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Co-digestion of *Hyptis suaveolens* (bushmint) and poultry manure for energy generation: Effects of pretreatment methods, modelling and process parameter optimization study

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ABSTRACT

The potentials of anaerobic co-digestion of poultry dropping with chemically pretreated and untreated Hyptis suaveolens (bushmint weeds) shoots for biogas generation, as well as the process optimization after using a combination of mechanical and thermo-alkaline pretreatment methods, were assessed in this study. Inoculum from cattle rumen content was used to anaerobically digest the shoots. A batch experiment was designed using the Central Composite Design (CCD). Standard procedures were used to assess the physicochemical parameters of the substrates and inoculum, as well as the components of the generated biogas. For the chemically pretreated and untreated tests, the experimental biogas yields were 0.7652 L/kg VS and 1.1396 L/kg VS, respectively. The methane and carbon dioxide content of biogas from both experiments was 69.08%; 17.64% and 62.13%; 21.39% respectively. The Response Surface Methodology (RSM) was employed in data optimization. The predicted biogas yield was 0.7652L/kg VS in the chemically treated experiment, and the model's coefficient of determination (R^2) was high (0.9159), indicating strong modelling and prediction accuracy for the chemically treated experiment. There was a 11.19% increase in methane gas yield in the chemically treated experiment over the untreated. The study recommended the worldwide usage of Hyptis suaveolens shoots for biofuel generation and several combinations of pretreatment methods to improve biogas yields.

INTRODUCTION

The world's over-reliance on fossil fuels is taking its toll on humanity in terms of environmental degradation, disease spread, and climate change/global warming via GHG emissions (Anjum *et al*., 2016; Guenther-Lübbers *et al*., 2016; Priebe *et al*., 2016). For this purpose, it is necessary to integrate cleaner production technology and appropriate policy implementation to address the global myriad environmental difficulties, particularly those related to energy generation and consumption (Klemes *et al*., 2012; Kalbar *et al*., 2016). Fossil fuels account for about 88 per cent of worldwide energy use, which is often accompanied by environmental challenges such as GHG emissions and contamination of soil, air, and water (Gonzalez-Garca *et al*., 2016). As a result of this circumstance, multiple research projects have been conducted on the creation of renewable and sustainable energies from various agricultural, industrial, and home resources, with the resulting fuels being reported to be ecologically beneficial and reducing GHG emissions (Mijakovski *et al*., 2016; Leonzio, 2016; Yong *et al*., 2016).

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

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

Hyptis suaveolens (pignut, bushmint weeds, or ewe jogbo, efinrin oso) is an invasive, a ‘rejected’ weed by risk assessment in Australia (PIER, 2016, GBIF, 2016). The most serious invaders annual herbs in the highlands of West-Central India that grow in disturbed habitats. It is a prolific seed producer and in dense infestations can yield up to 3000 seeds/m2, forming persistent propagule banks within a short period (Sharma *et al.,* 2009). It is regarded as an environmental weed in northern Queensland and northern Western Australia, while in the Northern Territory; it is listed as a noxious weed (Queensland Government, 2012). This species originates from the Neotropics, from Central America and the West Indies south to about the tropic of Capricorn. It has been introduced to the tropics and subtropics of the world (including some Pacific Islands), where it has become widely naturalized. Padalia *et al.* (2015) modelled the potential distribution of H. suaveolens and suggested that areas between 34° 02′ north and 28° 18′ south latitudes in the tropics are climatically suitable for this species, with West and Central Africa, tropical southeast Asia and northern Australia at high risk of invasion. This species is common in wetter tropical regions, but it can also occur in sub-tropical and semi-arid environments. At present, there are no control or proven management strategies in place to check the spread of the weeds in Nigeria and other countries. Therefore, this study is an attempt to utilize these weeds for energy generation since green plants are natural sinks for enormous energy as a result of photosynthesis. Their abundance and invasiveness in several locations around the world is an indication that veritable and environmentally-friendly usage needs to be sought for these weeds. This informed our choice of pawpaw peels as a substrate for biogas generation. Being a lignocellulosic biomass, it has a high potential for biodegradation during hydrolysis and fermentation by hydrolytic and acidogenic microorganisms.

Lignocelluloses are frequently employed for the generation of renewable and alternative energy due to their abundance/availability and key roles in GHG emissions reduction when properly exploited, globally (Auburger et al., 2016; Shane *et al.*, 2016). However, the high lignin content of these biomasses has remained a key barrier to their commercial use (Carrere *et al.*, 2016). Several pretreatment approaches, such as biological, mechanical, thermal, and chemical, have been studied to enhance the biodegradability of lignocellulosic biomasses to overcome this major obstacle (Caiet al., 2016; Lalak *et al.*, 2016; Li *et al.*, 2016a, b). These pretreatments often improve digestion efficiency, sludge reduction, digestate dewatering, and microbial diversity, all of which lead to increased methane output. Alkaline pretreatment has proven to be more suited for lignocelluloses than other treatments, particularly in terms of cost and increased methane ououtputDongyan et al. 2014). Furthermore, alkaline treatment has significant potential for the bioenergy industry's future because of its ability to be integrated with other methods such as thermal, ultrasound, and micro-wave to enhance biomass valorization and higher methane output than a single pretreatment approach (Jang and Ahn, 2013).

Livestock waste such as cow dung, and poultry waste generated in large volumes is on the increase with little or no proper disposal treatment thereby resulting in environmental problems which are also applicable to plant weeds and household wastes (Alia *et al.*, 2017). Animal manure has been found in significant quantities all throughout the world. Nigeria generates around 227,500 tons of fresh waste every day, with 1 kg of fresh animal waste producing approximately 0.03 m3 of gas each day (Dong *et al.*, 2009). This implies that Nigeria can theoretically create 6.8 million m^3 of biogas every day, which is comparable to around 3.9 million litres of petroleum in terms of energy. Because agricultural wastes and grasses are considered solid wastes, they are usually burned. These materials are now considered low-cost options for biotechnological biogas generation, thanks to the development of AD technology (Guenther-Lübbers et al., 2016; Othman *et al.*, 2016). Grasslands have already been discovered as high-energy substrates that are also effective at lowering greenhouse gas emissions, according to a previous study (Riggio et al., 2015). The creation of biogas from poultry faeces has been extensively researched, with mixed results. Significant setbacks have been observed as a result of its poor C/N ratio and high total ammonia concentration. Dalkilic and Ugurlu (2015) believe that co-digestion with other high-energy-yielding substrates is the best way to utilize it.

The digestate obtained at the end of the digestion period can be utilized as a soil improvement to increase soil fertility for agricultural produce output (Oladejo *et al.,* 2020). The optimization of bioprocess parameters is an important step for the success of the anaerobic digestion process (Kana *et al.*, 2012; Betiku *et al.*, 2015; Emeko et al., 2015). The aim of this research therefore was to evaluate the biogas-producing potentials of H. suaveolens biomass in co-digestion with poultry droppings. The process parameter optimization of the study was equally carried out using applicable models such as the Response Surface Methodology (RSM).

MATERIALS AND METHODS

Sample Collection and Preparation

Shoots of H. suaveolens biomass and fresh poultry droppings were collected from the Ladoke Akintola University of Technology Teaching and Research Farms and transported to the site of the experiment. Fresh cattle's rumen content was obtained from the Atenda slaughterhouse (abattoir) in Ogbomoso, Oyo State and used as inoculum for digestion. The use of rumen content as inoculum has been reported in many studies (Kana *et al.*, 2012; Alfa *et al.*, 2014a, b). Being a lignocellulosic material, H. suaveolens biomass was pre-treated using a modification of already described mechanical, thermal and chemical pretreatment methods (Alfa *et al.*, 2014a, b; Kim *et al.*, 2015). Only the mechanical approach was used to pretreat the first sample, labelled 'A.' The second sample, designated as 'B,' was a hybrid of mechanical, thermal, and chemical (Na OH) pretreatments, inoculated with cow rumen as previously described in Oladejo *et al.* (2020). A hammer mill was used to crush the biomass into 620 mm mesh sizes, which were then heated for one hour at 80 C in the CLIFTON, 88579 water bath (NICKELELECTRO Ltd., ENGLAND), as greater temperatures have been documented to have an unfavourable effect on the AD system (Liu et al., 2012). After that, a chemical preparation with 4 g/L sodium hydroxide was performed (NaOH). The choice of NaOH was based on previous studies that generated the best results for thermochemical pretreatment of AD substrates among other commonly used alkalis (Li *et al.,* 2015).

Analytical Procedure

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

Design of Experiment via central composite rotatable design (CCRD)

Central Composite Rotatable Design (CCRD) experimental design was employed to design the bioconversion of the biomass to biogas because of its success in improving bioprocessing systems (Betiku *et al.,* 2015; Emeko et al., 2015). Five-level-five factors design was applied, which generated 50 experimental runs including 42 non-centre points and 8 centre points to provide information regarding the interior of the experimental region thus making it possible to evaluate the curvature effect. The alpha value used was 2.37841.

Selected factors for biogas optimization were Temperature (°C): A, pH: B, Retention time (days): C, Total solids (g/kg): D and Volatile solids (g/kg): E. These factors were selected based on their importance in biogas generation and the chosen ranges are based on reports of earlier research. The optimal temperature for most mesophilic digestions has been reported to vary between 30 and 40 ^{0}C (McKennedy and Sherlock, 2015), pH of 6.5–8 has been reported to be best for methanogenesis (Zonta *et al.,* 2013, Olanipekun and Oladejo, 2022a), while the optimal retention time for mesophilic digestion has equally been reported to be within 20–30 days depending on the ambient temperature (Mao *et al.,* 2015). For total and volatile solids, it has been documented that for efficient operation of a liquid anaerobic system, the solid content must be less than 15% but not lower than 4% to avoid total failure (Jain *et al.,* 2015).

Experimental procedures for samples A and B

About 1500g of mechanically pretreated H. suaveolens (sample A) was mixed with 1500g of poultry waste and was further diluted with water in the ratio of 1:1 w/v to form a slurry thus making a total of 6000 cm^3. 574 cm^3 of the slurry was taken for physiochemical analysis. The capacity of the biodigester was 6000 cm^3 (6L) and hence a total slurry of 4226 cm^3 which occupied four-fifths of the biodigester while one-fifth was left for the collection of the gas.

For mechanically, and thermo-chemically pretreated sample B, 1000g of pretreated H. suaveolens was mixed with 1000g of poultry waste, to which one kg of inoculum was added and further diluted with water in the ratio of 1:1 w/v to form a slurry of 6000 cm^3, thus 4226 cm^3 volume of the slurry occupied four-fifth of the biodigester. The slurry was then pumped into each of the digestion tanks through four-fifths of the digester aperture.

Several metrics were assessed at various points throughout the AD to determine treatment efficiency. Daily measurements of generated biogas, as well as physicochemical parameters of feedstock and digestates, are recorded. The average temperature readings and pH values were taken from the daily readings twice and the average was recorded. The daily gas collection used the water displacement method previously described (Dahunsi et al., 2017). A Gas Chromatography-Mass Spectrometry/Electron Ionization (GC-MS/EI) model with a flame ionization detector was used to characterize produced biogas to measure methane and other compounds (FID).

Statistical Data Analysis

The data obtained from biogas generation from each of the digestion regimes was analysed statistically using response surface methodology, to fit the quadratic polynomial equation generated by the Design-Expert software version 9.0.3.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response variable to the independent variables, multiple regressions were used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using the test of significance and analysis of variance (ANOVA). The fitted quadratic response model is described by:

$$Y = \beta_O + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

where Y is the response variable, bo is the intercept, bi (i = 1, 2, k) is the first-order model coefficient, bij is the interaction effect, bii = the quadratic coefficients of Xi, and e is the random error. The model was validated with the same digesters using conditions predicted by the software. The deviations of actual values from the observed values were then plotted.

RESULTS AND DISCUSSION

Physicochemical analysis and biogas production

The results of the residual methane test indicated methane production starting from the second day of the experiment. The average methane content of the biogas ranged between 65% and 67%. The physical and chemical analyses of the substrate, both before and after digestion, as well as the inoculum, are presented in Tables 1 and 2. Throughout the digestion process, the pH of the substrate in all digesters remained slightly alkaline and within the experimental design range of 6.5 to 8, as determined by Response Surface Methodology (RSM). The temperature of the digesters was maintained within the mesophilic range (30–40°C) throughout the experiment, in accordance with the experimental design. Temperature readings fluctuated between 32.5°C and 36°C, consistently aligning with the design parameters. The results of all physical and chemical analyses revealed increases in the values of moisture content, total nitrogen, total phosphorus, potassium, sulfate, phosphate, magnesium, manganese, iron, zinc, aluminium, and copper, while reductions were observed in other parameters after digestion. This trend was previously noted by Olanipekun and Oladejo (2022b).

A significant reduction in the Chemical Oxygen Demand (COD) value of the digested substrate was observed, with a decrease of up to 71.875% across the various setups by the end of the digestion period. Biogas production in all experiments began between the 3rd and 4th day and continued until approximately the 20th to 23rd day, after which a decline was observed, and production gradually diminished until the end of the experiments, as shown in Figure 1 through gas chromatography analysis.

Table 1: Physiochemical analysis of substrates

S/N	PARAMETERS	INNOCULUM (I)	BUSHMINT SHOOT (BS)	POULTRY POOS (PP)	BSIPP (without chemically pretreatment)	BSIPPC (chemically Pretreated)	UNIT
1	pH	6.21	6.79	7.79	7.29	8.16	-
2	Total Alkalinity	240	360	175	235	340	Mg/L
3	Total Nitrogen	20.0	52.8	26.8	21.5	30.5	Mg/L
4	T.Phosphorus	2.15	4.82	2.65	2.35	3.64	Mg/L
5	Total Carbon	306.5	332.6	273.8	368.4	365.8	Mg/L
6	Potassium	3.5	5.8	3.7	3.4	4.2	Mg/L
7	Phosphate	96.8	245.0	98.7	130	185	Mg/L
8	Sulphate	56	97	64	56	77	Mg/L
9	Calcium	42	82	48	52	24	Mg/L
10	Magnesium	29	110	45	34	60	Mg/L
11	Manganese	0.0012	0.041	0.023	0.011	0.021	Mg/L
12	Iron	3.00	6.20	3.98	3.60	5.00	Mg/L
13	Zinc	12.0	26.4	14.49	16.5	23.0	Mg/L
14	Aluminum	0.25	0.80	0.37	0.36	0.39	Mg/L
15	Copper	1.70	4.45	2.45	2.15	2.75	Mg/L
16	BOD	236	146	235	268	256	Mg/L
17	COD	840	680	760	1116	1360	Mg/L
18	C/N	12:1	11:1	10:1	15:1	15:1	-
19	Wt of the sample	1556.4	1426.2	1559.7	4988.7	4988.2	(g)
20	Volume of the sample	1408.7	1408.7	1408.7	4226	4226	Cm^3
21	Moisture cont	83.2%	85.4%	80.6	79.4	79.6	%
22	% Total solids	16.8%	12.94	18.87	23.6	23.2	%
23	% Fixed solids	9.5%	13.87	13.8	13.43	13.39	%
24	% Volatile solids	84.94%	86.32	83.26	85.8	83.5	%

Analysis revealed the gas composition to be within the range of 62–69% methane and 17–21% carbon dioxide for the digestion of *Hyptis suaveolens* and poultry dropping. The biogas yields from samples A and B were 1.1396 and 0.7652 (L/kg VS), respectively (Figures 1), with 62.13% methane and 21.39% carbon dioxide for sample A (Table 3) and 69.08% methane and 17.64% carbon dioxide for sample B (Table 4). Overall, sample B outperformed A by 11.19% in terms of methane gas generation. Further statistical analysis and optimization was carried on experiment B because of the high methane yield.

Table 2: Physiochemical analysis of digestates

S/N	Parameters	BSIPP (without chemical pretreatment)	BSIPPC (chemically Pretreated)	Unit
1	pH	7.36	8.09	-
2	Total Alkalinity	220	325	Mg/L
3	Total Nitrogen	22.4	28.7	Mg/L
4	Total Phosphorus	2.33	3.59	Mg/L
5	Total Carbon	351.7	358.2	Mg/L
6	Potassium	3.0	3.7	Mg/L
7	Phosphate	115	152	Mg/L
8	Sulphate	53	69	Mg/L
9	Calcium	47	26	Mg/L
10	Magnesium	31	58	Mg/L
11	Manganese	0.010	0.020	Mg/L
12	Iron	3.54	4.87	Mg/L
13	Zinc	15.2	21.8	Mg/L
14	Aluminum	0.32	0.37	Mg/L
15	Copper	2.17	2.70	Mg/L
16	BOD	272	266	Mg/L
17	COD	1108	1286	Mg/L
18	C/N	12:1	12:1	-
19	Wt of the sample	4917.3	4827.1	(g)
20	Volume of the sample	4122	4149	Cm^3
21	% Moisture content	73.8	75.2	%
22	% Total solids	26.7	26.3	%
23	% Fixed solids	16.94	16.36	%
24	% Volatile solids	77.56	76.83	%

RSM optimization of biogas data

Table 5 shows the experimental design matrix by the Central Composite Rotatable Design (CCRD) for the five-level-five-factor response surface study for biogas generation from experiment B- BSIPPC. The experimentally observed and predicted yields as well as the residual values are shown in the table. From the results obtained, as shown in Table 6, the Model F-value of 42.12 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Furthermore, P-values less than 0.0500 indicate model terms are significant. In this case, AC, AE, BC, BE, CE, A^2, D^2, E^2 are significant model terms. are significant model terms. This is because values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The following is a description of the fit model statistics and the values obtained. The predicted R^2 of 0.9159 was very close to the adjusted R^2 of 0.9659 as normally expected because the difference is not more than 0.2. This therefore indicated that there was not a large block effect with the model and/or data. Also, adequate precision is used to measure the signal-to-noise ratio and usually, a ratio greater than 4 is always desirable. Therefore, the ratio of 15.4243 of this study indicated that the signal is adequate and this means the model is fit enough for the navigation of the design space.

The equation in terms of the coded factors (equ. 2) was used to make predictions about the response for given levels of each factor. By default, the high levels of the factors were coded as +1 and the low levels were coded as -1. The coded equation was also used for identifying the relative impacts of the factors through the comparison of the factor coefficients. From the results obtained as shown in Table 6, the recommended optimal conditions from the chemical pretreatment process from co-digestion of bushmint shoot, inoculum and poultry poo in this study are; temperature (40 ^{0}C), pH (6), retention time (30 days), total solids (4 g/Kg) and volatile solids (12 g/Kg), were statistically predicted as the best conditions for each factor with 0.898 or approx. 90 per cent. The projected biogas yield under these ideal conditions was 0.7652 L/kg VS.

CONCLUSIONS

As shown in this study, the co-digestion of *Hyptis suaveolens* (bushmint) shoots and poultry manure was suitable for biogas production. Biogas was produced in greater amounts and quality, particularly from sample B. This was due to the influence of inoculum (cow rumen) and the effects of alkaline pretreatment procedures. The digestates formed after digestion were mineral-rich, and their application as a soil improvement for crop growth could be beneficial. The RSM model was found to be effective in predicting gas generation from substrates during the process optimization and equation modelling investigation. *Hyptis suaveolens* shoots is an adaptable, fast-growing, precociously seeding weed with the ability to disseminate seeds up to 40 meters from the parent plant, as well as an exceptionally versatile nitrogen-fixing weed.

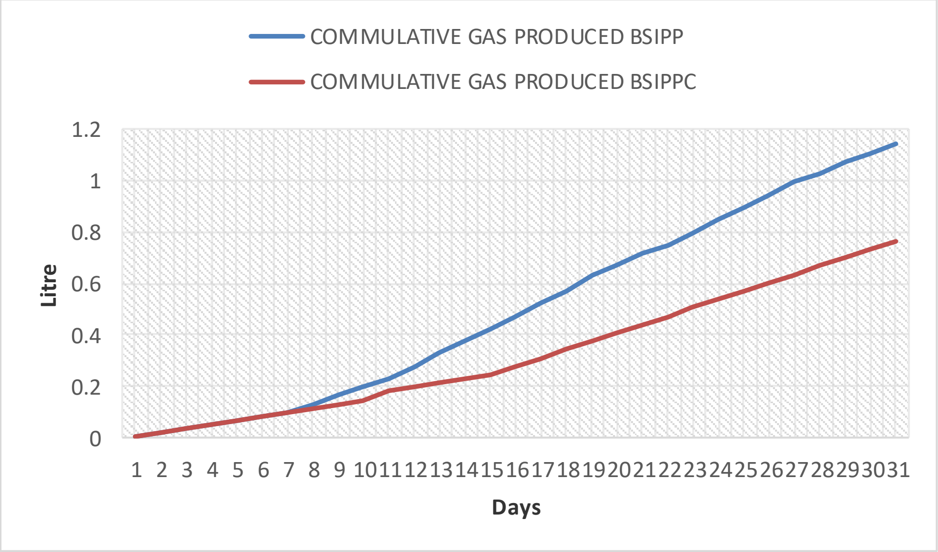

Figure 1: Graph of cumulative gas produced from BSIPP and BSIPPC against number of days

Table 3: Gas Chromatography-Mass Spectrometry Analysis of BSIPP

Peak	Retention time	Name of Gas	Molecular Formula	Molecular Mass	Peak Area (%)	% Composition
1	12.41	Helium	He	4	0.95	0.02
2	26.08	Nitrogen	N_2	44	5.70	2.32
3	27.11	Methane	CH_4	16	19.87	62.13
4	31.04	Standard	STD	STD	STD	STD
5	36.00	Carbon dioxide	CO_2	28	15.07	21.39
6	39.48	Hydrogen	H_2	2	3.77	0.03
7	40.32	Ethane	C_2H_6	30	17.02	0.58
8	42.00	Oxygen	O_2	32	9.44	0.21
9	50.99	Hydrogen Sulphide	H_2S	34	6.61	0.14
10	59.21	Argon/Oxygen composite-	Ar/O_2	18/32	4.12	0.10
11	63.98	Carbon monoxide	CO	28	5.68	0.12

Table 4: Gas Chromatography-Mass Spectrometry Analysis of BSIPPC

Peak	**Retention time**	**Name of Gas**	**Molecular Formula**	**Molecular Mass**	**Peak Area (%)**	**% Composition**
1	19.98	Helium	He	4	5.01	0.01
2	21.98	Methane	CH_4	16	16.06	69.08
3	23.05	Nitrogen	N_2	44	0.87	2.13
4	28.00	Standard	STD	STD	STD	STD
5	35.37	Hydrogen Sulphide	H_2S	28	5.24	0.44
6	40.29	Hydrogen	H_2	2	4.24	0.02
7	42.11	Ethane	C_2H_6	30	12.22	1.30
8	43.99	Oxygen	O_2	32	3.44	0.08
9	45.10	Carbon dioxide	CO_2	34	13.97	17.64
10	58.21	Argon/Oxygen composite-	Ar/O_2	18/32	6.94	0.09
11	76.00	Carbon monoxide	CO	28	3.48	0.07

Table 5: Experimental design matrix by central composite design (CCD) for five-level-five-factors response surface study for biogas yield for experiment B- BSIPPC

Run	A	B	C	D	E	Actual value L/kgVS	Predicted Value L/kgVS	Residual	Leverage
1	40	6	30	4	4	0.03256	0.0316	0.0009	0.810
2	35	4.6	25	8	8	0.01628	0.0171	-0.0008	0.866
3	30	6	20	12	12	0.03256	0.0311	0.0015	0.896
4	30	8	20	12	12	0.03256	0.0334	-0.0009	0.733
5	30	8	20	4	4	0.01628	0.0161	0.0002	0.716
6	30	6	20	4	12	0.03256	0.0326	0.0000	1.000(3)
7	30	6	20	4	4	0.03256	0.0311	0.0015	0.896
8	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
9	35	7	25	8	17.5	0.03256	0.0330	-0.0004	0.887
10	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
11	35	7	25	1.5	8	0.03256	0.0336	-0.0011	0.877
12	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
13	40	8	20	12	4	0.01628	0.0162	0.0001	0.898
14	35	9.4	25	8	8	0.01628	0.0166	-0.0003	0.838
15	40	8	30	4	12	0.03256	0.0320	0.0005	0.841
16	30	6	20	12	4	0.03256	0.0344	-0.0019	0.614
17	30	8	20	12	4	0.01628	0.0149	0.0014	0.833
18	30	8	30	4	12	0.03256	0.0311	0.0015	0.896
19	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
20	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
21	40	6	20	4	12	0.01628	0.0162	0.0001	0.898
22	30	6	30	12	4	0.03256	0.0311	0.0015	0.896
23	30	8	30	4	4	0.01628	0.0165	-0.0002	0.741
24	35	7	25	8	8	0.01628	0.0164	-0.0001	0.165
25	35	7	13.1	8	8	0.01628	0.0164	-0.0001	0.799
26	40	6	30	12	12	0.01628	0.0164	-0.0001	0.815
27	35	7	25	8	1.5	0.03256	0.0332	-0.0007	0.817
28	35	7	36.9	8	8	0.01628	0.0172	-0.0010	0.825
29	40	6	20	12	12	0.01628	0.0160	0.0002	0.741
30	23.1	7	25	8	8	0.03256	0.0336	-0.0011	0.877

Table 6: Analysis of variance (ANOVA) for Quadratic model and Test of significance and for all regression coefficient terms for biogas yield for experiment B- BSIPPC.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	0.0019	20	0.0001	42.12	< 0.0001	significant
A-Temperature	4.328E-06	1	4.328E-06	1.89	0.2027	
B-pH	1.611E-07	1	1.611E-07	0.0702	0.7970	
C-Retention time	5.014E-07	1	5.014E-07	0.2186	0.6512	
D-Total solids	1.824E-06	1	1.824E-06	0.7953	0.3957	
E-Volatile solids	3.153E-08	1	3.153E-08	0.0138	0.9092	
AB	0.0000	1	0.0000	4.63	0.0598	
AC	0.0001	1	0.0001	24.92	0.0007	
AD	5.037E-07	1	5.037E-07	0.2196	0.6505	
AE	0.0000	1	0.0000	20.08	0.0015	
BC	0.0000	1	0.0000	6.16	0.0348	
BD	0.0000	1	0.0000	4.51	0.0626	
BE	0.0003	1	0.0003	110.59	< 0.0001	
CD	3.712E-06	1	3.712E-06	1.62	0.2352	
CE	0.0000	1	0.0000	10.34	0.0106	
DE	8.489E-06	1	8.489E-06	3.70	0.0865	
A^2	0.0002	1	0.0002	69.01	< 0.0001	
B^2	2.766E-07	1	2.766E-07	0.1206	0.7363	
C^2	2.766E-07	1	2.766E-07	0.1206	0.7363	
D^2	0.0002	1	0.0002	82.20	< 0.0001	
E^2	0.0004	1	0.0004	187.76	< 0.0001	
Residual	0.0000	9	2.293E-06			
Lack of Fit	0.0000	4	5.160E-06			
Pure Error	0.0000	5	0.0000			
Cor Total	0.0020	29				
R^2	0.9894					
Adjusted R^2	0.9659					
Predicted R^2	0.9159					
Adeq Precision	15.4243					

Final Equation in Terms of Coded Factors:

$$Y = 0.0163987 - 0.000672826 * A - 0.000104368 * B + 0.000174326 * C + 0.000552193 * D - 4.61816e\text{-}05 * E + 0.00123156 * AB + 0.00309324 * AC + 0.000324105 * AD - 0.00285795 * AE + 0.00182993 * BC - 0.0011413 * BD + 0.00546431 * BE + 0.000897273 * CD - 0.00218042 * CE - 0.00119903 * DE + 0.0027657 * A^2 + 7.48436e\text{-}05 * B^2 + 7.48436e\text{-}05 * C^2 + 0.00328076 * D^2 + 0.00295277 * E^2$$

Y = (Biogas Yield, L/kg VS)

A = Temperature; B = pH; C = Retention time; D = Total solids: E = Volatile solids.

However, the current study has established the long-awaited solution to the weed's menace, as it should no longer be viewed as a stubborn plant but an energy crop because of its rich energy and biofertilizer-producing potential.

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