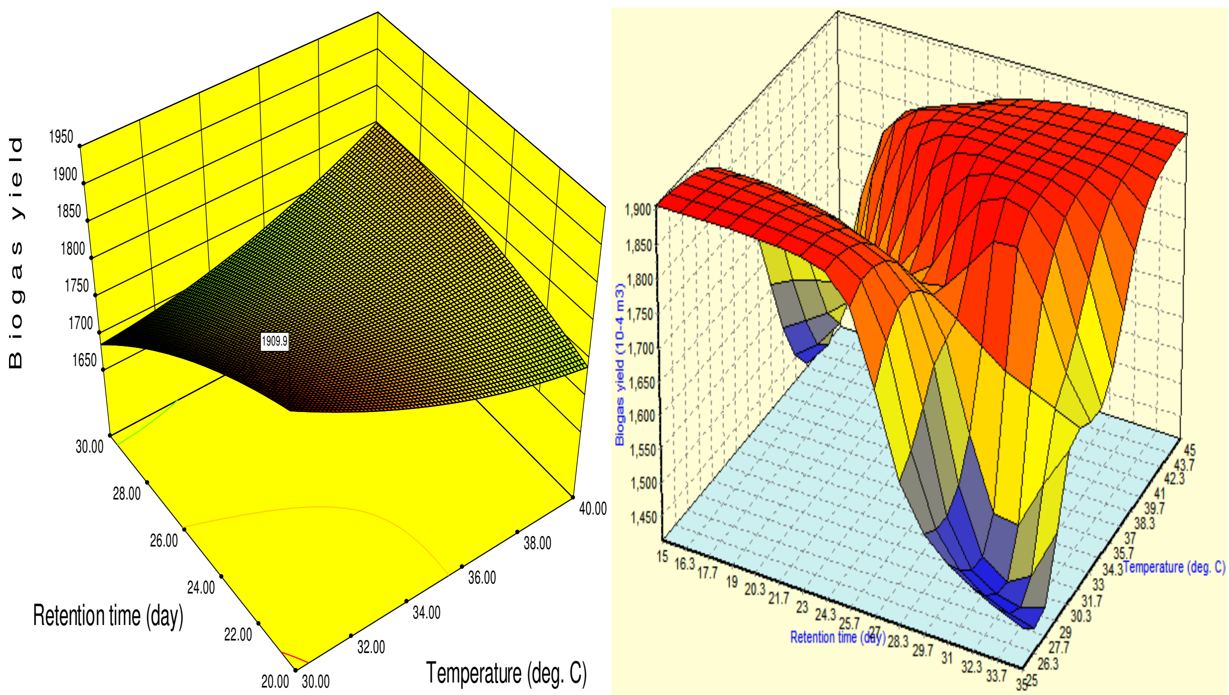
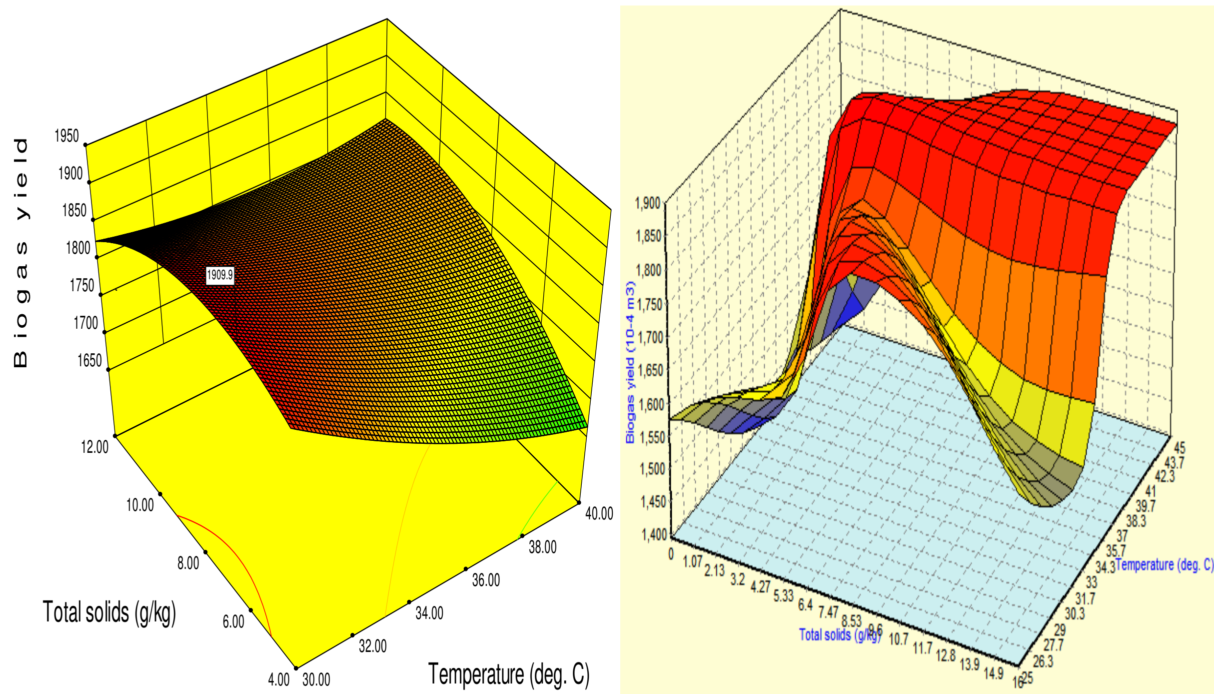

Biogas yield
pH
Temperature (deg. C)
Biogas yield (10-4 m3)
Temperature (deg. C)
pH

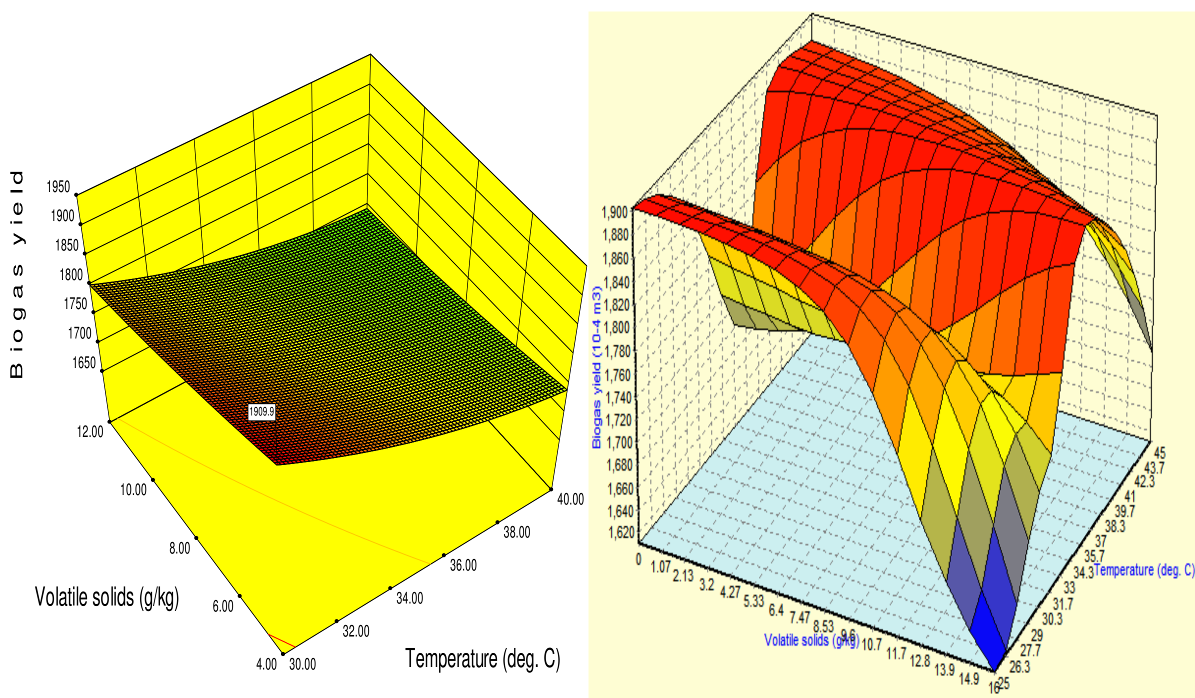
(a)


Biogas yield
Retention time (day)
Temperature (deg. C)
Biogas yield (10-4 m3)
Temperature (deg. C)
Retention time (day)

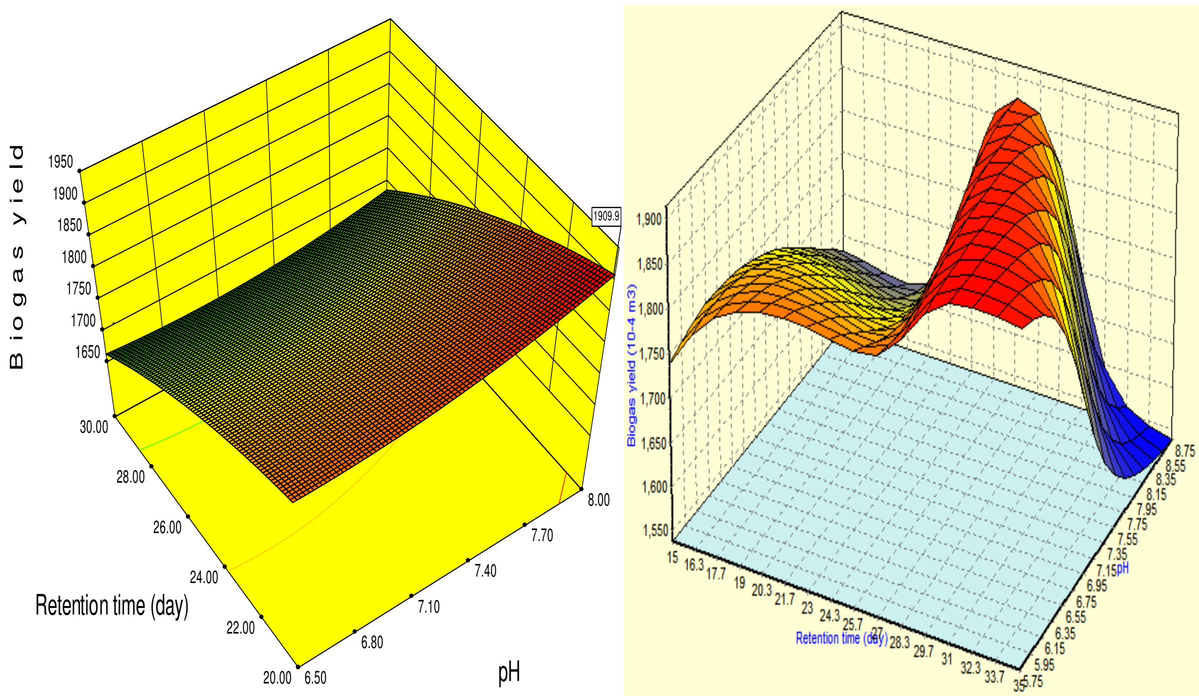
(b)


Biogas yield
1950
1900
1850
1800
1750
1700
1650
1909.9
12.00
10.00
8.00
6.00
4.00
Total solids (g/kg)
30.00
32.00
34.00
36.00
38.00
40.00
Temperature (deg. C)
Biogas yield (10-4 m3)
1,900
1,850
1,800
1,750
1,700
1,650
1,600
1,550
1,500
1,450
1,400
0 1.07 2.13 3.2 4.27 5.33 6.4 7.47 8.53 9.6 10.7 11.7 12.8 13.9 14.9 16
Total solids (g/kg)
25 26.3 27.7 29 30.3 31.7 33 34.3 35.7 37 38.3 39.7 41 42.3 43.7 45
Temperature (deg. C)

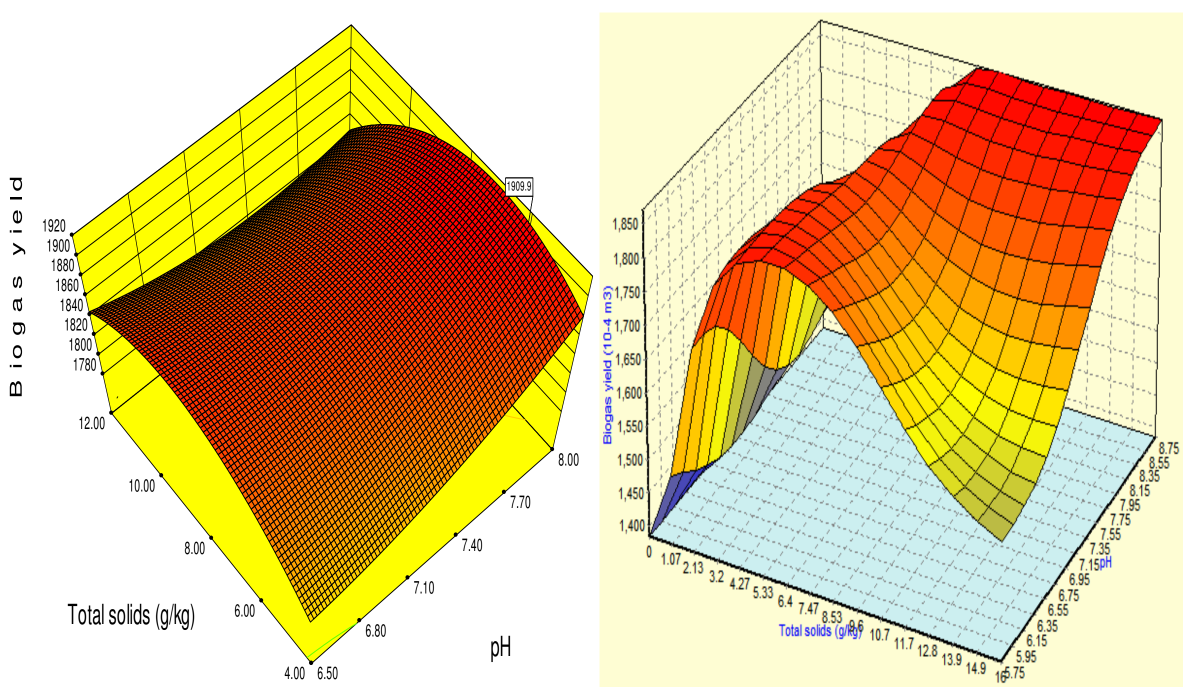
(c)


Biogas yield
1950
1900
1850
1800
1750
1700
1650
1909.9
12.00
10.00
8.00
6.00
4.00
Volatile solids (g/kg)
30.00
32.00
34.00
36.00
38.00
40.00
Temperature (deg. C)
Biogas yield (10-4 m3)
1,900
1,880
1,860
1,840
1,820
1,800
1,780
1,760
1,740
1,720
1,700
1,680
1,660
1,640
1,620
0 1.07 2.13 3.2 4.27 5.33 6.4 7.47 8.53 9.6 10.7 11.7 12.8 13.9 14.9 16
Volatile solids (g/kg)
25 26.3 27.7 29 30.3 31.7 33 34.3 35.7 37 38.3 39.7 41 42.3 43.7 45
Temperature (deg. C)

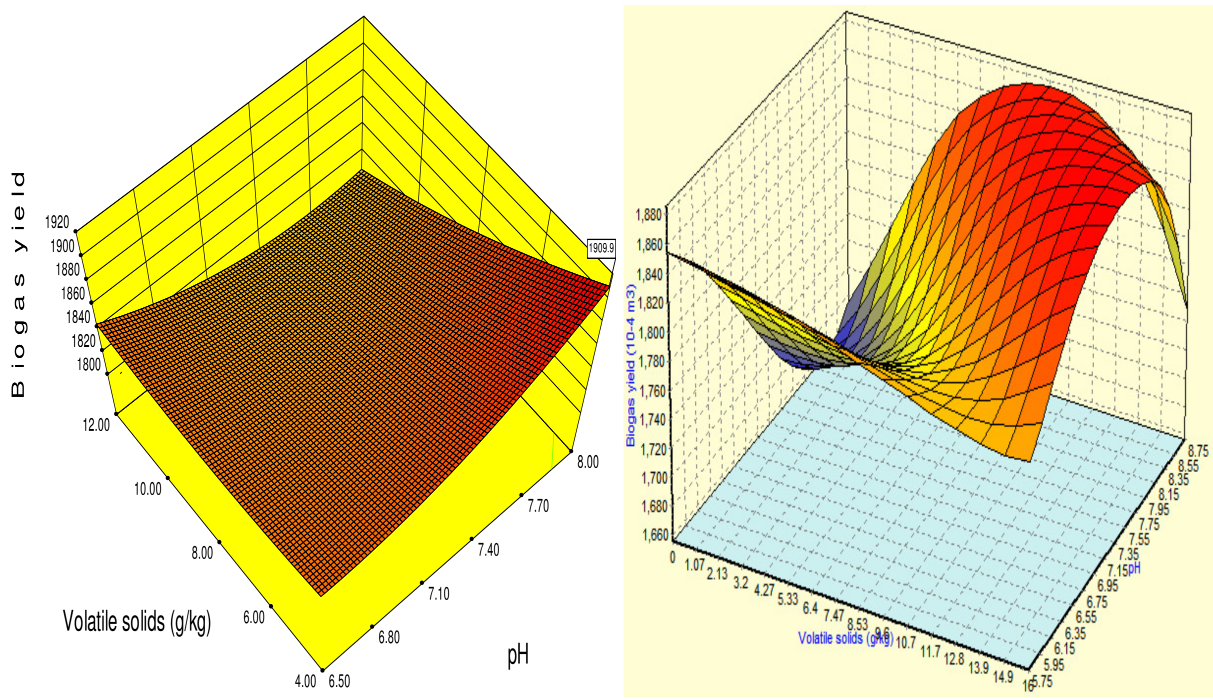
(d)


Biogas yield
1950
1900
1850
1800
1750
1700
1650
1909.9
30.00
28.00
26.00
24.00
22.00
20.00
Retention time (day)
6.50
6.80
7.10
7.40
7.70
8.00
pH
Biogas yield (10-4 m3)
1,900
1,850
1,800
1,750
1,700
1,650
1,600
1,550
Retention time (day)
pH

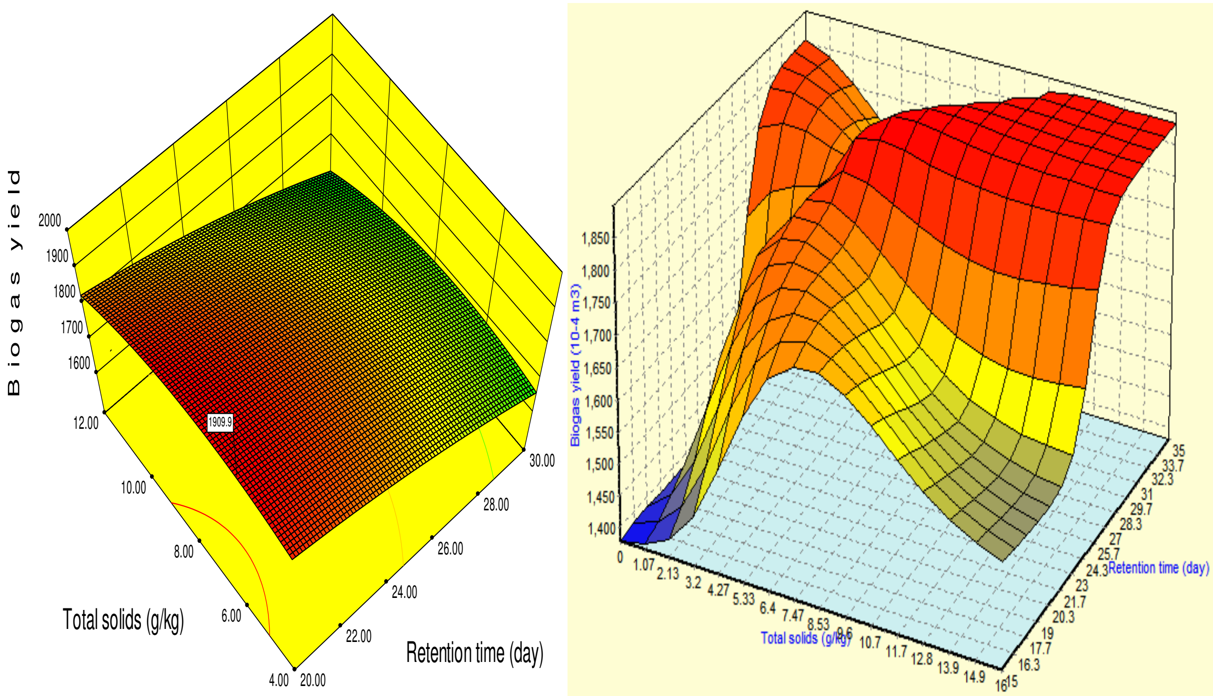
(e)


Biogas yield
1920
1900
1880
1860
1840
1820
1800
1780
1909.9
12.00
10.00
8.00
6.00
4.00
Total solids (g/kg)
6.50
6.80
7.10
7.40
7.70
8.00
pH
Biogas yield (10-4 m3)
1,850
1,800
1,750
1,700
1,650
1,600
1,550
1,500
1,450
1,400
Total solids (g/kg)
pH

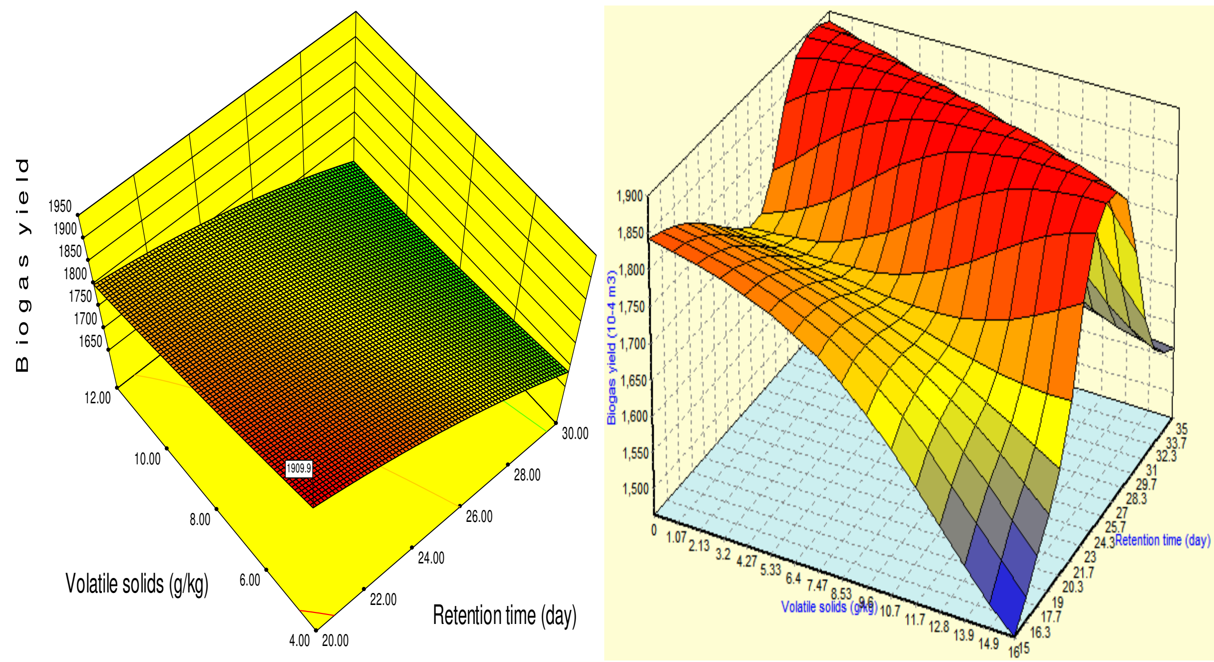
(f)


Biogas yield
Volatile solids (g/kg)
pH
1909.9
Biogas yield (10-4 m3)
Volatile solids (g/kg)
pH

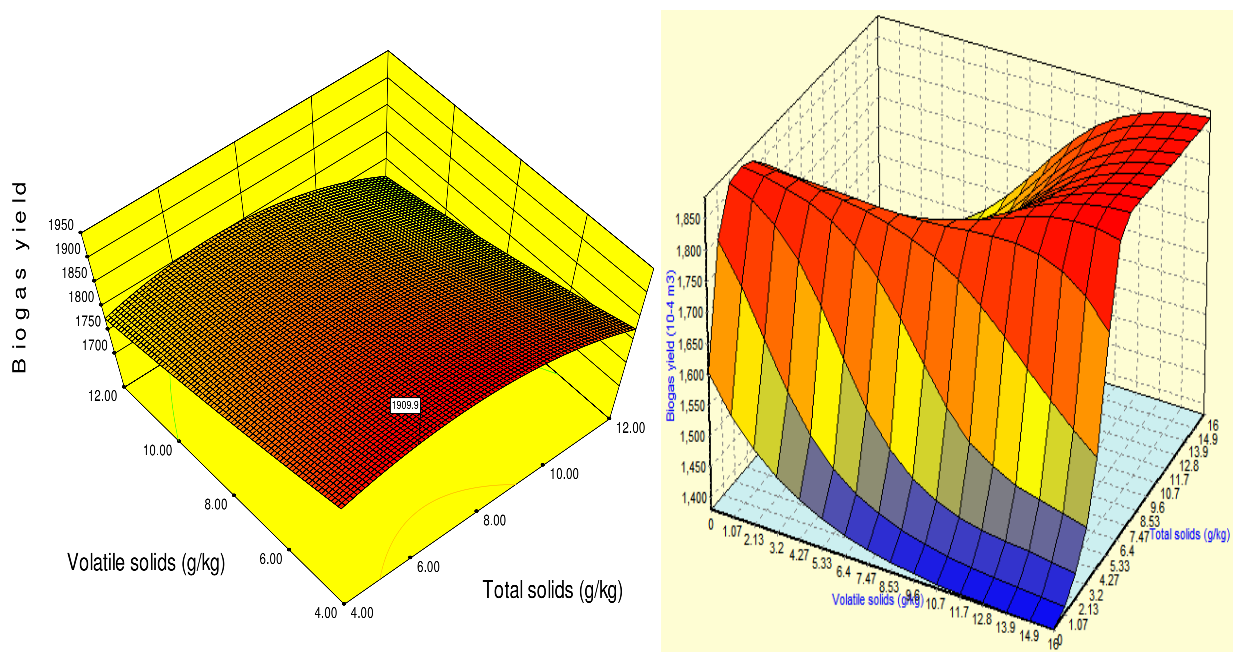
(g)


Biogas yield
Total solids (g/kg)
Retention time (day)
1909.9
Biogas yield (10-4 m3)
Total solids (g/kg)
Retention time (day)

(h)



(i)



(j)

Figure 3(a-j): 3D Curvatures' plots of RSM (Left) and ANNs (Right) optimization of biogas generation from *Telfairia occidentalis* fruit rind and poultry manure (Digestion 'O')

Figure 4(on next page)

Importance level of each parameter

Each point indicates the order of importance of all the five variables employed in the optimization study

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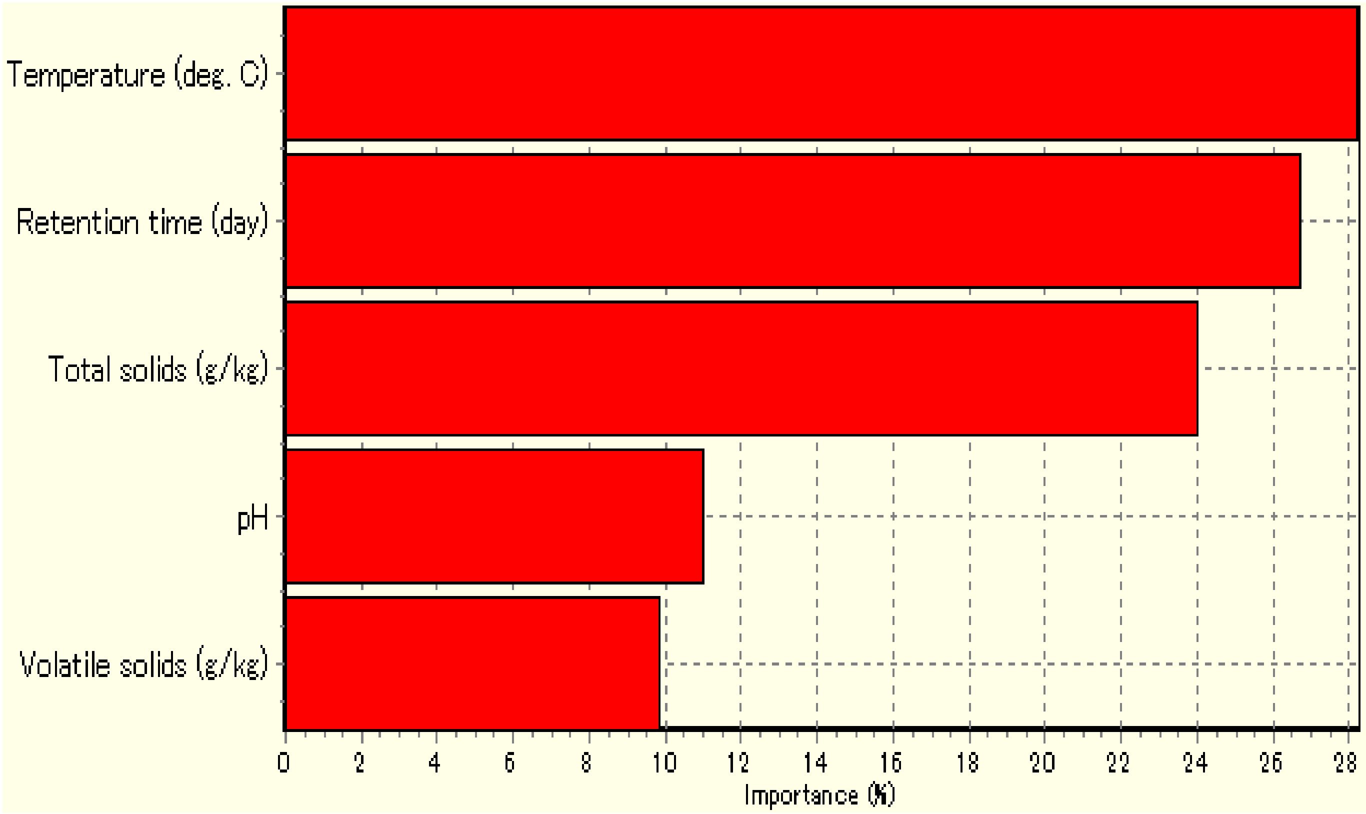

Figure 4: ANNs' importance level of each independent variable employed in the optimization