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Synergy of Siam weed (*Chromolaena odorata*) and poultry manure for energy generation: Effects of pretreatment methods, modeling and process optimization

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HIGHLIGHTS

- Co-digestion of Siam weed and poultry manure generated huge biogas.
- The study established the appropriate pre-treatment method for the substrates.
- Modeling and optimization was done using the Response Surface Methodology.
- The optimized conditions for maximal biogas yield were established.

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ABSTRACT

The co-digestion of *Chromolaena odorata* with poultry manure was evaluated in this study. Two samples of the weed: (A: which was pre-treated with mechanical, chemical and thermal methods) and (B: which was pretreated using mechanical and chemical methods only) were separately digested with poultry manure. Biogas generation started from the 2nd to 4th and 4th to 7th day for samples 'A' and 'B' respectively. The most desired actual biogas yield from samples 'A' and 'B' were 3884.20 and 2544.70 (10^{-4} m^3/kg VS) respectively and the gas composition was 68 ± 2% Methane and 20 ± 2% Carbon dioxide for sample A while it was 62 ± 3% Methane and 22 ± 2% Carbon dioxide for sample B. In all, there was a 38.06% increase in gas generation in 'A' over 'B'. The coefficient of determination (R^2) for the Response Surface Methodology (RSM) model (0.9009) was high suggesting high accuracy in the modeling and prediction. The worldwide usage of *C. odorata* is encouraged.

1. Introduction

Globally regarded as 'one of the world's worst weed', Siam weed (*C. odorata*) is an invasive plant species known for its negative impact on agricultural systems, the economy and biodiversity conservation in its areas of dominance (Perrings et al., 2010). As common for most invasive plant species, *C. odorata* constitutes huge threat to both the natural and derived ecosystems in its introduced habitats. It is known for its capability to smother existing native plant communities and by so doing has generated huge attractions in different agricultural systems worldwide (Adebayo and Uyi, 2010).

C. odorata has been noted to have originated from Mexico, the Caribbean and Brazil all in tropical Central and South America, from where it has spread to other localities due to its effective short- and long-distance dispersal mechanisms. It is often found in disturbed land areas, grasslands, fallow areas and forestry plantations where it forms pure stands when fully established (Gautier, 1992). *C. odorata* was introduced to Southern Nigeria in 1937 from Sri Lanka. Presently, it has reached alarming population in Nigeria (Uyi et al., 2013) and other African countries like Cameroon, Ghana etc (Djietror et al., 2011a, 2011b). It has over the last few decades been regarded as one of the worst weeds in Nigeria and West Africa.

C. odorata have in the past been put to some uses including as a fallow species in crop rotations, as medicine, in soil fertility improvement and as potential pesticide (Alisi et al., 2011). Several

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control measures ranging from chemical, mechanical and biological have been applied to control the plant due to its presence over large areas and its invasive nature (Zachariades et al., 2013). However, none of the methods have been practically found sustainable in terms of cost (Uyi et al., 2014). At present, there are no control or proven management strategies in place to check the spread of the weed in Nigeria and other countries. Therefore, this study is an attempt to utilize this weed for energy generation since green plants are natural sinks for enormous energy as a result of photosynthesis. Its abundance and invasiveness in several locations around the world is an indication that a veritable and environmental-friendly usage needs to be sought for the weed.

Anaerobic digestion (AD) is a technology for the conversion of organic materials into biogas which is subsequently used for electricity and mechanical energy generation, heating and other forms of energy utilization (Leite et al., 2016). The biogas generation in AD is brought about by the hydrolysis and subsequent fermentation of feedstock by diverse group of microorganisms carrying out several biochemical reactions in an anoxic environment (Chuichulcherm et al., 2016; He et al., 2016; Ismail and Talib, 2016).

Agricultural wastes and grasses are usually burnt off since they are mostly seen as solid wastes. However, the advent of the AD technology now makes these materials constitute low cost suitable candidates for the biotechnological production of biogas (Guenther-Lübbers et al., 2016; Othman et al., 2016). Previous investigations have specifically identified grasses as rich energy substrates and highly effective in greenhouse gas control than first generation biofuel resources when fully exploited for biogas production (Riggio et al., 2015). Biogas generation from poultry manure has been extensively investigated on with some success recorded. However, major setbacks were reported due to its low C/N ratio and high amount of total ammonia. The best approach to its usage therefore is co-digestion with other high energy-yielding substrates as earlier suggested by Dalkilic and Ugurlu (2015).

Prior to anaerobic co-digestion, pretreatment of substrates using different methods such as mechanical, chemical, thermal and others has been widely reported as an efficient approach to increase their accessibility to microbial bioconversion and also improve methane production (Lalak et al., 2016; Li et al., 2016a, b; Serrano et al., 2016). This is necessary because most lignocellulosic materials are highly recalcitrant to biodegradation and subsequent bioconversion by AD microorganisms due to their abundant lignin and cellulose matrix (Carrere et al., 2016). Similarly, optimization of process parameters is important in bioenergy generation (Montingelli et al., 2016) and the Response Surface Methodology (RSM) has been successfully utilized in the modeling of biogas from different substrates (Emeko et al., 2015). This study therefore aims at evaluating the usage of one of the world's worst weed (*C. odorata*) for biogas generation in co-digestion with poultry dropping. This is the very first time Siam weed will be reported as a viable substrate for bioenergy generation coupled with use of different pretreatment combinations. Since no permanent solution has been documented for the weed's invasion across the world and the challenges it poses to Agriculture, this research proposes a permanent solution to this barrier after which the plant will no longer be regarded as a weed but an energy biomass.

2. Methodology

2.1. Sample collection for the study

The vegetative portion of *C. odorata* and fresh poultry manure were both collected from the Teaching and Research Farms of Landmark University, Omu-Aran, Kwara State, Nigeria where the experiment also took place. In order to have adequate microbial flora in the AD systems, the inoculum (bovine rumen contents) were collected from the slaughter slab of Landmark University's Cafeteria. Considering the lignocellulosic nature of the plant, it was pre-treated using two different methods in order to establish the most appropriate pre-treatment method for the biomass. The first sample labeled 'A' was pre-treated using the combination of mechanical, thermal and chemical (Na OH) pre-treatment earlier described (Dahunsi et al., 2016a,b). In doing this, a hammer mill was employed to crush the biomass into mesh sizes of $\leqslant$20 mm and was then heated in the CLIFTON, 88579 water bath (NICKEL-ELECTRO Ltd., ENGLAND) at 80 °C for one hour since thermal pre-treatment at higher temperature has been reported to have adverse effect on the AD system (Liu et al., 2012). Chemical pre-treatment was then followed with 3 g/L sodium hydroxide (Na OH). The choice of Na OH was premised on earlier reports that among other widely used alkalis, Na OH has produced the best result for thermo-chemical pre-treatment of AD substrates (Li et al., 2015). The second sample labeled 'B' was pre-treated using the described mechanical and chemical (Na OH) methods only. The twenty-five-litre volume digesters earlier used (Dahunsi et al., 2016a,b) were employed in this study. Each digester setup comprises an air-tight tank furnished with a mechanical iron stirrer in-built for appropriate substrate mixing. A liquid displacement apparatus for gas collection was equally attached to the digestion tank (Dahunsi and Oranusi, 2013).

2.2. Design of experiment

The CCRD was employed in the experimental design for the AD of pre-treated samples of *C. odotata* and poultry manure to biogas because of its efficiency in biofuel process improvement (Emeko et al., 2015). A total of 50 experimental runs were generated via the five-level-five-factors design used. Based on their importance in the success of AD process, five independent factors (Temperature (°C), pH, Retention time (days), Total solids (g/kg) and Volatile solids (g/kg) designated as $\boldsymbol{X_1}$, $\boldsymbol{X_2}$ $\boldsymbol{X_3}$ $\boldsymbol{X_4}$ and $\boldsymbol{X_5}$ respectively were selected for the biogas modeling and optimization study. In most previous studies till date, temperature for most mesophilic AD has been varied between 30 and 40 °C (Tampio et al., 2016). Likewise, pH range of between 6.5 and 8 has been severally reported to be best for AD microbial operations. The range of retention time for mesophilic AD has equally been given to be from 20 to 30 days based on the ambient temperature. It has been documented that liquid AD efficiency is achieved when the total solids content is $<15\%$ and $\geqslant 4\%$ (Tampio et al., 2016). It was therefore on the basis of the prescribed values in literature that the working ranges were chosen in this optimization study (Table 2) so as to document the optimal process conditions for the most efficient AD using *C. odorata* and poultry manure as substrates.

2.3. Biochemical and residual methane potential tests

In order to determine the potential methane production of the substrates at standard temperature and pressure (STP), the biomethane potential test was carried out following already prescribed methods (Dahunsi et al., 2016a,b). The experiment ran anaerobically in a batch system for 30 days using two 250 ml flasks for the experiment and a blank making three in all and in triplicate with an inoculum to substrate ratio of 3 according to earlier protocol (Ghasimi et al., 2016a,b). Collection of produced gas from the digesters was constantly carried out and the methane content was analyzed chromatographically. The same method was employed for carrying out the Residual methane test carried out solid digestates.

2.4. Digestion

For the two pre-treated samples of *C. odorata* (A and B), 4 kg each were separately mixed with 4 kg of poultry dropping (Dahunsi et al., 2016a). Each combination was further diluted with water in the ratio 1:1 w/v to form slurry and to which one kg of inoculum was added thus making a total of 17 L. The slurry was then charged into each of the digestion tanks through an opening occupying three quarter of the digester space. Several parameters were evaluated at intervals during the AD in order to assess treatment efficiency. These include daily measurement of produced gas, weekly assessment of microbial dynamics and physiochemical analyses of feedstock and digestates. Average temperature readings were recorded from the daily values taken twice while weekly pH measurement was done and the average recorded. The water displacement method earlier described was employed in daily gas collection. Characterization of produced biogas to quantify the methane and other constituents were done with the aid of a HP 5890 Gas Chromatography (Avondale, USA) coupled with a Hayesep Q column (13 m × 0.5 m × 1/800) and a flame ionization detector (FID).

2.5. Analytical procedure

Before commencement of the AD process, analyses of important physical and chemical parameters of the cattle rumen content (inoculum) and the pre-treated substrates were carried out. These were done in the laboratory of Environmental Engineering, Landmark University. A total of 18 important parameters were evaluated using the Palintest$^{(R)}$ Photometer 7100 (PHOT.1.1.AUTO.71) and Photometer 7500 (PHOT.1.1.AUTO.75) advanced digital-readout colorimeter (England) with same procedure. The operation of the photometer was done at 0.5 absorbance and wavelength of 450 nm and all samples were evaluated in triplicates as earlier described (Dahunsi et al., 2016a,b). For COD determination, the Standard Methods for the Examination of Water and Wastewater (APHA, 2012; Dahunsi et al., 2014) was adopted.

2.6. Microbial assessment

2.6.1. Aerobic bacterial and fungal enumeration

Standard methods for total aerobic plate enumeration were followed in isolating and characterizing the aerobic organisms associated with the fermenting materials in all the digesters. This was carried out in the laboratories of Biological Sciences Department (Microbiology), Covenant University, Ota, Nigeria using different specific media including nutrient agar, eosin methylene blue agar, peptone water and MacConkey agar. Samples were collected from the specialized sampling port on each digester and kept refrigerated prior analyses. Analyses were done weekly in triplicates and both phenotypic methods and use of appropriate rapid API kits (BioMerieux) were used in characterizing the presumptive isolates according to earlier method (Dahunsi et al., 2016a,b). For fungal isolation, samples were cultured on Potato dextrose agar. After this, features including the microscopic and macroscopic characteristics of the hyphal mass, cell spores morphology, the pattern of the fruiting bodies etc were used for identification (Tsuneo, 2010).

2.6.2. Anaerobic organism's enumeration

Anaerobes especially facultative *Clostridium* species and others were isolated by first culturing the samples on Reinforced Clostridia medium followed by further culturing on blood agar in an anoxic condition at 37 °C for 5–7 days. The Brain Heart Infusion agar was specifically used and developed colonies were counted and recorded (Ayandiran et al., 2014). Pure cultures were obtained after series of sub-culturing on distinct colonies and were further kept in fresh slants. Morphological and biochemical techniques were employed to confirm the presumptive isolates which were further characterized using appropriate rapid API kits (Ayandiran and Dahunsi, 2016).

2.6.3. Enumeration of methanogen (archaea)

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

2.7. Optimization and statistical data analysis

At the end of the digestion processes, the Response Surface Methodology was used to statistically analyse all the biogas generation data as a way of fitting the polynomial equation already produced via the version 9.0.3.1 of the 'Design-Expert software'. Multiple regressions were employed in fitting the coefficient of the polynomial model so as to correlate both response and independent factors. Tests of significance and ANOVA were used to evaluate the fit quality of the model and the fitted quadratic model is shown in the equation below:

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

where: Y = the response variable; b_o = intercept value; b_i (i = 1, 2, k) = the first order model coefficient; b_{ij} = the interaction effect; b_{ii} = the quadratic coefficients of X_i while e = the random error. Validation of RSM model was carried out using same digesters and the predicted values after which the plots of the deviations of actual and observed values were constructed.

3. Results

3.1. Stability of digesters and performance evaluation

The results of residual methane test showed that gas generation commenced at the second experimental day with analyses showing average methane content of 65 ± 1.5%. Table 1 show results for physicochemical analyses of the pre-treated samples of *C. odorata*, the inoculum and those of the digestates. The recorded values for the physical parameters (pH and temperature) were within the designed ranges for the experiment. pH remained at slightly alkaline range (6.5–8) (Fig. 1) throughout though with an initial fall to strongly acidic range during the first week while temperature readings of all digesters were within 30–40° all through the experimental period. The AD was found to bring about enormous degradation of the fermented biomass as the values of most evaluated chemical parameters increased significantly in the digestates. Also, total solids analyses revealed the poultry manure alone being heavier than the combination of *C. odorata* and poultry manure and the inoculum. However, in

Table 1
Physical and chemical characteristics of mixture of *Chromolaena odorata* shoot with poultry dropping and cattle rumen content.

Parameters	The mixture before digestion	Poultry droppings	Rumen content	Digestate A	Digestate B
pH	7.60 ± 0.12	6.90 ± 0.22	7.91 ± 0.02	7.50 ± 0.02	7.61 ± 0.01
Total Solids (g/kg)	110.48 ± 2.01	280.24 ± 1.02	90.52 ± 0.11	97.61 ± 0.10	103.13 ± 0.11
Volatile Solids (g/kg)	97.97 ± 4.01	180.71 ± 1.13	90.44 ± 2.12	90.67 ± 2.00	95.53 ± 1.00
Ash Content (%)	8.93 ± 0.03	18.29 ± 2.11	5.56 ± 0.13	6.33 ± 0.01	5.14 ± 0.01
Moisture Content (%)	88.52 ± 3.07	71.76 ± 2.80	90.48 ± 2.12	91.94 ± 1.00	86.43 ± 1.00
Total Carbon (g/kg TS)	290.10 ± 5.08	292.10 ± 3.10	265.21 ± 4.10	250.54 ± 0.22	272.42 ± 0.20
Total Nitrogen (g/kg TS)	40.00 ± 0.02	61.00 ± 1.12	48.00 ± 1.12	57.00 ± 1.02	45.10 ± 1.01
COD (mg/kg TS)	269 ± 3.95	228.98 ± 3.00	168.21 ± 1.12	79 ± 3.02	85 ± 2.00
Total Phosphorus (g/kg TS)	4.80 ± 0.02	7.90 ± 0.12	6.30 ± 0.13	7.06 ± 1.02	5.55 ± 1.02
Potassium (g/kg TS)	7.40 ± 0.02	9.00 ± 0.00	7.20 ± 0.12	8.60 ± 0.03	7.69 ± 0.02
Phosphate (g/kg TS)	2.30±.01	3.80 ± 0.10	3.00 ± 0.12	3.50 ± 0.02	3.13 ± 0.01
Sulphate (g/kg TS)	118.00 ± 2.11	164.00 ± 3.02	134.00 ± 5.09	154.00 ± 2.01	126.02 ± 2.02
Calcium (g/kg TS)	162.00 ± 2.21	44.00 ± 0.02	80.00 ± 1.22	52.00 ± 1.00	73.50 ± 1.10
Magnesium (g/kg TS)	70.00 ± 1.02	150.00 ± 2.10	96.00 ± 2.12	130.00 ± 2.01	107.22 ± 1.11
Manganese (g/kg TS)	0.022 ± 0.00	0.040 ± 0.01	0.028 ± 0.01	0.034 ± 0.01	0.022 ± 0.01
Iron (g/kg TS)	0.96 ± 0.31	1.46 ± 0.02	1.18 ± 0.11	1.34 ± 0.21	1.17 ± 0.01
Zinc (g/kg TS)	29.00 ± 0.12	51.00 ± 2.02	38.00 ± 0.14	44.00 ± 0.02	33.40 ± 0.02
Aluminium (g/kg TS)	0.58 ± 0.40	0.62 ± 0.30	0.80 ± 0.02	0.64 ± 0.01	0.61 ± 0.01
Copper (g/kg TS)	3.90 ± 1.01	5.80 ± 0.72	4.80 ± 0.05	5.50 ± 1.01	4.87 ± 1.00

n = 60; COD = Chemical Oxygen Demand.

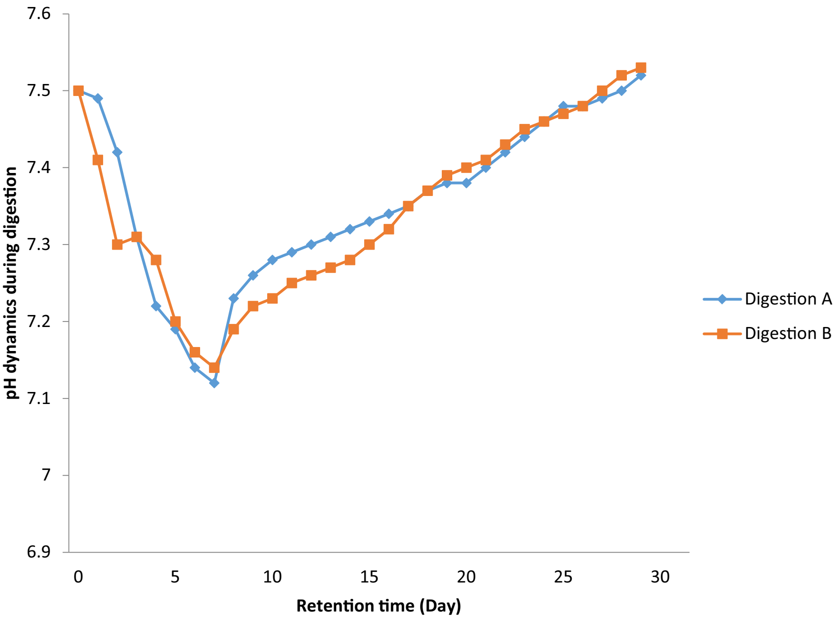

Fig. 1. The figure shows the pH dynamics in the anaerobic co-digestion of *Chromolaena odorata* shoot and poultry dropping.

terms of nutrient and mineral element, the poultry dropping was the richest. Another significant observation was the significant reduction in COD values of the two substrate (58.6 and 50.2%) for samples ‘A’ and ‘B’ recorded in their digestates. Biogas generation started from second to fourth and fourth to seventh experimental days for samples A and B and remained increasing till the nineteenth and twenty third days before declining as shown in Fig. 2. The most desired actual biogas yield from samples ‘A’ and ‘B’ were 3884.20 and 3544.70 (10^{-4} m^3/kg VS) respectively. Analyse of the gas via Gas chromatography shows the composition to be within 68 ± 2% Methane and 20 ± 2% Carbon dioxide for sample A while it was 62 ± 3% Methane and 22 ± 2% Carbon dioxide for sample B.

3.2. Microbial dynamics

Several aerobic bacteria, fungi, facultative anaerobes and methanogens were identified at the different stages of the AD setups. In all, the population of aerobic bacteria and fungi increased steadily till about the 12th day of experiment before reduction was noticed. On the other hand, population of facultative anaerobes and methanogens experienced initial decrease before steady increase towards the end of the experiments. The aerobes include *Bacillus megaterium*, *Bacillus circulans*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Proteus vulgaris*, *Proteus mirabilis* and *Enterococcus faecalis.* Implicated anaerobes include *Bacteroides fragilis*, *Clostridium clostridioforme*, *Clostridium histolytica* and *Clostridium spp.* Fungal isolates include *Aspergillus niger*, *Mucor*, *Rhizopus stolonifer* and *Penicillium* while four methanogens were implicated at various stages of the digestions. The highest TPC for aerobic bacteria was 2.7 x 10^{11} cfu/ml while the lowest was 5.0×10^9 cfu/ml. The highest fungal TPC was 1.9×10^8 cfu/ml while the lowest was 1.0×10^4 cfu/ml. For anaerobes, the highest and lowest TPC were $1.8 \times 10^{1\circ}$Cfu/ml and $1.0 \times 10^{1\circ}$Cfu/ml. The highest methanogenic TPC was 2.4×10^{12} cfu/ml while the lowest was 9.0×10^9 cfu/ml.

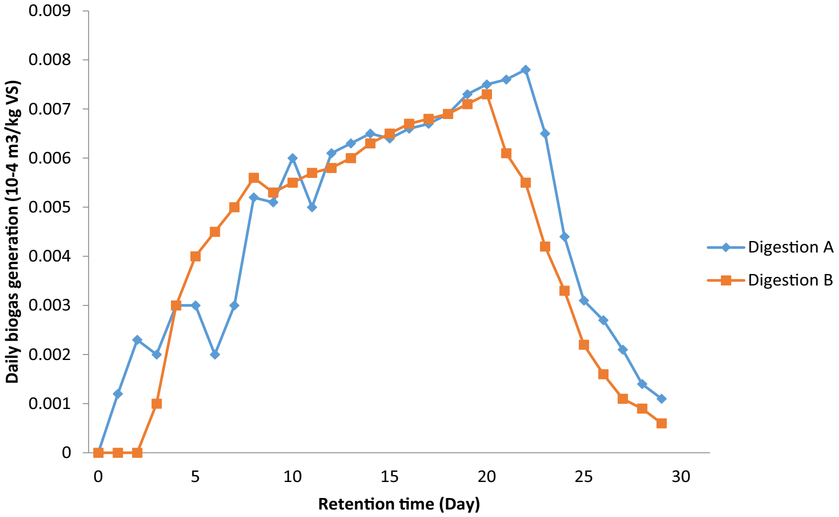

Fig. 2. The figure shows the average daily biogas yield in the anaerobic co-digestion of *Chromolaena odorata* shoot and poultry dropping.

3.3. Data optimization via RSM

Table S1 shows the factors and their response surface levels for the AD of *C. odorata* shoot and poultry manure while Table 2 shows the experimental design matrix by CCRD for the study. Features of the table include the experimentally observed and predicted biogas yields and their residuals. Determination of the coefficients of the model equation and their statistical significance were done, the significance test and the ANOVA for all regression coefficients are shown in Table 3. Based on the low p-values ($p < 0.05$) recorded some of the model terms, they showed significance and have contributed immensely to the reported biogas yield. For both samples 'A' and 'B', the Model F-values of 4.09 and 3.88 implies the model is significant with the linear terms X_3, X_4, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_2X_5 and X_4^2 being the most significant for sample 'A' and X_3, X_5, X_2X_3, X_2X_4 and X_2X_5 for sample 'B'. In the same vein, the Adequate Precision values of 11.950 and 9.902 for 'A' and 'B' indicates that the model can be adequately used for biogas prediction from the substrates. In checking the model's 'goodness of fit', the determination coefficient (R^2) was used and resulting 'Lack of Fit' F-values (7.90 and 6.89) for 'A' and 'B' implies non significance. Fig. 3 showed graph of predicted against the actual biogas yield while the equation below shows the relationship between the biogas yield (Y) and the coded values (X_1, X_2, X_3, X_4 and X_5) of the five independent variables and their respective interactions for sample 'A':

$$\begin{aligned}Y = 3946.63 &- 70.12x_1 - 4.78x_2 + 188.22x_3 + 226.97x_4 \\ &+ 68.39x_5 + 198.06x_1x_2 + 217.61x_1x_3 - 225.14x_1x_4 \\ &- 3.28x_1x_5 - 214.91x_2x_3 - 208.82x_2x_4 - 136.20x_2x_5 \\ &+ 60.86x_3x_4 + 172.88x_3x_5 + 269.89x_4x_5 + 30.60x_1^2 \\ &+ 48.90x_2^2 - 77.99x_3^2 - 280.98x_4^2 + 45.53x_5^2 \qquad (2)\end{aligned}$$

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

Fig. S1(i–x) (Supplementary materials) shows the 3-dimensional graphical plots of the regression equation shown above (Eq. (2)). By solving the equation, the optimal values of each of the five factors optimized in the AD of *C. odorata* shoot and poultry dropping was obtained. The optimal condition for each factor was statistically predicted as $\boldsymbol{X_1}$ = 30.00 (°C), $\boldsymbol{X_2}$ = 7.50, $\boldsymbol{X_3}$ = 30.00 (day), $\boldsymbol{X_4}$ = 12.00 (g/kg) and $\boldsymbol{X_5}$ = 10.00 (g/kg) with 0.970 i.e. 97% and 0.999 i.e. 99.9% desirability for 'A' and 'B' respectively. Under these optimal conditions, the predicted biogas yield was 4178.81 and 3888.50 (10^{-4} m^3/kg VS) for samples 'A' and 'B' respectively. These same set of conditions were applied to three replicates in as a way of verifying the model's prediction and the average biogas yield obtained was 4152.22 and 3833.13 (10^{-4} m^3/kg VS) for 'A' and 'B' and these are close to the predicted values.

4. Discussion

Suitable pH must be constantly maintained during anaerobic digestion in order to provide the necessary ambience for the adequate operation of microorganisms and their subsequent bioconversion of intermediate acids to methane (Zahedi et al., 2016). A pH range of 6.5–8.0 has been documented as efficient for anaerobic digestion processes. pH readings throughout the digestion supported the above submissions as they remained at slightly alkaline range. The mesophilic temperature range was equally maintained in all the digesters in the course of the AD. Temperature is a major factor affecting the success of anaerobic digestion as the various arrays of bacteria carrying out the bioconversion of substrate are known to operate at specified extremes of temperature (Jain et al., 2015; Mckennedy and Sherlock, 2015). Failure to establish such temperature ranges lead to permanent failure of the AD system. Furthermore, mesophilic temperature ensures better stability of substrate conversion as well as providing higher bacteria richness and efficiency (Kwietniewska and Tys, 2014).

The bulkiness of the poultry manure (Table 2) could be traced to the poultry diets which are mostly made of protein and carbohydrate matters with large molecular weights. Another factor is the increased moisture content of the mixture of the weed and poultry manure due to dilution prior to AD. The characteristics of the *C. odorata* shoot are comparable with those of *Cymbopogon citratus* (Alfa et al., 2014a,b) and *Tithonia diversifolia* (Our unpublished work) earlier utilized in biogas generation. The *C. odorata* shoot samples are adequately rich in nutrients and mineral elements usually needed by microorganisms for growth and substrate bioconversion in a fermentation process. The high nutrient and elemental composition of the plant could be due to its high capacity for bioaccumulation of nutrients and metals from the rhizosphere (Alisi et al., 2011). Analyses of the two anaerobic digestates obtained in this study showed increased nutrient status for all analysed parameters than their initial levels prior to digestion with digestate from 'A' having higher nutrient status than 'B'. There was significant increase in values of most major (Nitrogen, Phosphorus and Potassium) and minor (Magnesium, Manganese, Iron, Zinc,

Table 2
Experimental design matrix by central composite rotatable design (CCRD) for five-level-five-factors response surface study for biogas generation from *Chromolaena odorata* shoot and poultry dropping.

	Independent Factors					Digestion A			Digestion B		
Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield (10^{-4} m^3/kg VS)	Predicted biogas yield (10^{-4} m^3/kg VS)	Desirability	Actual biogas yield (10^{-4} m^3/kg VS)	Predicted biogas yield (10^{-4} m^3/kg VS)	Desirability
1	30.00	7.50	30.00	12.00	10.00	3884.2	4178.81	0.970	2544.7	2588.5	0.999
2	30.00	7.50	30.00	11.83	12.00	4000.5	4157.60	0.964	2877.5	3146.6	0.998
3	30.00	7.51	30.00	11.77	11.99	3872.2	4043.30	0.960	2452.5	2655.6	0.997
4	30.00	7.50	29.63	11.93	12.00	3875.4	4218.95	0.954	2889.9	3201.4	0.996
5	30.00	7.50	29.71	12.00	11.86	4196.4	4512.12	0.952	3096.4	3132.4	0.993
6	30.01	7.50	30.00	12.00	11.63	3900.3	4209.00	0.951	2450.7	2550.9	0.992
7	30.00	7.50	29.76	11.92	11.71	3673.2	3884.49	0.945	2495.6	2532.2	0.991
8	30.00	7.50	29.33	11.99	12.00	4201.1	4882.75	0.944	2151.4	3302.3	0.990
9	30.01	7.50	30.00	11.98	11.23	3502.8	3832.75	0.931	3362.1	3415.5	0.989
10	30.00	7.50	29.88	10.74	12.00	2600.8	2799.13	0.922	2350.4	2511.3	0.988
11	30.00	7.50	30.00	11.99	10.87	3834.1	4168.09	0.914	2733.8	2840.4	0.987
12	30.00	7.50	30.00	10.53	11.92	3572.2	3766.86	0.914	2302.8	2425.1	0.984
13	30.01	7.64	30.00	11.33	12.00	3521.1	3743.44	0.908	2451.5	2561.5	0.977
14	30.00	6.56	30.00	12.00	10.91	3991.1	4119.87	0.901	2421.4	2479.8	0.975
15	30.00	6.50	28.83	12.00	11.40	3980.6	4206.31	0.898	2420.4	2444.4	0.970
16	30.02	6.50	28.10	12.00	11.97	4000.9	4694.82	0.895	2410.2	2435.9	0.966
17	31.85	6.51	29.48	12.00	12.00	3852.2	3924.91	0.876	2332.6	2466.8	0.965
18	30.41	6.50	27.91	11.99	12.00	2861.1	2896.27	0.874	2652.6	2782.5	0.962
19	30.00	6.52	27.59	12.00	12.00	4190.1	4600.42	0.870	2462.4	2560.0	0.959
20	30.00	6.52	30.00	12.00	9.85	2932.9	2976.07	0.863	2276.4	2309.5	0.953
21	30.00	6.50	27.20	11.91	12.00	3473.0	3551.58	0.857	2743.9	2944.3	0.951
22	32.90	6.51	30.00	12.00	12.00	2300.1	2528.59	0.851	3803.5	2868.3	0.950
23	30.00	6.50	30.00	11.17	9.57	3968.0	4077.18	0.837	2468.3	2981.1	0.947
24	30.05	6.72	30.00	9.91	12.00	3891.0	3957.08	0.832	3031.8	3152.6	0.945
25	32.77	6.51	29.15	12.00	12.00	3862.1	3851.91	0.831	2466.5	2502.2	0.942
26	30.00	6.50	30.00	10.76	9.48	3294.9	3422.35	0.823	2395.5	2532.5	0.936
27	30.63	6.97	29.98	12.00	12.00	6094.0	6175.93	0.811	2493.2	2466.5	0.925
28	30.00	7.09	30.00	11.98	12.00	3905.7	4032.08	0.799	2101.2	2148.3	0.922
29	30.00	6.50	29.60	12.00	8.08	3722.0	3753.25	0.778	2212.1	2373.4	0.920
30	30.08	7.25	30.00	11.39	12.00	3832.2	3918.19	0.743	3222.1	3322.2	0.917
31	35.00	8.00	22.09	5.43	10.00	3900.3	4209.00	0.741	2421.6	2515.4	0.915
32	37.00	7.65	30.00	5.61	8.31	3673.2	3884.49	0.740	2401.3	2484.7	0.912
33	36.00	8.00	29.93	12.00	5.34	4201.1	4882.75	0.736	2315.1	2471.4	0.906
34	36.88	6.50	30.00	8.62	4.00	3502.8	3832.75	0.732	2002.1	2111.7	0.903
35	37.00	6.50	30.00	8.58	6.74	2808.8	2990.13	0.731	2341.5	2441.7	0.901
36	36.51	8.00	30.00	6.33	4.00	3834.1	4168.09	0.730	2122.5	3273.5	0.888
37	30.47	6.53	20.08	11.92	4.05	3572.2	3766.86	0.729	2142.0	3210.4	0.885
38	39.98	6.61	29.93	9.53	4.73	3521.1	3743.44	0.728	2471.2	2500.8	0.883
39	34.59	6.55	29.57	9.34	4.23	3991.1	4119.87	0.725	2347.3	2417.3	0.881
40	32.21	6.52	28.06	11.54	4.02	3980.6	4206.31	0.722	2025.6	2115.8	0.877
41	30.00	7.53	27.97	9.26	4.06	4000.9	4694.82	0.717	2269.4	2303.7	0.874
42	30.18	6.77	27.33	10.17	4.01	3852.2	3924.91	0.715	2357.4	2444.2	0.871
43	37.90	6.73	26.98	9.29	4.05	2861.1	2896.27	0.714	2812.3	3011.7	0.866
44	36.99	8.00	20.20	8.33	11.92	4190.1	4600.42	0.713	2106.2	2201.7	0.864
45	30.56	6.57	27.35	7.54	4.17	2932.9	2976.07	0.712	2174.5	2192.6	0.860
46	30.26	7.89	26.98	8.44	4.00	3473.0	3551.58	0.710	2304.7	2411.5	0.857
47	30.20	7.15	2.93	9.11	4.31	3600.1	3728.59	0.709	2202.3	2302.7	0.854
48	35.91	6.54	27.90	5.97	4.71	3968.0	4077.18	0.708	2202.5	2217.4	0.851
49	31.59	6.51	27.66	8.92	5.99	3791.5	3937.08	0.706	2001.2	2018.2	0.840
50	30.09	6.50	20.03	10.47	4.02	3962.1	4051.91	0.705	2111.0	2121.3	0.837

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

Aluminium and Copper) elements required for organisms growth. This further suggests the use of such digestates as efficient fertilizers/soil conditioner for increasing soil fertility and crop yield enhancement and which can adequately replace chemical inorganic fertilizers in the long run (Westphal et al., 2016). Some of the aerobic organisms implicated in all the digesters have been established in mesophilic AD processes (Dahunsi and Oranusi, 2013; Dahunsi et al., 2016a,b) whereas some are newly encountered. All aerobes reached their highest populations during the third to fourth weeks of digestions because the pH of the digestion media were very acidic at the period. Most aerobes (Bacteria and fungi) thrive mostly in acidic medium. However, most of the facultative anaerobes are newly encountered in anaerobic systems which is due to the composition and hygienic status of both the fermented biomass and rumen contents which serve as sources of organisms. Gas generation was higher in digestion ‘A’ than ‘B’ in quantity and methane content. The highest gas yield in digestion ‘A’ could be attributed to the combination of three pre-treatment (mechanical, thermal and chemical) methods which proved more effective than two pre-treatments (mechanical and Na OH chemical method) employed in digestion ‘B’. A major factor that brought about the clear difference in digestion ‘A’ was thermal pre-treatment via heating. Heating brought about the adequate breakdown or softening of the lignocellulsic matrix of the biomass thus rendering it malleable for microbial degradation and subsequent bioconversion in the digesters. There was a 38.06% increase in gas generation in experiment ‘A’ over ‘B’ and this could easily be linked to the use of thermal treatment in the former. Generally,

Table 3
Test of significance and Analysis of variance (ANOVA) for all regression coefficient terms for biogas generation from *Chromolaena odorata* shoot.

Source	df	Digestion A				Digestion B			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value
X_1	1	1.180	1.180	0.91	0.3663	1.830	1.830	0.67	0.0651
X_2	1	547.60	547.60	4.20	0.9497	508.7	508.7	3.23	0.0746
X_3	1	8.503	8.503	6.52	**0.0310**	7.622	7.622	7.39	**0.0392**
X_4	1	1.236	1.236	9.48	**0.0132**	7.155	7.155	4.17	0.5050
X_5	1	1.122	1.122	0.86	0.3777	2.168	2.168	0.69	**0.0472**
X_1X_2	1	6.277	6.277	4.81	0.0559	5.106	5.106	4.62	0.0661
X_1X_3	1	7.576	7.576	5.81	**0.0392**	6.025	6.025	5.99	0.0762
X_1X_4	1	8.110	8.110	6.22	**0.0342**	7431	7431	5.36	0.5160
X_1X_5	1	172.53	172.53	1.32	0.9718	165.7	165.7	2.04	0.1772
X_2X_3	1	7.390	7.390	5.67	**0.0412**	2628	2628	2.44	**0.0361**
X_2X_4	1	6.977	6.977	5.35	**0.0460**	7.029	7.029	5.13	**0.0453**
X_2X_5	1	2.968	2.968	2.28	0.1656	3.418	3.418	3.08	**0.0431**
X_3X_4	1	59265	59265	0.45	0.5171	2.995	2.995	1.22	0.1190
X_3X_5	1	4.782	4.782	3.67	0.0877	4.589	4.589	4.87	0.1633
X_4X_5	1	1.165	1.165	8.94	**0.0152**	1.113	1.113	10.31	0.0651
X_1^2	1	24966	24966	0.19	0.6720	16516	16516	0.16	0.5550
X_2^2	1	63759	63759	0.49	0.5020	57338	57338	1.70	0.1082
X_3^2	1	1.622	1.622	1.24	0.2936	3.058	3.058	0.29	0.6553
X_4^2	1	2.105	2.105	16.15	**0.0030**	1106	1106	11.03	0.6253
X_5^2	1	55283	55283	0.42	0.5312	82933	82933	0.51	0.8612
Model	20	1.067	5.335	4.09	0.0174	2.410	4.565	3.88	0.0341
Residual	9	1.173	1.304			6.191	1.244		
Lack of Fit	6	1.104	1.839	7.90	0.0590	4.155	1.796	6.89	0.0902
Pure Error	3	802.20	267.40			655.12	254.52		
R-Squared		0.9009				0.8991			
Adequate Precision		11.950				9.902			

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

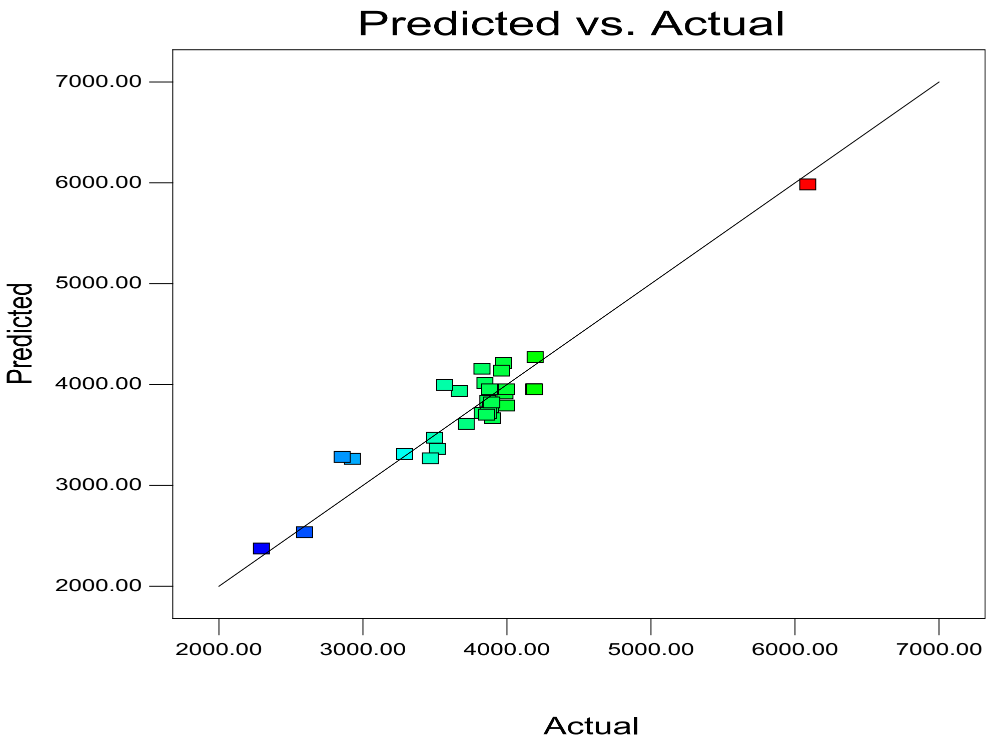

Fig. 3. The figure shows the graph of predicted against the actual biogas yield for the anaerobic co-digestion of *Chromolaena odorata* shoot and poultry dropping.

biogas generation from these mixture was far higher than was obtained from *Carica papayas* peels and poultry dropping (Dahunsi et al., 2016a) and *Telfairia occidentalis* fruit peels (Dahunsi et al., 2016b).

The F-values of the model with corresponding low p-values of 0.0174 and 0.0341 obtained for samples 'A' and 'B' implies that the model is significant. The R^2 value of 0.9009 and 0.8991 for samples 'A' and 'B' implies that the sample variation of 90.09 and 89.91% for the biogas yield from samples 'A' and 'B' is caused by the interactions of the five factors employed in the study. Both values are greater than the 0.80 prescribed for a model's 'goodness of fit'. The 'Adequate Precision' is a based on the ratio of the model's signal and noise and a value of $\geqslant 4$ is preferred for the good fit of a model. The 'Adequate Precision' values of 11.950 and 9.902 for samples 'A' and 'B' are far higher than the prescribed value (4.0) indicates suitability of the model. All the p-values $p < 0.05$ contributes to the suitability of the model for the interactions between the five selected factors. The 'lack-of-fit' terms (0.0590 and 0.0902) were not significant and such is desirable since a non-significant 'lack of fit' is desirable in using a model for the theoretical prediction of the biogas production.

The nature of all the 3-dimensional plots for the optimization of the reaction factors showed less interactions for plots i, ii, iv, and vii while moderate relationships were obtained for plots iii, vi, viii, ix and x. Similar interactions has been reported (Emeko et al., 2015). The predicting capability of RSM model was evaluated to determine its fitness for biogas prediction and modeling for the current substrate. The root mean squared error (RSME) and R^2 values were used. From these, the RSME for samples 'A' and 'B' were 237.40 and 254.52 while the R^2 was 0.9009 i.e. 90.09% and 0.8991 i.e. 89.91% for 'A' and 'B'. This shows that the RSM model gives high accuracy and efficiency in the generation of biogas from *C. odorata* shoot and poultry manure using different pre-treatment methods.

5. Conclusion

The co-digestion of *C. odorata* and poultry manure proved suitable for biogas production as shown in this study. Higher quantity and quality of biogas was equally produced. The optimization/-modeling study revealed that RSM model is efficient in predicting gas production from the substrates. Several failures have been recorded in attempt to control/minimize the invasiveness of Siam weed in different cropping systems. The current study has established the much-awaited solution to the menace of the weed as it should no longer be regarded as a weed but an energy crop because of its rich energy and biofertilizer producing potential.

Conflict of interest

No conflict of interest is declared. All authors agreed to this submission.

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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.2016.11.123.

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